Branched-Chain Amino Acids and Mitochondrial Biogenesis: An Overview and Mechanistic Summary

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1. Introduction

Branched-chain amino acids (BCAA) are essential in the diet and promote several vital cell responses which may have benefits for health and athletic performance, as well as disease prevention. While BCAA are well-known for their ability to stimulate muscle protein synthesis, their effects on cell energetics are also becoming well-documented, but these receive less attention. In this review, much of the current evidence demonstrating BCAA ability (as individual amino acids or as part of dietary mixtures) to alter regulators of cellular energetics with an emphasis on mitochondrial biogenesis and related signaling is highlighted. Several studies have shown, both in vitro and in vivo, that BCAA (either individual or as a mixture) may promote signaling associated with increased mitochondrial biogenesis including the upregulation of master regulator of mitochondrial biogenesis peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), as well as numerous downstream targets and related function. However, sparse data in humans and the difficulty of controlling variables associated with feeding studies leave the physiological relevance of these findings unclear. Future well-controlled diet studies will be needed to assess if BCAA consumption is associated with increased mitochondrial biogenesis and improved metabolic outcomes in healthy and/or diseased human populations.

1.1. Potential Mechanisms of Action

Mechanistically, BCAA have been shown to increase anabolic signaling via activation of mammalian/mechanistic target of rapamycin (mTOR).[5–16] mTOR exists as two independent complexes, mTORC1 (attributed to regulating skeletal muscle hypertrophy),[17] and the lesser studied mTORC2. Importantly, both appear to be sensitive to nutrient signals,[18] though mTORC1 activation is most often examined for the anabolic response of muscle to stimuli such as leucine. Leucine may activate mTORC1 directly via acetyl-CoA,[13] or by inhibiting SESTRIN2[19] or secretion associated Ras related GTPase 1B (SAR1B)[20] which negatively regulate the GAP activity towards gators (GATOR) complex (a positive regulator of mTOR), or by suppressing AMP-activated protein kinase (AMPK) activity which opposes mTORC1 action.[11] Interestingly, loss of mTORC1 function can result in diminished mitochondrial content and function. For example, cultured C2C12 myotubes treated with the mTOR-inhibitor rapamycin exhibit reduced mRNA expression of regulators of mitochondrial biogenesis (peroxisome proliferator-activated receptor gamma coactivator 1 alpha (Ppargc1a), nuclear respiratory factor 1 (Nrf1), and estrogen related receptor alpha (Esrra)) and mitochondrial respiratory components (ATP synthase (Atp5g1), cytochrome c oxidase subunit 5a (Cox5a), cytochrome c (Cycs), and isocitrate dehydrogenase 3 alpha (Idh3a)), but not peroxisome proliferator-activated receptor gamma coactivator 1 beta (Ppargc1b) expression.[21] Moreover, reduced mTORC1 activity led to significantly lower mtDNA content and

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oxygen consumption.\[21\] The same report assessed tuberous sclerosis 2 (TSC2) knock out cells (which cause a constitutive activation of mTOR), which resulted in upregulation of mitochondrial biogenic and respiratory component gene expression including \(Ppargc1a, Ppargc1b, Nrfl1, Esra, Atp5g1, Cox5a, CyC, and Idh3a,\) as well as mtDNA content and oxygen consumption.\[21\] Also in line with these observations, mTORC1 knockdown (via mTOR and Raptor knockdown) suppressed expression of mitochondrial genes.\[21\] Ultimately, the authors showed mTORC1-mediated mitochondrial biogenesis was controlled by interactions between master regulator of mitochondrial biogenesis peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1\(\alpha\)) and transcription factor yin-yang 1 (YY1), namely the up-regulation of PGC-1\(\alpha\) (which promotes YY1 expression).\[21\] Thus, given leucine is a known activator of mTORC1, it could be that leucine/BCAA mediated upregulation of mitochondrial biogenic signaling could be coordinated through YY1 activation. Importantly, leucine’s ability to promote \(Ppargc1a\) expression in an mTOR-dependent fashion has been shown in C2C12 myotubes, during which indicators of both protein synthesis and mitochondrial biogenesis (indicated by targeted protein or mRNA expression) were abolished by concurrent treatment with rapamycin.\[22\]

