Branched-chain amino acids in metabolic signalling and insulin resistance

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Abstract | Branched-chain amino acids (BCAAs) are important nutrient signals that have direct and indirect effects. Frequently, BCAAs have been reported to mediate antiobesity effects, especially in rodent models. However, circulating levels of BCAAs tend to be increased in individuals with obesity and are associated with worse metabolic health and future insulin resistance or type 2 diabetes mellitus (T2DM). A hypothesized mechanism linking increased levels of BCAAs and T2DM involves leucine-mediated activation of the mammalian target of rapamycin complex 1 (mTORC1), which results in uncoupling of insulin signalling at an early stage. A BCAA dysmetabolism model proposes that the accumulation of mitotoxic metabolites (and not BCAAs *per se)* promotes β-cell mitochondrial dysfunction, stress signalling and apoptosis associated with T2DM. Alternatively, insulin resistance might promote aminoacidaemia by increasing the protein degradation that insulin normally suppresses, and/or by eliciting an impairment of efficient BCAA oxidative metabolism in some tissues. Whether and how impaired BCAA metabolism might occur in obesity is discussed in this Review. Research on the role of individual and model-dependent differences in BCAA metabolism is needed, as several genes (*BCKDHA, PPM1K, IVD* and *KLF15*) have been designated as candidate genes for obesity and/or T2DM in humans, and distinct phenotypes of tissue-specific branched chain ketoacid dehydrogenase complex activity have been detected in animal models of obesity and T2DM.

Introduction

Branched-chain amino acids (BCAAs; that is, leucine, isoleucine and valine) are essential amino acids and BCAA supplementation or BCAA-rich diets are often associated with positive effects on the regulation of body weight, muscle protein synthesis and glucose homeostasis. Leucine is a particularly important nutrient signal: levels of this BCAA increase in the circulation after consumption of a protein-containing meal. Despite these effects on metabolic health, studies have highlighted that along with blood sugar, insulin and certain inflammatory markers, increased fasting concentrations of circulating BCAAs are associated with an increased risk of type 2 diabetes mellitus (T2DM) and insulin resistance in humans and in some rodent models. This consistent observation in cross-sectional and prospective human studies, along with limited studies suggesting that BCAA supplementation leads to deterioration in insulin sensitivity, has prompted two questions. Firstly, are BCAAs or BCAA-rich diets harmful, helpful or neutral with respect to insulin and glucose homeostasis? And secondly, what is the aetiology of altered blood levels of BCAAs in the insulin-resistant state? This Review provides evidence that under most conditions, alterations in fasting blood levels of BCAAs in the obese insulin-resistant state result from changes in the rate of appearance and clearance of these metabolites, coupled with decreased activity of catabolic enzymes in some tissues compared with the insulin-sensitive state. Perturbations in BCAA levels probably reflect the insulin resistant and T2DM 'patho-phenotypes' and BCAAs themselves are probably not necessary or sufficient to trigger disease.

BCAAs and metabolic health

The results from a number of interventional studies have suggested that increasing dietary levels of BCAAs should have a positive effect on the parameters associated with obesity and T2DM, such as body composition, glycaemia levels and satiety. Direct and indirect mechanisms for these positive effects have been proposed. For example, leucine seems to have direct effects on hypothalamic and brainstem processes involved in satiety.[1–22]

In the gastrointestinal tract and in fat deposits, BCAAs regulate the release of hormones (for example, leptin, GLP-1 and ghrelin) that can potentially affect food intake and glycaemia levels.[15,16,20,23–25] BCAAs and insulin are anabolic signals that alter the growth of energy-consuming tissues, mediated in part through their ability to activate the mammalian target of rapamycin complex 1 (mTORC1) and protein kinase Cε (PKCε),[26–34] as well as by decreasing protein breakdown through unknown mechanisms.[27] Synthesis of muscle protein is thought to underlie the higher level of diet-induced thermogenesis (energy wasting) associated with protein

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Key points

- Branched-chain amino acids (BCAAs) have beneficial nutrient signalling effects but paradoxically are associated with obesity, insulin resistance and type 2 diabetes mellitus (T2DM)
- BCAAs might be a marker of, rather than, a cause of insulin resistance, as insulin resistance increases the rate of appearance of BCAAs and is linked to reduced expression of mitochondrial BCAA catabolic enzymes
- Alternatively, two mechanisms have emerged indicating that a causative link exists between increased plasma concentrations of BCAAs and T2DM or insulin resistance
- In the first mechanism, persistent activation of the mammalian target of rapamycin complex 1 signalling pathway uncouples the insulin receptor from the insulin signalling mediator, IRS-1, which leads to insulin resistance
- In the second mechanism, abnormal BCAA metabolism in obesity results in accumulation of toxic BCAA metabolites that in turn trigger the mitochondrial dysfunction and stress signalling associated with insulin resistance and T2DM
- Factors that alter expression of genes involved in the BCAA metabolic pathway (or post-translational modification of the encoded proteins) are associated with obesity and T2DM; three genes in the pathway are candidate genes for obesity and/or T2DM

consumption or BCAA infusion than that associated with other nutrients.[35–40] Supplementation of BCAAs also seem to result in health benefits in a number of liver diseases.[41–45] As a consequence, supplementation with BCAAs or a BCAA-rich diet is believed to improve metabolic health; an increase in the recommended dietary allowance for protein has been proposed, which would effectively increase dietary levels of BCAAs.[7,13,20,22,46–51] Nevertheless, the idea that BCAAs or their supplementation might have a positive role in preventing metabolic disease is controversial.

Circulating BCAA levels and poor health

A number of observational studies indicate that elevated circulating levels of BCAAs are associated with poor metabolic health. Given the above cited studies suggesting health benefits of high levels of dietary BCAAs, it might seem paradoxical that levels of BCAAs tend to be increased, not decreased, in insulin-resistant obesity and T2DM (Figure 1).[52–71] Such increases are consistently observed in patients with T2DM or obesity and in some rodent models of obesity or T2DM.[59,63,72–75] Furthermore, increased levels of BCAAs have been linked to the metabolic syndrome and cardiovascular disease.[59,60,76–78] In clinical studies, increased blood levels of BCAAs positively correlate with insulin resistance,[63,79] HOMA index[79] and levels of HbA_{1c} (Figure 1).[66,79] Several longitudinal studies in different cohorts have reported that increased blood levels of BCAAs are predictive of future insulin resistance or T2DM,[54,62] which has led to speculation about a potential causative role for BCAAs. Although these associations are consistently observed in human populations, the mechanisms underlying the relationship remain to be fully established. Another issue is that when circulating levels of BCAAs increase, it is possible that they compete with the uptake of amino acid precursors of dopamine and 5-hydroxytryptamine in the brain.[80–83] Although speculative, loss of these precursors could contribute to the increased risk of depression in individuals with obesity.[84,85]

Processes affecting circulating BCAA levels

To understand the seemingly conflicting findings regarding circulating BCAA levels and health it is helpful to appreciate the processes that contribute to BCAA rates of appearance (R_a) and disappearance (R_d) in the blood. Processes contributing to R_a include food intake and tissue protein degradation. However, in the case of the frequently reported association between blood levels of BCAAs and insulin resistance, the phenotype is not due to recent (for example, within a few hours) overeating, as almost all studies were conducted in the overnight-fasted state. That is not to say that overeating is not a culprit in obesity, but rather that other processes affecting the BCAA R_a and/or R_d are responsible for the plasma BCAA concentration in fasting individuals, including those with obesity.