Interestingly, mTORC2 has been reported to be involved in mitochondrial biogenesis\[17\] and can be directly activated by AMPK during nutrient deprivation.\[18\] Leucine has been shown to promote glucose uptake and Akt (Ser-473) activation in an mTORC2-dependent fashion in cultured C2C12 myotubes treated with leucine at 2 mM for 45 min (shown via silencing (siRNA) of Rictor, a component of mTORC2).\[22\] That being said, the report by Liu et al.\[23\] did not investigate targets related to mitochondrial biogenesis. Another important note however is that both mTORC1 and mTORC2 are sensitive to inhibitors such as rapamycin, and therefore rapamycin inhibition experiments should be interpreted with this in mind. It should also be noted that mTORC1 activity may function in a tissue-specific fashion regarding its influence on mitochondrial content and related signaling. For example, adipose-specific Raptor (a component of mTORC1) knockout increases mitochondrial gene expression, expression, browning, and leanness in vivo.\[24\] Thus, the exact role of mTORC1 and mTORC2 in the regulation of mitochondrial biogenesis is complex and worthy of further investigation.\[17\]

Additionally, suppression of amino acid transports L-type/large neutral amino acid transporter 1 (LAT1) and system A transporter 2 (SNAT2) via a methylaminoisobutyric acid has also been shown to reduce mRNA expression of both Sirtuin 1 (Sirt1) and \(Ppargc1a\) in C2C12 myotubes.\[25\] Moreover, PGC-1\(\alpha\) knockout mice given leucine showed diminished mTOR-mediated protein synthesis, suggesting PGC-1\(\alpha\) is required for mTORC1 action.\[26\]

There is also direct evidence linking BCAA to increased mitochondrial biogenic signaling and function in several tissue types. For example, leucine and/or BCAA have been linked with several potential metabolic benefits including mitochondrial biogenesis (and related signaling at the mRNA and protein level) and metabolism.\[22,27–38\] although when studied individually, the effects may be uniquely attributable to leucine (and its metabolites).\[22,29\] Leucine appears to induce mitochondrial biogenesis through upregulation of PGC-1\(\alpha\)\[27–29\] which coordinates mitochondrial biogenesis through increases in nuclear respiratory factors (NRF1/2).\[40–43\] mitochondrial transcription factor A (TFAM),\[44–46\] and the sirtuins (SIRT1/3).\[47–50\] In fact, leucine and BCAA have been shown to up-regulate AMPK, PGC-1\(\alpha,\) and the sirtuins,\[47,27,31,51–57\] as well as peroxisome proliferator activated receptor beta (PPAR\(\beta/\delta\)).\[28,32\] all of which can contribute to increased mitochondrial biogenesis. A summary of the potential mechanisms linking BCAA to mitochondrial biogenesis are summarized in Figure 1.

### 1.1.1. Current Evidence Linking BCAA to Mitochondrial Biogenesis and Metabolism (In Vitro)

As discussed in detail below, treatment with leucine has been repeatedly shown to increase markers of both mitochondrial biogenic signaling\[22,27–29,31,32,34,35,38–60\] and/or function\[27–29,31,32,34,35,38,51,59,60\] in 3T3-L1 adipocytes,\[29\] C2C12 myocytes,\[29,51\] C2C12 myotubes,\[27,28,32,34,35,38,60\] porcine skeletal muscle satellite cells,\[31,34\] and mouse primary myocytes.\[59\]

These findings suggest leucine, in particular, has the ability to stimulate signaling or outcomes associated with increased mitochondrial content and/or function. However, some studies have shown that leucine treatment in conjunction with palmitate resulted in unaltered markers of mitochondrial biogenesis.\[14,38\] Unlike leucine treatment, treatment with isoleucine\[59,61\] or valine\[59,59–61\] resulted in no mitochondrial changes suggesting the effects on mitochondrial content and/or function are unique to leucine. Several experiments have specifically explored the effects of leucine on mitochondrial biogenesis in several in vitro models. For example, C2C12 myotubes were treated with leucine at 10 mM for 1 h, and leucine (but not valine, alanine, or phenylalanine) upregulated mRNA of \(Ppargc1a,\) mitochondrial transcription factor A (TFAM), ATP synthese (Atp5a), and CyC (which was abolished by concurrent rapamycin), supporting the mTOR signaling pathway hypothesis.\[22\] Similarly, 0.5 mM leucine treatment for up to 48 h significantly increased mitochondrial content, biogenesis-related gene expression (\(Ppargc1a\) and \(Sirt1\)), and AMPK phosphorylation compared to alanine and valine.\[27\] The same report showed leucine increased SIRT1 activity, fatty acid oxidation, and NAD\(^+\) content.\[27\] Mechanistically, EX527 (a selective SIRT1 inhibitor) reversed the effects of leucine on AMPK phosphorylation, mitochondrial content, and expression of \(Ppargc1a,\) Sirtuin 3 (\(Sirt3\)), and cytochrome c oxidase subunit 5b (\(Cox5b\)) (mitochondrial biogenesis-related gene markers), as did compound C.\[27\] These observations suggest a dependence on the SIRT1/AMPK axis as a mechanism for leucine-mediated mitochondrial biogenesis.\[27\] Likewise, leucine at 0.5 mM for 48 h increased mitochondrial biogenesis regulatory genes (\(Sirt1, Ppargc1a, Nrfl1\), component genes (NADH dehydrogenase (NDufa1), cytochrome c oxidase subunit 7c1 (\(Cox7c\)), uncoupling protein 3 (\(Ucp3\)), and mitochondrial mass in C2C12 myocytes.\[29\] Comparable findings were observed in C2C12 myotubes treated with leucine at either 100 or 500 mM for up to 48 h showing increases in PGC-1\(\alpha\) and mitochondrial oxygen consumption.\[51\] Sun and Zemel\[29\] further showed leucine increased mitochondrial mass in 3T3-L1 adipocytes. Moreover, leucine treatment was shown to function in part through SIRT1, as \(Sirt1\) siRNA reduced the stimulating effect of leucine on \(Sirt1, Ppargc1a,\) and \(Nrfl1\) gene expression.\[29\] Other reports
exploring the effects of leucine at either 0.5 mM or 1.5 mM for 24 h with and without palmitate on C2C12 myotubes demonstrated leucine at 1.5 mM increased expression of Ppargc1a, Nrf1, Tfam, Sirt1, Ppara, Cpt1b, and peroxisome proliferator-activated receptor gamma (Pparg), while only some of these effects were observed in cells treated with 0.5 mM or co-treatment with palmitate at 0.75 mM. In addition to dose-dependence, Wu et al. also showed several of these effects were abolished following concurrent mTORC1 inhibition with rapamycin. Similar reports have shown that 2 mM leucine for 24 h can induce mitochondrial biogenesis and function (increased Ppargc1a mRNA and other downstream targets, as well as increased oxygen consumption) in a manner dependent on lipid content, with upregulation in mitochondrial biogenesis inhibited by concurrent palmitate treatment. Conversely, neither myocytes nor myotubes treated with leucine, isoleucine, or valine at 5 mM acutely for 1 h showed any effect on mitochondrial oxygen consumption, though leucine-treated myotubes displayed reduced glycolytic metabolism attributed to increased BCAA catabolism.