Regulation of protein degradation can occur through changes in autophagy or proteasomal-mediated degradation. Rates of protein degradation in muscle and liver can be inhibited by insulin, insulin-like growth factor 1 (IGF-1) and BCAAs via impairment of autophagy mediated by mTORC1 and AKT (also known as PKB)[27,86–92] and the ubiquitin proteasomal pathway.[29,87,93–99] Indeed, whereas insulin is able to stimulate protein synthesis in newborn pigs,[100] in adult humans there is what has been called a 'specific effect' of insulin on protein degradation.[101] Consistently, amino acids but not insulin stimulated protein synthesis in leg muscle, whereas insulin but not amino acids attenuated the breakdown of proteins in the leg muscles of humans.[99] BCAAs and insulin usually act additively or synergistically to activate mTORC1. This additivity, in addition to the more specific action of insulin on protein degradation, might explain the finding that despite elevated BCAA levels, protein degradation is frequently increased in fasting individuals with obesity and insulin resistance, and in those with poorly controlled T2DM.[61,73,102–106] It is tempting to speculate that elevated protein degradation might be attenuated by providing additional BCAAs in the diet (rather than by avoiding them), as even overnight infusion of BCAAs caused sustained decreases in muscle protein degradation.[107]

The major processes affecting the BCAA R_d include protein synthesis, excretion and BCAA catabolism and/or oxidation. As already mentioned, insulin and amino acids stimulate protein synthesis during growth in newborn pigs.[100] In weight-stable adult humans, this effect of insulin is either less important or not apparent (even though protein degradation is affected), whereas amino acids and IGF-1 stimulate protein synthesis in adult humans.[101] Counterintuitively, in insulin-resistant obesity and untreated T2DM, several studies have suggested that synthesis of muscle protein is either unchanged or increased.[61,102–106] In these situations, muscle mass can decrease because protein degradation is increased more than protein synthesis. BCAA excretion could also be affected by insulin-resistant obesity, owing to the increased levels of circulating amino acids,[73] but the degree to which this process is affected in humans remains to be established. BCAA oxidation is discussed further later.
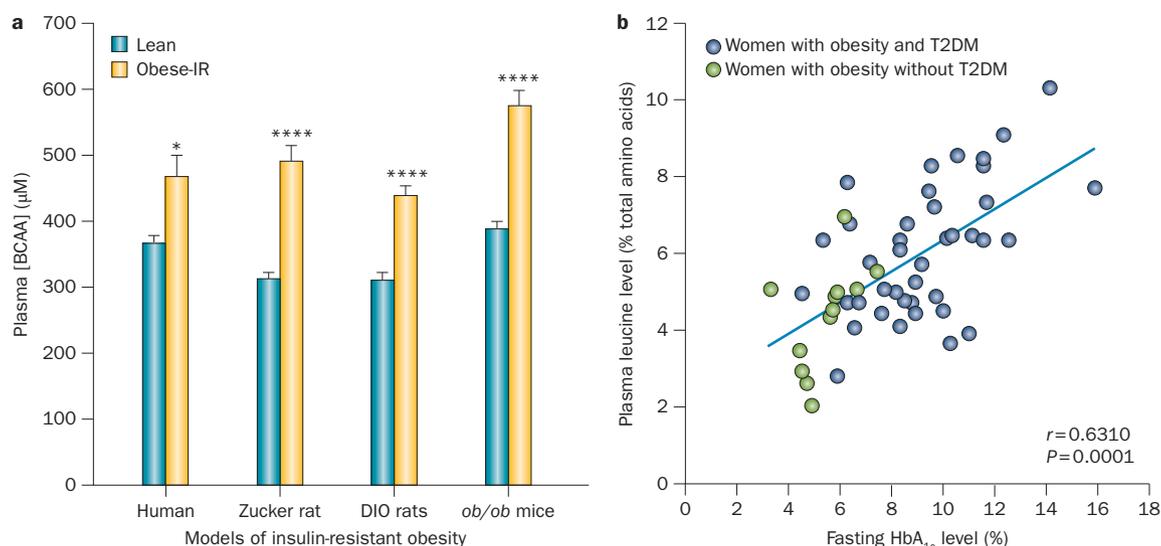
Figure 1 | Plasma BCAA levels and insulin-resistant obesity. **a** | Association between plasma BCAA levels and insulin-resistant obesity in humans, obese Zucker rats, mice with diet-induced obesity (DIO) and *ob/ob* mice. Data were compiled from elsewhere and redrawn.[63,72,73,75] **b** | Correlation between plasma levels of leucine and fasting levels of HbA$_{1c}$ in African–American women with obesity and T2DM (blue circles) and those with obesity but no T2DM (green circles). Abbreviations: BCAA, branched-chain amino acid; IR, insulin resistant; T2DM, type 2 diabetes mellitus. Adapted from Fiehn, O. *et al. PLoS ONE* **5**, e15234 (2010),[66] which is published under a Creative Commons Licence owned by PLOS ©.

Branched-chain aminoacidaemia and T2DM

Despite evidence that elevated levels of BCAAs predict future insulin resistance or T2DM, it is still unclear whether BCAAs are a causative factor in insulin resistance and T2DM or just a biomarker of impaired insulin action. In terms of the uptake of amino acids by the central nervous system, it is also unclear whether obesity causes depression or whether reverse causality exists;[108] causation versus association is an important distinction. If BCAAs improve satiety, cholesterolaemia, glycaemia and lean mass, supplementation with BCAAs might be of therapeutic value. Two potential mechanisms explaining how BCAAs might contribute to insulin resistance in obesity and T2DM have emerged (Figures 2 and 3). The first mechanism proposes that an excess of dietary BCAAs activates mTORC1 signalling, which leads to insulin resistance and T2DM. The second mechanism has its origins in studies of maple syrup urine disease (MSUD) and organic acidurias. This alternative mechanism proposes that in animal models or in individuals with impaired BCAA metabolism (referred to as BCAA dysmetabolism), increased levels of BCAAs are a biomarker of impaired metabolism; however, BCAA dysmetabolism also leads to the accumulation of toxic metabolites that cause mitochondrial dysfunction in pancreatic islet β cells (or elsewhere) and is associated with insulin resistance and T2DM.

Role of mTORC1

Persistent nutrient signalling might cause insulin resistance by BCAA activation of the mTORC1 signalling pathway (Figure 2).[59,60,109,110] Persistent activation of the serine kinases S6K1 and mTORC1 promotes insulin resistance through serine phosphorylation of insulin receptor substrate (IRS)-1 and IRS-2, which might occur in response to persistent hyperinsulinaemia or aminoacidaemia. In essence, the theory proposes that over time, the increased demand for insulin from impaired insulin action, along with inflammation and lipotoxicity associated with insulin resistance, elicits hyperinsulinaemia and exhaustion of the β cells. Eventually euglycaemia can no longer be maintained and T2DM becomes evident.