Other studies have compared the effect of leucine versus its catabolite by products. For example, the leucine-catabolite \( \beta \)-hydroxy-\( \beta \)-methylbutyrate (HMB) was compared against leucine using cultured C2C12 myotubes and showed supplementation of 0.5 mM for Leu or 50 \( \mu \)M for HMB for 24 h significantly increased mitochondrial mass, mitochondrial respiration capacity, and mRNA expression of mitochondrial biogenesis-related genes. And while both treatments increased mitochondrial function (oxygen consumption), only HMB increased Ppargc1a mRNA. Zhong et al. also demonstrated the effects of both leucine and HMB were dependent on PPAR\( \beta \delta \), which is similar to other observations by Schnuck et al. using cultured myotubes treated with 2 mM leucine for 24 h. Other reports have shown both leucine’s and its catabolites’ abilities to stimulate mitochondrial biogenic signaling in vitro. For example, one report treated myotubes with leucine at 250 \( \mu \)M or 500 \( \mu \)M, or HMB at 25 \( \mu \)M or 50 \( \mu \)M for 48 h and showed increased palmitate oxidation across all groups versus control cells. The same report also showed treatment with HMB at 25 \( \mu \)M or 50 \( \mu \)M, or leucine or the leucine-derived alpha-keto acid/alpha-ketoisocaproic acid (KIC)
at 125 μM or 250 μM increased mitochondrial staining in myotubes, with the lowest doses all linked to increased Ppargc1a, Nrf1, and Ucp3 expression. The experiments by Stancilfe et al. showed the silencing of branched-chain amino acid transaminase 2 (BCAT2, responsible for the degradation of BCAA to their alpha-ketoic acid metabolites) abolished leucine-mediated upregulation of increased Ppargc1a, Nrf1, and Ucp3 suggesting involvement of the leucine catabolites. Conversely, Schnuck et al. showed HMB at lower concentrations for a shorter duration (either 6.25, 12.5, or 25 μM for 24 h) reduced Ppargc1a, Nrf1, Tfam, and Sirt3 mRNA expression, as well as reduced protein expression of Nrf1, PPARα, PPARβ/δ and acutely depressed pAMPK expression (although PGC-1α, TFAM, SIRT1, and SIRT3 protein expression, and cell metabolism remained unaltered). Despite somewhat conflicting results, some have speculated the mechanism by which leucine increases mitochondrial content may be in part due to the degradation of leucine to either HMB or KIC.

In primary porcine skeletal muscle satellite cells, leucine treatment with 2 mM for 3 days increased protein expression of slow myosin heavy chain (MyHC), SIRT1, pAMPK, PGC-1α, and Cyt C, as well as enhanced mRNA levels of AMPKα1 (Prkaa1), Tfam, mitochondrial transcription factor b1 (Tfb1m), Cytc, mitochondrial NADH dehydrogenase 1 (mt-Nd1), cytochrome c oxidase (Cox1), and Atp5g. Leucine treatment also increased the activity of malate dehydrogenase and succinate dehydrogenase. Mechanistically, the addition of Prkaa1 siRNA, AMPK inhibitor compound C, or SIRT1 inhibitor EX527 all blocked the effects of leucine on mitochondrial gene expression, strongly suggesting a role of SIRT1 and AMPK activation in the effect of leucine on mitochondrial biogenesis. Comparable findings have also been obtained using porcine skeletal muscle satellite cells treated with up to 4 mM leucine for 24 h, which increased mRNA expression of adiponectin (Adipoq), adiponectin receptor (Adipor1), AMPKα2 (Prkaa2), Ppargc1a, Nrf1, Atp5g, and Tfam, mtDNA levels, and protein expression of pAMPK and PGC-1α; effects which could be mitigated with concurrent Adipor1 silencing via siRNA. Leucine treatment for 3 days at low levels (50 μM) has also been shown to increase molecular regulators of mitochondrial biogenesis including Ppargc1a, PGC-1α, Nrf1, TFAM, and SIRT1 expression in C2C12 myotubes. And although not in skeletal muscle, 160 μM leucine treatment for 2 h increased mitochondrial size and fusion in primary mouse and rat cardiomyocytes.

Similar to leucine, cells treated with different ratios of BCAA mixtures for various durations also displayed increased mitochondrial biogenesis and/or function in C2C12 myotubes, H1-1 cardiomyocytes, mouse primary cardiomyocytes, and HepG2 cells. D’Antona et al. treated HL-1 cardiomyocytes with an optimized BCAA mixture (containing a BCAA ratio of 2:1:1, leucine:isoleucine:valine, along with other essential amino acids) which increased expression of Ppargc1a and Nrf1, mitochondrial respiratory components, and ATP content. Next in a mechanistic approach, the group used either mTORC1 inhibition with rapamycin or eNOS silencing to demonstrate the dependence of BCAA on eNOS. C2C12 myotubes treated with a wide-continuum of BCAA concentrations at a ratio of 2:1:1 showed increased mitochondrial staining at 2 and 20 mM (per leucine content) but not 0.2 mM, suggesting a dose-dependency. Perplexingly, 20-mM-treated cells displayed reduced basal mitochondrial respiration. Moreover, the addition of insulin resistance diminished the increase in mitochondrial staining by BCAA treatment, and reduced basal and peak mitochondrial respiration, as well as caused a significant reduction in Ppargc1a, Cs, Cox5a, and Atp5b. Another study using isolated mouse primary cardiomyocytes showed either leucine or valine, or their alpha-ketoic acid derivatives KIC or alpha-ketosiovalerate (KIV), respectively, or a BCAA mixture dose-dependently increased mitochondrial fatty acid oxidation and peroxisome proliferator-activated receptor alpha (PPARα) protein expression in a dose-dependent fashion, which was accompanied by increased carnitine palmitoyl transferase 1b (CPT1B) and fatty acid translocase expression but not PGC-1α (please see Table 1 for specific treatment concentrations). Conversely, neither isoleucine or the alpha-ketoic acid derivative of isoleucine, a-keto-β-methylvalerate (KMV) altered PPARα expression or fatty acid oxidation. Similarly, the HL-1 cardiomyocytes treated with a separate BCAA mixture (α5) also exhibited increased markers of mitochondrial biogenesis, including mRNA expression of Ppargc1a, Nrf1, Tfam, Cytc, and Cox4, as well as increased protein expression of Cyt C and cytochrome c oxidase (COX IV). The same report showed that doxorubicin-mediated suppression of mitochondrial biogenic signaling (all of the aforementioned targets), could be rescued by the α5 BCAA mixture. D’Antona et al. also showed BCAA treatment as 1 %wt:vol (2:1:1-leucine:isoleucine:valine) of myotubes increased citrate synthase (CS) activity and oxygen consumption, as well as ATP production. Additionally, the same report showed myotubes treated with rosuvastatin (a statin therapy commonly used to treat hypercholesterolemia) and the BCAA mixture exhibited rescued CS activity, oxygen consumption, and ATP production. Tedesco et al. examined the effects of an amino acid mixture on HepG2 cells and found increased Ppargc1a, carnitine palmitoyl transferase 1 (Cpt1), and eNOS mRNA expression, in addition to elevated SIRT1, PGC-1α, Cyt C, and COX-IV protein expression. Collectively, these data suggest leucine, its catabolite HMB, and/or combinations of BCAA may induce signaling associated with increased mitochondrial biogenesis in vitro, though these effects may be dependent on dose, duration of treatment, the composition of media/substrates such as palmitate, and cell type (though importantly, excess palmitate may be cytotoxic, possibly explaining some of the aforementioned palmitate dependence as viability studies were not reported for all experiments). Table 1 summarizes much of the current evidence examining the effect BCAA on mitochondrial biogenesis and function in vitro experimental models.

1.1.2. Current Evidence Linking BCAA to Mitochondrial Biogenesis and Metabolism (In Vivo)

In addition to in vitro data supporting a role for BCAA in mitochondrial biogenic signaling, several reports have also investigated the effects of individual or combination BCAA treatments on mitochondrial biogenesis and function in vivo. While valine and isoleucine are currently under-investigated, leucine treatment has repeatedly resulted in increased mitochondrial biogenic signaling and/or function in several...
Table 1. Current evidence on the effect of BCAA on various indicators of mitochondrial biogenesis and related metabolism in metabolically consequential cell types using in vitro experimental models.