Currently, it is unclear if this putative effect of BCAAs occurs in humans and to what extent other potential mediators besides BCAAs contribute to this scenario. Furthermore, while some experimental evidence supports this model, a number of observations do not support the concept of BCAA activation of mTORC1 as being necessary and sufficient to elicit insulin resistance. Firstly, although increases in BCAAs are associated with mTORC1 signalling in skeletal muscle, this pathway is also activated by exercise, a known factor in the prevention and regression of insulin-resistant and T2DM phenotypes. Secondly, supplementing or increasing circulating levels of BCAAs is associated with metabolic improvements despite increased mTORC1 signalling.[21,111] Thirdly, in contrast to studies where large doses of leucine were orally administered,[28] it is unclear whether the small changes in BCAA levels observed in patients with obesity and insulin-resistance are sufficient to independently affect serine phosphorylation of IRS-1 and IRS-2 (or to what extent other factors such as insulinaemia or inflammatory mediators might contribute to these phosphorylations). For example, individuals with morbid obesity have raised levels of BCAAs that normalize after gastric bypass surgery;[72,112–114] however, mTORC1 activation in muscle did not change after gastric bypass surgery in a longitudinal study.[113] Fourthly, isoleucine has been reported to reduce plasma levels of glucose by stimulating glucose uptake in skeletal
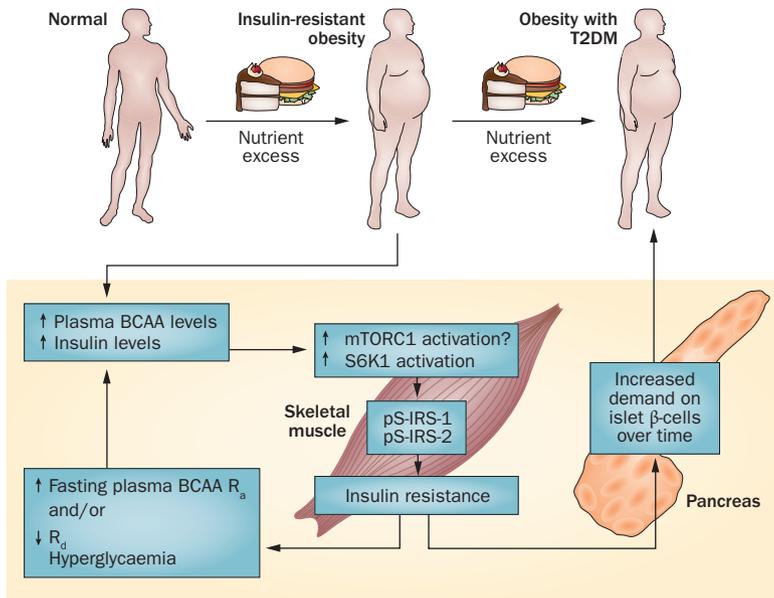
Figure 2 | Persistent activation of mTORC1 links increased plasma BCAA levels to insulin resistance. According to this theory,[59,109,110] excess nutrients that lead to obesity also result in frequent prandial increases in plasma levels of leucine, which together with insulin activate mTORC1 and S6K1. Persistent activation leads to serine phosphorylation of IRS-1 and IRS-2, which interferes with signalling and might target IRS1 for proteolysis via a proteasomal pathway.[109,110] The resulting insulin resistance increases demand on insulin to dispose of excess glucose. Insulin resistance might increase the R_a of BCAAs from protein degradation. Long-term demand for insulin secretion, along with other factors such as lipotoxicity, might negatively affect the function of islets (for example, an initial compensatory increase in β-cell numbers and mass and islet mass, followed by apoptosis), ultimately resulting in a failure to produce sufficient quantities of insulin and leading to the onset of T2DM. Abbreviations: BCAA, branched-chain amino acid; IRS, insulin receptor substrate; mTORC1, mammalian target of rapamycin complex 1; R_a, rate of appearance; R_d, rate of disappearance; S6K1, ribosomal protein S6 kinase β1; T2DM, type 2 diabetes mellitus.

muscle by an unknown mechanism.[115,116] Similarly, an analogue of isoleucine (4-hydroxyisoleucine) increased the glucose R_d in euglycaemic hyperinsulinaemic clamp studies.[117] Finally, in adipose tissue, mTORC1 is important for the increase in fat cell mass associated with the positive effects of PPAR-γ agonists on whole body insulin sensitivity.[118]

Although the focus of the mTORC1 hypothesis is on activation of mTORC1 in skeletal muscle, to the best of our knowledge none of the more recently described candidate genes for obesity, HbA_{1c} and T2DM seem to be involved in activation of the mTORC1 signalling pathway.[119–121] Most T2DM candidate genes are thought to exert their actions in pancreatic islets, not in skeletal muscle.[119] However, rather than causing T2DM, activation of mTORC1 in β cells has been linked to avoidance of T2DM, which is associated with compensatory increases in islet and β-cell mass.[122–128] Adverse effects of the mTORC1 blocker, sirolimus, have consistently included two hallmarks of the metabolic syndrome, hyperglycaemia and dyslipidaemia, along with new onset diabetes after transplantation.[122–125,129] Those actions might be secondary to the ability of rapamycin to inhibit mTORC1 and to interfere with mTORC2 assembly

over time, which in turn can affect AKT activity.[126–128] Both the impaired peripheral resistance that occurs in response to chronic rapamycin treatment and a second effect on islets that impairs their ability to produce sufficient insulin are thought to underlie new onset diabetes after transplantation.[129] Thus, a number of studies do not support the notion that sustained activation of mTORC1 promotes islet dysfunction or T2DM.[122]

BCAA dysmetabolism
In addition to the 'persistent activation of mTORC1' mechanism, there is a second hypothetical mechanism explaining how increased levels of BCAAs might be causally linked to insulin resistance and T2DM. Impairments in BCAA metabolism (BCAA dysmetabolism) could result in the accumulation of potentially toxic intermediates that impair cellular function. Individuals or animal models with a phenotype of impaired or incomplete BCAA metabolism might thereby have increased susceptibility to insulin resistance or T2DM. According to this model,[75] an accumulation of toxic BCAA metabolites (rather than BCAAs themselves) contributes to β-cell mitochondrial dysfunction and eventually the apoptosis of β cells that accompanies T2DM (Figure 3). Nevertheless, elevated levels of BCAAs would be evident with such dysfunction.

The BCAA metabolic pathway
The first step in the metabolism of BCAAs in most peripheral tissues, except the liver, is catalysed by the mitochondrial isoform of branched-chain-amino-acid transaminase, BCAT(m), encoded by the *BCAT2* gene. One piece of evidence supporting a putative mechanism of BCAA dysmetabolism derives from the phenotype of *BCAT2*−/− mice. Deletion of *BCAT2* largely prevents BCAA metabolites from forming in peripheral tissues. Rather than exhibiting insulin resistance as might be expected from the mTORC1 persistent activation mechanism (Figure 2), *BCAT2*−/− mice exhibit greatly improved glycaemic control, insulin sensitivity, adiposity and lipid profiles, despite overall increased mTORC1 signalling and increased energy expenditure (Box 1).[111] One caveat is that at least some of the improvements in glycaemic control in BCAT−/− mice probably result from the loss of gluconeogenic precursors, a reflection of the important role that muscle transaminases have in generating gluconeogenic substrates for the liver (Box 1).