| Experimental model            | Treatment                  | Main findings                                                                 | Reference       |
|-------------------------------|----------------------------|-------------------------------------------------------------------------------|-----------------|
| 3T3-L1 adipocytes            | Leu 0.5 mM, 24 h           | ↑mitochondrial mass/respiration                                                | Sun 2009(29)    |
| C2C12 myocytes               | Leu 0.5 mM, 48 h           | ↑Sirt1, Pparg1a, Nrf1, Ndufa1, Cox7c, Ucp3 mRNA expression; ↑mitochondrial mass/respiration | Sun 2009(29)    |
| C2C12 myocytes               | Leu 0.1–0.5 mM, 24–48 h    | ↑PGC-1α, Cyt C protein expression; ↑mitochondrial content/respiration         | Vaughan 2013(31) |
| C2C12 myocytes               | Leu 5 mM, 1 h              | ↔mitochondrial respiration                                                    | Suzuki 2020(61) |
| C2C12 myotubes               | Leu 5 mM, 1 h              | ↔mitochondrial respiration                                                    | Suzuki 2020(61) |
| C2C12 myotubes               | Leu 10 mM, 1 h             | ↑Pparg1a, Tjaf, Atp5o, Cycs, ↔Ndufa5 mRNA expression                          | Sato 2018(22)   |
| C2C12 myotubes               | Leu 0.5 mM, 1–48 h         | ↑Pparg1a, Sirt1 mRNA expression; ↑pAMPK protein expression; ↑mitochondrial content, SIRT1 activity, fat oxidation, NAD content | Liang 2014(27)  |
| C2C12 myotubes               | Leu 0.5 mM, 24 h           | ↑Nrf1, Ucp2, Sirt1, Cpt1b, ↔Pparg1a, Paara, Paarg, ↑Ucp3, Tjaf mRNA expression; ↔mitochondrial content | Wu 2019(38)     |
| C2C12 myotubes               | Leu 1.5 mM, 24 h           | ↑Pparg1a, Nrf1, Tjaf, Sirt1, Paara, Cpt1b, ↔Pparg1a, Ucp2, Ucp3 mRNA expression; ↔mitochondrial content | Wu 2019(38)     |
| C2C12 myotubes               | Leu 0.5 mM + PAM 0.75 mM, 24 h | ↑Sirt1, Nrf1, Tjaf, Cyt C, Cpt1b, ↑pAMPK protein expression; ↑mitochondrial content | Wu 2019(38)     |
| C2C12 myotubes               | Leu 1.5 mM + PAM 0.75 mM, 24 h | ↑Sirt1, Nrf1, Tjaf, Cyt C, ↑pAMPK protein expression; ↑mitochondrial content | Wu 2019(38)     |
| C2C12 myotubes               | Leu 2 mM, 24 h             | ↑PGC-1α, NRF1, TFAM, Cyt C, PPARα, PPARβ/δ protein expression; ↑mitochondrial content/respiration | Schnuck 2016(24) |
| C2C12 myotubes               | Leu 0.5–2 mM, 24 h         | ↑Pparg1a, Sirt3, Cs, ↔Nrf1, Tjaf mRNA expression; ↑mitochondrial respiration | Johnson 2019(34) |
| C2C12 myotubes               | Leu 0.5–2 mM + PAM 0.5 mM, 24 h | ↑Pparg1a, Nrf1, Tjaf, Sirt3, Cs mRNA expression; ↑mitochondrial respiration | Johnson 2019(34) |
| C2C12 myotubes               | Leu 2 mM, 24 h             | ↑Pparg1a, Nrf1, Tjaf, Sirt3, Cs mRNA expression; ↑mitochondrial respiration | Rivera 2020(35) |
| C2C12 myotubes               | Leu 50 μM, 3 days          | ↑PGC-1α, Nrf1, TFAM, SIRT1 protein expression                                 | Jin 2022(38)    |
| C2C12 myotubes               | Leu 0.5 mM, 24 h           | ↑Sirt1, Nrf1, Tjaf, ↔Pparg1a mRNA expression; ↑mitochondrial content/respiration | Zhong 2018(32)  |
| C2C12 myotubes               | Leu 125 μM, 48 h           | ↑Pparg1a, Nrf1, Ucp3 mRNA expression; ↑mitochondrial content                 | Stancliffe(60)  |
| C2C12 myotubes               | Leu 250 μM, 48 h           | ↑mitochondrial content/palmitate oxidation                                    | Stancliffe(60)  |
| C2C12 myotubes               | Leu 500 μM, 48 h           | ↑mitochondrial content/palmitate oxidation                                    | Stancliffe(60)  |
| C2C12 myotubes               | HMB 50 μM, 24 h            | ↑Pparg1a, Sirt1, Nrf1, Tjaf mRNA expression; ↑mitochondrial content/respiration | Zhong 2018(32)  |
| C2C12 myotubes               | HMB 6.25 μM, 24 h          | ↑Pparg1a, Tjaf, ↔Nrf1, Sirt3 mRNA expression                                  | Schnuck 2016(42) |
| C2C12 myotubes               | HMB 12.5 μM, 24 h          | ↑Pparg1a, Tjaf, ↔Nrf1, Sirt1, Sirt3 mRNA expression; ↔glucose/palmitate oxidation | Schnuck 2016(42) |
| C2C12 myotubes               | HMB 25 μM, 24 h            | ↑Pparg1a, Sirt1, Nrf1, Tjaf mRNA expression; ↔PGC-1α, TFAM, SIRT1, SIRT3, ↓Nrf1, PPARα, PPARβ/δ, pAMPK protein expression ↔glucose/palmitate oxidation | Schnuck 2016(42) |
| C2C12 myotubes               | HMB 12.5 μM, 48 h          | ↑mitochondrial content                                                        | Stancliffe 2012(40) |
| C2C12 myotubes               | HMB 25 μM, 48 h            | ↑mitochondrial content/palmitate oxidation                                    | Stancliffe 2012(40) |
| C2C12 myotubes               | HMB 50 μM, 48 h            | ↑mitochondrial content/palmitate oxidation                                    | Stancliffe 2012(40) |
| C2C12 myotubes               | KIC 125 μM, 48 h           | ↑Pparg1a, Nrf1, Ucp3 mRNA expression; ↑mitochondrial content                 | Stancliffe 2012(40) |
| C2C12 myotubes               | KIC 250 μM, 48 h           | ↑mitochondrial content/palmitate oxidation                                    | Stancliffe 2012(40) |
| C2C12 myotubes               | KIC 500 μM, 48 h           | ↑mitochondrial content/palmitate oxidation                                    | Stancliffe 2012(40) |
| C2C12 myotubes               | Leu 0.5 mM with 10 nM sildenafil or icarin, 24–48 h | ↑fat oxidation/mitochondrial content                                        | Fu 2015(87)     |
| Porcine skeletal muscle satellite cells | Leu 2 mM, 72 h           | ↑Pkaa1, Tjaf, Tβ1m, Cysc, mt-Ndu1, Cox1, Atp5g mRNA expression; ↑slow MyHC, SIRT1, pAMPK, PGC-1α, Cyt C protein expression; ↑MDH/SDH activity | Chen 2019(13)   |
| Porcine skeletal muscle satellite cells | Leu 4 mM, 24 h           | ↑Adipoq, Adipor1, Pkaa2, Pparg1a, Nrf1, Atp5g, Tjaf mRNA expression; ↑pAMPK, PGC-1α protein expression; ↑mtDNA content | Xiang 2021(54)  |