After BCAT(m), the next step in the BCAA metabolic pathway is rate-controlling and the first irreversible step in BCAA metabolism. This step is catalysed by the multienzyme mitochondrial branched-chain α-ketoacid dehydrogenase complex (BCKDC), which results in the oxidation of BCAAs to their respective ketoacids (Figure 4). BCKDC activity is inhibited by phosphorylation at a single site by the specific kinase, branched-chain α-ketoacid dehydrogenase kinase (BCKDK, Figure 4). Conversely, BCKDC is activated by the mitochondrial isoform of protein phosphatase 1K (PPM1K, also known as PP2CM, Figure 4). A number of metabolic factors altered in obesity, insulin resistance and T2DM affect
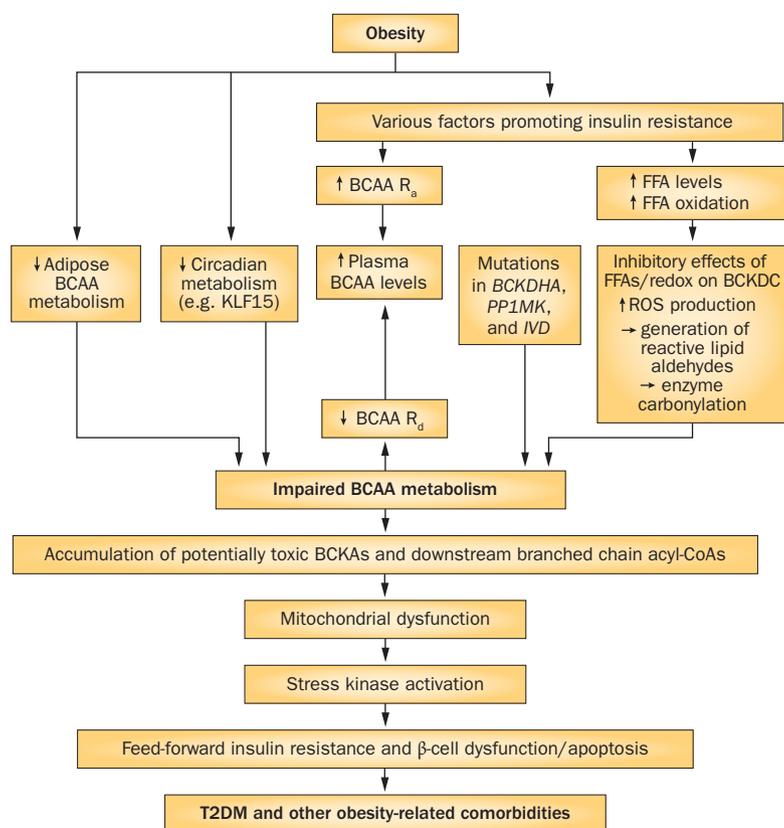
Figure 3 | BCAA dysmetabolism links elevated plasma levels of BCAAs and FFAs to T2DM and obesity-related comorbidities. The schematic shows how obesity might affect a number of factors contributing to elevated circulating BCAA levels via effects on the R_a or R_d of BCAAs. Loss of steps in BCAA metabolism could lead to the accumulation in tissues of BCKAs and BCAA-related acyl-CoAs. Accumulation of these species in inherited disorders can be mitotoxic and might lead to T2DM and other obesity-related comorbidities. A caveat is that while the metabolites of BCAAs are potentially toxic in maple syrup urine disease and organic acidurias, their role in T2DM-associated mitochondrial dysfunction or in activation of stress kinases is unknown. Alternatively, reduced or incomplete valine and isoleucine catabolism could attenuate anaplerosis from these substrates, contributing to anaplerotic stress in one or more tissues affected by T2DM. Abbreviations: BCAA, branched-chain amino acid; BCKA, branched-chain α-keto acid; BCKDC, branched-chain α-keto acid dehydrogenase complex; CoA, coenzyme A; FFA, free fatty acid; KLF15, Krueppel-like factor 15; R_a, rate of appearance; R_d, rate of disappearance; ROS, reactive oxygen species; T2DM, type 2 diabetes mellitus.

the activity and expression of these enzymes (Table 1). Mutations in genes encoding subunits of BCKDC or in *PPM1K* can lead to MSUD—a potentially lethal disease that results in elevated levels of BCAAs and branched-chain α-ketoacids (BCKAs, Box 1), the latter of which are widely believed to be the toxic factor in the disease.

The putative BCAA dysmetabolism mechanism is also supported by studies in which either several BCKAs or the α-ketoacid of leucine, α-ketoisocaproate (α-KIC), are added to cells, and by data from mouse models of altered BCKDC metabolism.[130–141] Consistently, the addition of BCKAs to glial cells or to the cerebral cortex increases lipid peroxidation and oxidative stress leading to mitochondrial bioenergetic dysfunction.[130,131,141] The ability of BCKAs to cause mitochondrial dysfunction extends beyond the brain. For example, BCKAs inactivate

pyruvate dehydrogenase in rat liver and strongly inhibit pyruvate dehydrogenase and α-ketoglutarate dehydrogenase in the heart[132,133,141] and cause apoptosis when added to fibroblast and neural cells isolated from patients with MSUD.[134,135] Further support for the BCAA dysmetabolism mechanism is derived from studies on human or mouse cells with altered BCKDC activity (Box 1). Cells from patients with classic or intermittent MSUD and mouse cells with disrupted expression of *PPm1K* and *BCKDK* exhibit toxic cellular changes, including mitochondrial dysfunction and/or apoptosis (Box 1 and Figure 4). For example, in cells expressing a *BCKDK* transgene, cell death occurs when leucine is added, presumably due to its rapid conversion to, and the subsequent accumulation of, α-KIC.[136] Consistently, mutation or deletion of *PPM1K* results in a mild increase in levels of BCAAs in humans and mice, which nevertheless increases lipid peroxidation, lipotoxicity, oxidative stress, mitochondrial transition pore opening and apoptosis, along with the activation of the stress kinases JNK and p38.[137–140] All of the above-mentioned factors are generally believed to be involved in insulin resistance and in the pathogenesis of T2DM. However, it must be emphasized that blood levels of BCKAs in these conditions are modest compared with those in individuals with MSUD, and elevations in circulating levels of BCKAs in individuals with insulin resistance and in animal models of obesity are not universally observed.[63]

The products of BCKDC activity are branched-chain acyl-Coenzyme A (CoA) species that are further metabolized by multiple mitochondrial-matrix enzymatic steps, eventually leading to the formation of lipogenic, ketogenic or gluconeogenic substrates (in liver), such as acetoacetyl-CoA, acetyl-CoA and propionyl-CoA (Figure 4). Acyl-CoAs can be converted to acylcarnitines, which in turn can be transported out of the mitochondria and cells. Acylcarnitines can be assayed in plasma or urine as reporters of the status of mitochondrial metabolism involving CoA species. A number of BCAA-derived acylcarnitines are increased in individuals with insulin-resistant obesity or T2DM.[59,60,73,113,142–144] Clinically, urine and blood levels of acylcarnitines are used to detect organic acidurias. Similar to MSUD, organic acidurias can result in mitochondrial dysfunction, albeit the mechanism for the latter is not entirely obvious.[145] In part, the idea that accumulations of acyl-CoA species are potentially toxic originates from the disorders caused by these inborn errors of metabolism. However, as with BCKAs, it is uncertain whether levels of acyl-CoA species reach sufficient concentrations in insulin-resistant states to cause mitochondrial dysfunction.

Impaired mitochondrial BCAA metabolism
One of the most robust and consistent changes in obesity, regardless of the model, is a marked decrease in adipose tissue expression of genes involved in BCAA metabolism;[63,72,74,143,146–150] however, the mechanisms underlying these changes remain to be defined. Changes in adipose tissue concentrations of these proteins in obesity coincide with the above-cited reduced gene expression

BCAT(m)

- *Bcat2⁻/⁻* mice have increased levels of BCAAs and decreased levels of BCKAs[111]
- *Bcat2⁻/⁻* mice have improved glycaemic control (indicating a role of BCAT(m) in gluconeogenesis), glucose tolerance and insulin sensitivity[111]
- *Bcat2⁻/⁻* mice display decreased adiposity and increased thermogenesis[111]

BCKDH complex

- Human mutations in components of the BCKDH complex lead to MSUD[183–186]
- Individuals with MSUD and MSUD animal models exhibit both elevated levels of BCAAs and BCKAs[166,184–187]
- Addition of BCKAs, which are elevated in MSUD, to cells results in oxidative stress and mitochondrial dysfunction[130–133,188]
- Fibroblasts and neural cells derived from individuals with MSUD undergo apoptosis when BCKAs are added[134,135]
- *BCKDHA* has been described as a primary susceptibility gene for both obesity and T2DM[120]

BCKDK

- *BCKDK* transgenic overexpressing cells undergo cell death when leucine is added[136]
- Increasing BCKDK protein levels in animals would be expected to increase plasma concentrations of both BCAAs and BCKAs, but no viable BCKDK transgenic animals have been reported

PPM1K

- *Ppm1k⁻/⁻* mice have increased levels of BCAAs and BCKAs[137,151]
- Mutation in human *PPM1K* leads to an intermittent form of MSUD[138]
- *PPM1K* has been described as a T2DM susceptibility gene in human islets[165]
- Allelic variation near *PPM1K* was associated with poorer glycaemic control and body weight response in the POUNDS LOST trial[167]
- Cells from an individual with defective *PPM1K* and *Ppm1k⁻/⁻* mice display changes frequently linked to obesity, insulin resistance and T2DM, such as increased lipotoxicity and lipid peroxidation, increased oxidative stress (ROS generation and mitochondrial permeability transition pore opening), increased apoptosis and stress kinase activation (JNK and p38)[137–140,166]

Abbreviations: BCAA, branched-chain amino acid; BCAT(m), branched-chain amino acid transaminase, mitochondrial; BCKA, branched-chain α-keto acid; BCKDH; branched-chain α-keto acid dehydrogenase; JNK, c-Jun N-terminal kinase; MSUD, maple syrup urine disease; p38, mitogen-activated protein kinase p38; ROS, reactive oxygen species; T2DM, type 2 diabetes mellitus.

levels, at least in the first two steps in metabolism that have been examined in Zucker rats and *ob/ob* mice. In the Zucker rat, the losses in BCAT(m) and BCKDC E1-α are quite large even when changes in adipose tissue mass are considered.[72,73] Thus, it has been proposed that decreased BCAA metabolism in fat contributes to increased plasma levels of BCAAs in individuals with insulin-resistant obesity or untreated T2DM,[63,72,73,143] and that visceral adipose tissue, in particular, might have an important role in this regard.[63] Consistent with the latter view, BCAA catabolic enzyme gene expression in visceral adipose tissue strongly correlates with insulin sensitivity and is reduced in individuals with the metabolic syndrome and obesity compared with control individuals with similar levels of obesity but who do not have the metabolic syndrome.[63,150]

However, there is a caveat for this proposal given that whole-body BCAA metabolism demonstrates considerable interorgan dependence. A demonstration of interorgan dependence comes from studies in patients with MSUD as well as from mouse models of MSUD. In these studies, transplantation of normal tissue (such as liver or adipose tissue) largely compensates for the loss of BCAA metabolism in the other tissues of the metabolically impaired mice or patients with MSUD and this leads to considerable reductions in plasma levels of BCAAs.[74,151,152] This reduction is assisted, in part, by the fact that BCKDC is activated in the transplanted tissue by the elevated circulating α-KIC levels found in patients with MSUD. Thus, in individuals with intact metabolism, a high BCAA intake should be well-tolerated because of the reserve capacity of BCKDC in the body and the fact that BCKDC is activated by excess substrate under normal conditions.[153] Loss of BCAA metabolism in one organ, such as fat, might be associated with normal or increased metabolism in other tissues, unless those other tissues exhibit some form of mitochondrial dysfunction, as is common in insulin-resistant obesity or T2DM. As described later, two such phenotypes have been observed in animal models of obesity associated with changes in hepatic BCKDC.[75]

The issue of whether or not organs other than adipose tissue have altered BCAA metabolism in insulin resistant or T2DM states is starting to be addressed. For example, spectral analysis has revealed decreased expression of two enzymes involved in valine and isoleucine metabolism in muscle of patients with T2DM (Figure 4).[154] Similarly, in Goto–Kakizaki rats, expression of 3-hydroxyisobutyrate dehydrogenase, the protein encoded by the *Hibadh* gene in rats, was reduced by >50% in skeletal muscle (Figure 4).[155] This protein catalyses a step in valine metabolism (Figure 4) and impairment of this enzyme in humans is associated with an accumulation of 3-OH-butyryl-CoA and 3-OH-butyrylcarnitine; loss of this enzymatic step can have toxic effects.[156] In another study, male individuals with obesity and T2DM, but not similarly affected female individuals, exhibited considerably reduced BCKDC protein concentrations.[157]

However, several challenges exist in addressing whether whole-body or tissue-specific BCAA metabolism is increased or decreased in states of insulin-resistant obesity and T2DM. Interpretive problems arise when comparing tissues or even whole-body metabolism from individuals with different body compositions (a problem that has also affected the calorimetry field[158]). For example, the size of the liver is approximately twofold higher in obese compared with lean Zucker rats (livers from obese Zucker rats are also more lipid laden than those from lean Zucker rats) and the contribution of adipose tissue increases even more and the muscle mass declines further in obese Zucker rats than in lean Zucker rats.[73] In the case of tissues from individuals with and without obesity, should each tissue be considered as a 'pool' of enzyme activity? Thus an important question is which denominator or normalizer is appropriate when measuring tissue levels of BCAA enzymes when the body size and composition are different.

Other issues also present themselves when analysing whole-body BCAA metabolism in individuals with T2DM or insulin resistance, with or without obesity. In addition to the R_d from oxidative metabolism, several processes contributing to the R_a and R_d of BCAAs are likely to be altered. If these changes are dramatic they can confound efforts to develop a simple interpretation
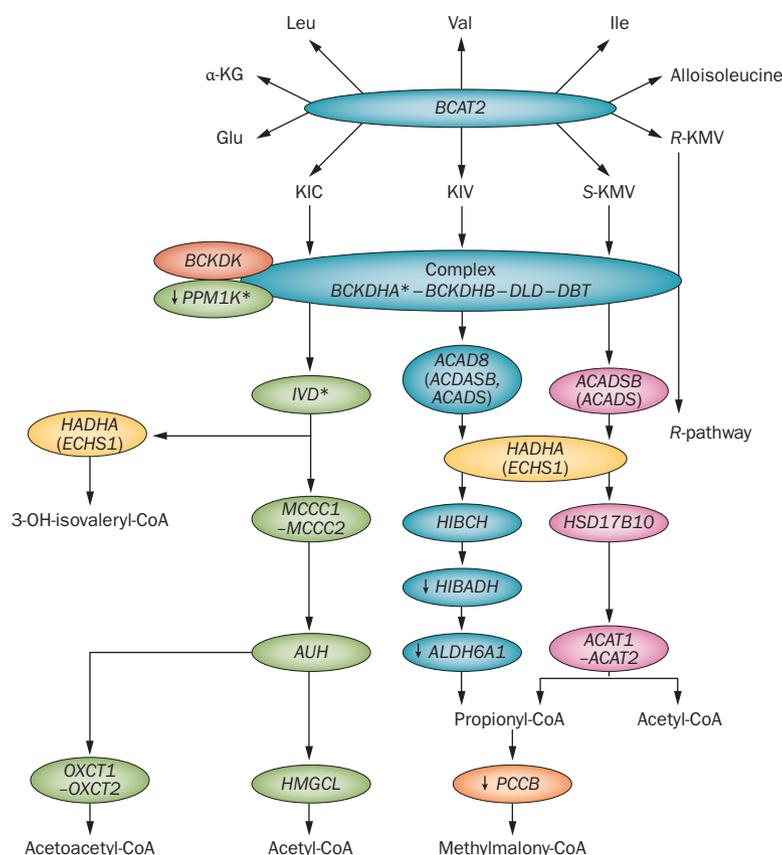
Figure 4 | Mitochondrial genes attributed to BCAA metabolism. The genes involved in mitochondrial BCAA metabolism are shown. Note that BCAT(m) activity is essentially absent from the liver. During reversible BCAT(m) metabolism, an intermediate of isoleucine can tautomerize, leading to alloisoleucine formation.[182] Alloisoleucine formation increases when BCKDC activity is impaired and might be useful for identifying individuals with impaired BCKDC activity,[75] in addition to its usual use in identifying those with maple syrup urine disease. *Indicates an obesity and/or T2DM susceptibility gene.[120,165] ↓ Indicates a literature finding of decreased gene or protein expression observed in human islets or skeletal muscle biopsies from individuals with T2DM, except for *HIBADH*, which is decreased in skeletal muscle of Goto–Kakizaki rats.[154,155,165] Coloured oval shapes represent genes implicated in BCAA metabolism. Abbreviations: BCAA, branched-chain amino acid; BCAT(m), branched-chain-amino-acid aminotransferase, mitochondrial; BCKDC, branched-chain α-ketoacid dehydrogenase complex; KIC, α-ketoisocaproate; KIV, 2-ketoisovalerate; KMV, α-keto-β-methylvalerate. Modified with permission from Herman, M. A. *et al. J. Biol. Chem*. **285**, 11348–11356 (2010)[74] © The American Society for Biochemistry and Molecular Biology.

of BCAA oxidation data.[73] Another issue in whole-body metabolism is related to the choice of using either leucine or α-KIC specific activities for leucine isotope studies. After priming the bicarbonate pool with isotope, α-KIC rather than leucine-specific activity or enrichment is frequently used to monitor muscle or whole-body protein synthesis and turnover,[159,160] as α-KIC is believed to better represent the intracellular pool of leucine in skeletal muscle (the frequent focus of this methodology and where there is considerable BCAT(m) activity to interconvert BCAAs and BCKAs).[159] However, considerable BCKA oxidation takes place in the liver, which lacks BCAT(m). In Zucker rats, the obese:lean ratios of BCAA and BCKA levels are different in plasma and muscle;[73] although the obese:lean BCAA level ratio is similar in

liver, the ratio for BCKA levels is far more increased in liver than in plasma and skeletal muscle. Neglecting the specific activity problem, another issue is still what denominator should be used for calculating rates of leucine oxidation and appearance, and the tissue-specific rates of protein synthesis when body compositions are different. Thus, the body composition factors comparing individuals with and without obesity create interpretive dilemmas that need to be considered.

With regard to branched-chain α-keto acid dehydrogenase (BCKDH) activity or phosphorylation (inactivation), there currently seems to be two responses evident in animal models of insulin-resistant obesity (Figure 5). The first phenotype is exemplified by animal models of insulin-resistant obesity, such as *Lep^ob/ob* mice, obese Zucker rats, ZDF rats and Otsuka Long–Evans Tokushima Fatty rats.[72,73,161,162] Along with the usual reductions in BCAA metabolic pathways in adipose tissue, these animals exhibit impaired active or total BCKDC activity in the liver. Considering that BCKDC activity is regulated by phosphorylation, it is possible to measure its activity in isolated tissues, in comparison to how much the activity might be if the enzyme was fully active—the total activity. Total activity is measured after the enzyme is treated with a phosphatase that removes the inhibitory phosphorylations on the E1-α subunit of BCKDC in homogenates. The impaired hepatic activity of BCKDC in obese Zucker rats is explained in part by increased expression of BCKDK. In obese Zucker rats, a global reduction in BCKDC activity is evident in fat, liver, kidney, skeletal muscle and heart, compared with activity in lean animals.[72] This generalized diminution is associated with greatly increased levels of BCKAs and alloisoleucine—the pathognomonic biomarker of MSUD.[75]

The second putative phenotype is demonstrated by rodents with diet-induced obesity. Whereas this model is characterized by reduced levels of BCAA enzymes in fat,[63] other studies show that liver BCKDH activity is actually increased and could compensate for the reduced activity in fat.[163] Consistently, the lean versus obese differences in plasma levels of BCAAs are much lower in the model of diet-induced obesity than in Zucker rats.[163] Extrapolating, it is tempting to speculate that individuals could also have different phenotypes in this regard. It has been proposed that human studies focusing more carefully on alloisoleucine might be useful in determining where these two phenotypes manifest in patients with insulin-resistant obesity or T2DM.[75] This approach would be more practical than measuring BCKDC activity in muscle and liver biopsy samples. However, in a biopsy study, human livers from male individuals with obesity or obesity and T2DM had lower expression levels of BCKDC protein than control lean male individuals; female individuals were not affected.[157] Thus, compared to the diet-induced obesity model[163] the decrease in BCKDC in male Zucker rats[72,73] might more closely model male human obesity and T2DM. The finding that female individuals did not show any changes in BCKDC levels is consistent with the idea that different

Table 1 | Short and long-term regulation of BCKDC activity

Regulator	Duration of effect	Effect on BCKDK (activity or expression)	Effect on total BCKDC activity (or subunit expression)	Expected or known effect on BCKDC actual activity or activity state	Organ, tissue, cell affected or *in vitro* findings
Clofibrate	Short term and long term	Inhibition of enzyme activity (directly) and ↓ protein levels	↑ Expression of subunits and ↑ total activity	↑ Percentage of active enzyme	Liver; other PPAR-α agonists have long-term effects but no allosteric effects[189]
Exercise	Short term	Inhibition of binding to complex	No change	Activation	Skeletal muscle[190] and liver[191] (↓ binding of BCKDK to BCKDC)
Glucocorticoids	Long term	↓ Protein levels and ↓ gene expression[192]	No change	Activation	Kidney cell line (BCKDK studies); other studies on liver hypothesized effects of methylpredisone due to ↑ plasma levels of leucine[193]
High-protein diet	Long term	↓ Protein levels	↑	↑	Liver[194–196]
Insulin	Long term	↑ Protein levels	No change	↓ or no change	No change in healthy animals following insulin administration or in 3T3-L1 cells stimulated with insulin;[63] ↓ BCKDH activity in liver of methylpredisone-treated rats correlates with plasma levels of leucine;[193] clone 9 cells: effects attenuated by mTORC1 and PI3-K inhibition[197]
Low-protein diet or protein starvation	Long term	↑ Protein levels	↓	↓	Liver[194,196,198,199]
Medium-chain fatty acids (MCFAs)	Short term	Inhibition observed with octanoate on purified kinase	NA	↑ is expected, but in cells MCFAs also elicit ↓ BCKDC activity via ↑ NADH:NAD+ ratio and acetyl-CoA[143]	Purified kinase;[200] uncertainty between the balance of kinase vs FFA inhibition
Oleate or palmitoylcarnitine	Short term	NA	NA	↓	Liver cells and muscle cells[98,175–177]
Sepsis, IL-1 and TNF	Long term	NA	NA	↑	Skeletal muscle: effect prevented by cortisone treatment[201]
Starvation*	Short term and long term	Short term: ↑ BCAA R$_a$ and BCKAs inhibit BCKDK Long term: ↑ BCKDK activity in liver	No change	Activation	Liver[202,203]
STZ-induced diabetes mellitus	Long term	Skeletal muscle: ↓ BCKDK protein levels without affecting gene expression Cardiac muscle: ↑ protein levels ↑ and gene expression Liver: ↓ mass of BCKDK; no change in gene expression	Skeletal muscle: ↑ activity, ↑ protein levels of E1-α, E1-β and E2 subunit, and ↑ gene expression Cardiac muscle: ↓ activity, no effect on protein levels or gene expression of subunits Liver: ↑ total BCKAD activity with diabetes, ↑ mass of each BCKDH subunit, no change in gene expression	Skeletal muscle: ↑ activity state and ↓ rate of inactivation Cardiac muscle: ↓ activity state and ↑ rate of inactivation Liver: diabetes increases the activity state	Liver, heart and muscle[202,204–207]
T$_3$	Long term	↑	Not affected	↓	Liver[208]
α-KIC	Short term	Inhibition	Not affected	↑	All tissues tested showed inhibition of the kinase by α-KIC[198,209]
PPAR-γ agonists	Long term	No effect	↑ Protein levels and ↑ gene expression	↑ Predicted	Adipose tissue[63,210]

*Starvation for all food and just protein or low-protein diets have opposite effects. Abbreviations: BCKDC, branched-chain α-ketoacid dehydrogenase complex; BCKDK, branched-chain α-ketoacid dehydrogenase kinase; α-KIC, α-ketoisocaproate; mTORC1, mammalian target of rapamycin complex 1; FFA, free fatty acid; NA, not available; PPAR, peroxisome proliferator-activated receptor; PI3-K, phosphoinositide 3-kinase; STZ, streptozotocin; TNF, tumour necrosis factor.

phenotypes in hepatic BCKDC might occur in various obesity models. Further studies on how BCKDC activity is affected in other tissues of individuals with obesity and T2DM and between different animal models and the effect of sex is needed.

Finally, other evidence that BCAA catabolism is altered in obesity is derived from the observed increases in levels of BCAA-related acylcarnitines mentioned earlier.[59,60,73,113,142–144] One interpretation of these increases is that they reflect increased BCAA metabolic flux,[59]
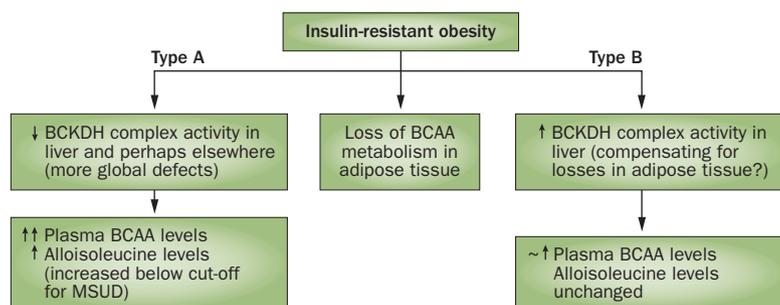
Figure 5 | Patterns of altered BCAA metabolism observed in animal models of obesity. Losses of adipose tissue BCAA metabolic gene and protein expression in obesity have consistently been observed. In a rat model of diet-induced obesity,[63] reduced levels of BCAA metabolizing enzymes in adipose tissue seem to be compensated for by increased hepatic BCKDH activity[163] (termed a type B response). In contradistinction, hepatic BCKDH was also attenuated in other models such as ZDF rats,[161,162] Zucker fa/fa rats[72,73], ob/ob mice[72] and Otsuka Long–Evans Tokushima Fatty rats[162] (termed a type A response). Multiple peripheral tissues were examined and found to be affected in Zucker rats.[73] These distinct phenotypes are important because uncompensated loss of BCAA metabolism in multiple peripheral tissues could result in a higher range of plasma BCAAs that, when observed in states of obesity, associate to a greater extent with insulin resistance, levels of glycaemia and future T2DM. Alloisoleucine elevations below the level used to screen for MSUD have been proposed as a strategy to distinguish between these phenotypes.[75] Levels of BCAAs were considerably increased in models in which metabolism was impaired (↑↑). Abbreviations: BCAA, branched-chain amino acid; BCKDH, branched-chain α-keto acid dehydrogenase; MSUD, maple syrup urine disease. Adapted with permission from John Wiley and Sons © Olson, K. C. et al. Obesity (Silver Spring) **22**, 1212–1215 (2014).[75]

a reasonable hypothesis given that the substrates for BCAA metabolism are increased in states of obesity. However, a caveat is that, as mentioned earlier, acylcarnitine profiles are used clinically, not to determine accelerated metabolism, but rather to detect defects in metabolism (for example, organic acidurias). Notably, in human skeletal muscle mitochondria isolated from individuals with insulin-resistant obesity, spectral analysis shows a reduction of ~50% in transcript levels of *PCCB* (encoding propionyl-CoA carboxylase β chain, mitochondrial) and *ALDH6A1* (encoding methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial), consistent with impaired BCAA metabolism (Figure 4).[154] Further studies are needed to understand the mechanisms underlying the increased levels of BCAA-related acylcarnitines in states of T2DM and insulin-resistant obesity.

Altered BCAA metabolism in disease states

Attenuation of complete mitochondrial BCAA metabolism, if it should occur in states of insulin resistance or T2DM, could occur by several mechanisms (Figure 3), the simplest of which involves altered gene expression, mutations or epigenetic factors that affect gene expression. Seven independent computational disease-prioritization methods have been applied to 9,556 positional candidate genes for obesity and T2DM.[120] This approach identified nine primary candidate genes for T2DM and five for obesity, together with 94 secondary candidates for T2DM and 116 for obesity. *BCKDHA*, the gene encoding the regulated subunit of BCKDC was only one of two primary susceptibility genes identified that affected the risk of both T2DM and obesity (Figure 4).[120] *IVD*, encoding

isovaleryl-CoA dehydrogenase, which catalyses the next step in leucine metabolism, was identified as a secondary T2DM susceptibility gene (Figure 4).

In 2009, PPM1K was identified as the BCKDHA phosphatase.[137,164] A later report, attempting to identify T2DM susceptibility genes,[165] examined global gene expression profiles of 63 islet donors with or without T2DM and compared this to 48 known genes located near known risk variants of T2DM (Figures 3 and 4). Decreased expression of *PPM1K* was observed in islets from individuals with T2DM. *PPM1K* was selected as one of the top 20 candidate genes for further study. Knockdown of *PPM1K* in rat INS-1 cells impaired glucose-stimulated insulin secretion and activated apoptosis (Figures 3 and 4, Box 1).[165] Thus, *PPM1K* has been designated as a T2DM susceptibility gene in islet β cells. In another study, elevated circulating valine levels and the ratio of BCAA levels to the phenylalanine plus tyrosine concentration were found to be associated with a human single-nucleotide polymorphism called rs1440581.[166] RS1440581 is located upstream of *PPM1K*. Subsequently, an analysis of POUNDS LOST trial participants on an energy-restricted high-fat diet who had the C allele of rs1440581 had poorer weight loss outcomes as well as less improved insulin sensitivity than those missing this allele. (Box 1 and Figure 3).[167] Further studies are needed to determine if the rs1440581 polymorphism does indeed affect *PPM1K* expression.

Obesity and/or insulin resistance are known to disrupt circadian homeostasis.[168–172] Krueppel-like factor 15 (encoded by the *KLF15* gene) is a master regulator of glucose and amino acid metabolism (including BCAA metabolism)[119,173] that is differentially expressed in the muscle and fat of overweight individuals with insulin resistance[174] and is thought to regulate circadian nitrogen homeostasis. KLF15 or idiosyncratic responses to it could be another factor in altering BCAA metabolism in insulin-resistant obesity (Figure 3).

Other factors altered by states of obesity and T2DM regulate BCKDC activity in the short and long term (Table 1). Unfortunately, many of these factors have only been studied in a single tissue; an important limitation, as tissue specific regulation has been observed for some factors (Table 1). Thus, it is difficult to predict how some of these regulators affect BCKDC activity in other tissues. It is not immediately obvious from looking at these regulators how the known changes in insulin-resistant states and T2DM might additively work to alter BCAA metabolism or bring about different BCKDC activity phenotypes (Figure 5). However, an interesting point is that long-chain fatty acids and their metabolites, which are elevated in insulin-resistant states and T2DM, inhibit BCKDC activity either by affecting redox states or acetyl-CoA concentrations.[98,143,175–181] Increased free fatty acid metabolism has also been linked to the increased generation of reactive oxygen species, which leads to the formation of reactive lipid aldehydes.[178–181] Proteomic studies have revealed that enzymes in the BCAA metabolic pathway are carbonylated (for example, by the action of 4-hydroxynonenals) following oxidative stress, which could potentially impair their enzymatic activity (Figure 4).[178–181] The increased

availability of free fatty acids and their ability to directly or indirectly inhibit BCAA metabolism in mitochondria (for example, through oxidative stress associated carbonylation) could be a factor linking increased free fatty acid levels to the BCAA dysmetabolism model presented here (Figure 3), and discussed elsewhere.[143]

Conclusions

Although a number of studies have suggested that BCAA supplementation or BCAA-rich diets are beneficial for promoting lean body mass in obesity or catabolic disorders, or for increasing satiety for body weight loss, acceptance of this idea has been tempered by the associations between increased circulating levels of BCAAs and insulin-resistant obesity and T2DM, as well as the observations that these increases might predict future insulin resistance or T2DM. Two mechanisms have been proposed that could explain how elevated levels of BCAAs might be linked to metabolic disease. One involves the persistent activation of the nutrient sensing complex, mTORC1. However, numerous observations indicate that BCAA-associated mTORC1 activation is not necessary or sufficient to trigger insulin resistance. A BCAA dysmetabolism model suggests that those individuals or animal models with the highest plasma levels of BCAAs (that correlate more closely with metabolic dysfunction)

might have impaired BCAA metabolism that adds to the contribution of BCAAs resulting from protein degradation in insulin resistant states. In contrast to the mTORC1 mechanism, the BCAA dysmetabolism model assumes that it is the accumulation of BCAA metabolites that result in dysfunction and that the increased BCAA levels are simply reporters of that accumulation. Inborn errors of metabolism and accumulation of BCAA metabolites can cause mitochondrial dysfunction associated with stress kinase activation and β-cell apoptosis—factors that are frequently associated with insulin resistance and T2DM.

Alternatively, BCAA dysmetabolism or incomplete oxidation (of isoleucine and valine, in particular) might promote anaplerotic stress and an anaplerosis–cataplerosis imbalance that contributes to suboptimal mitochondrial function in states of T2DM.[66,142] These concepts remain speculative but warrant further study. Even though rodent models of obesity and humans with insulin resistance universally exhibit reduced levels of BCAA metabolic enzymes in fat compared with metabolically healthy controls, (Figure 5) there is emerging evidence that these reductions in adipose tissue BCAA metabolic capacity might either extend to other tissues (phenotype A) or be compensated for by increased BCKDC activity in the liver (phenotype B). If these phenotypes exist in humans (as seems to be the case on the basis of the results of a recent study[157])some individuals might have more global reductions in BCAA metabolic capacity that could contribute to increasing circulating levels of BCAAs to the higher ranges that have an increased association with the development of future T2DM and insulin resistance. To determine whether different BCAA metabolic phenotypes affecting multiple tissues exist in humans, a strategy using alloisoleucine has been proposed.[75] Further research is needed to elucidate the contributions of various processes, such as tissue uptake, oxidation and lean mass catabolism, in mediating the increased levels of BCAAs found in individuals with insulin resistance and T2DM and to understand the molecular mechanisms underlying the cellular responses to dysfunctional BCAA metabolism.

In summary, although mechanisms have been proposed that might explain how increased BCAA levels could lead to insulin resistance or obesity, it should be appreciated that increased BCAA levels are more likely to be a marker of loss of insulin action and not, themselves, causative.

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C.J.L. and S.H.A. researched data for the article, made substantial contributions to discussions of the content, wrote the article and reviewed and/or edited the manuscript before submission.