(Continued)
Table 1. (Continued).

| Experimental model | Treatment | Main findings | Reference |
|--------------------|-----------|---------------|-----------|
| Male C57BL/6 mice (cardiomyocytes) | Leu 160 μM (media), 2 h | ↑ mitochondrial fusion/volume; ↓ mitochondrial number | Morio 2021<sup>[54]</sup> |
| Male Wistar rats (cardiomyocytes) | Leu 160 μM (media), 2 h | ↑ mitochondrial fusion/volume; ↓ mitochondrial number | Morio 2021<sup>[54]</sup> |
| Mouse primary cardiomyocytes | Leu 0.208, 0.416, 0.832, 1.664 mM, 12 h | ↑ PPARα protein expression/fatty acid oxidation | Li 2020<sup>[59]</sup> |
| Mouse primary cardiomyocytes | KIC 0.208, 0.416, 0.832, 1.664 mM, 12 h | ↑ PPARα protein expression/fatty acid oxidation | Li 2020<sup>[59]</sup> |
| C2C12 myocytes | Ile 5 mM, 1 h | --- mitochondrial respiration | Suzuki 2020<sup>[63]</sup> |
| C2C12 myotubes | Ile 5 mM, 1 h | --- mitochondrial respiration | Suzuki 2020<sup>[63]</sup> |
| Mouse primary cardiomyocytes | Ile 0.107, 0.208, 0.416, 0.832 mM, 12 h | --- PPARα protein expression/fatty acid oxidation | Li 2020<sup>[59]</sup> |
| Mouse primary cardiomyocytes | KMV 0.107, 0.208, 0.416, 0.832 mM, 12 h | --- PPARα protein expression/fatty acid oxidation | Li 2020<sup>[59]</sup> |
| C2C12 myocytes | Val 5 mM, 1 h | --- mitochondrial respiration | Suzuki 2020<sup>[63]</sup> |
| C2C12 myotubes | Val 5 mM, 1 h | --- mitochondrial respiration | Suzuki 2020<sup>[63]</sup> |
| C2C12 myotubes | Val 125 mM, 48 h | --- mitochondrial content | Standcliffe 2012<sup>[60]</sup> |
| C2C12 myotubes | Val 250 mM, 48 h | --- mitochondrial content | Standcliffe 2012<sup>[60]</sup> |
| C2C12 myotubes | Val 0.5–2 mM, 24–48 h | --- Ppargc1a, Nrf1, Tfam, Cs mRNA expression; --- PGC-1α, NRF1, CS protein expression; --- mitochondrial content/respiration | Rivera 2020<sup>[99]</sup> |
| Mouse primary cardiomyocytes | Val 0.117, 0.234, 0.418, 0.936 mM, 12 h | ↑ PPARα protein expression/fatty acid oxidation | Li 2020<sup>[59]</sup> |
| Mouse primary cardiomyocytes | KIV 0.117, 0.234, 0.418, 0.936 mM, 12 h | ↑ PPARα protein expression/fatty acid oxidation | Li 2020<sup>[59]</sup> |
| C2C12 myotubes | BCAA 2:1:1 (Leu:Val) 1 %wt:vol, 24 h | ↑ CS activity, oxygen consumption, ATP production | D’Antona 2016<sup>[65]</sup> |
| C2C12 myotubes | BCAA 2:1:1 (Leu:Val) 1 %wt:vol + Rvs 50 μM, 24 h | ↑ rescued CS activity, oxygen consumption, ATP production | D’Antona 2016<sup>[65]</sup> |
| C2C12 myotubes | BCAA 2:1:1 (Leu:Val) 0.2 mM Leu content, 6 days | --- Ppargc1α, Nrf1, Tfam, Cs, Atp5b mRNA expression; --- mitochondrial content/basal and peak respiration | Rivera 2021<sup>[146]</sup> |
| C2C12 myotubes | BCAA 2:1:1 (Leu:Val) 2 mM Leu content, 6 days | --- Ppargc1α, Nrf1, Tfam, Cs, Atp5b mRNA expression; --- mitochondrial content/basal and peak respiration | Rivera 2021<sup>[146]</sup> |
| C2C12 myotubes | BCAA 2:1:1 (Leu:Val) 20 mM Leu content, 6 days | --- Ppargc1α, Nrf1, Tfam, Cs, Atp5b mRNA expression; --- mitochondrial content/basal and peak respiration | Rivera 2021<sup>[146]</sup> |
| C2C12 myotubes | BCAA 2:1:1 (Leu:Val) 0.2 mM Leu content, 6 days + IR | --- Ppargc1α, Nrf1, Tfam, Cs, Atp5b mRNA expression; --- mitochondrial content/basal and peak respiration | Rivera 2021<sup>[146]</sup> |
| C2C12 myotubes | BCAA 2:1:1 (Leu:Val) 2 mM Leu content, 6 days + IR | --- Ppargc1α, Nrf1, Tfam, Cs, Atp5b mRNA expression; --- mitochondrial content/basal and peak respiration | Rivera 2021<sup>[146]</sup> |
| C2C12 myotubes | BCAA 2:1:1 (Leu:Val) 20 mM Leu content, 6 days + IR | --- Ppargc1α, Nrf1, Tfam, Cs, Atp5b, ↓ Ppargc1α, Cs mRNA expression; --- mitochondrial content/basal and peak respiration | Rivera 2021<sup>[146]</sup> |
| HL-1 cardiomyocytes | BCAA 2:1:1 (Leu:Val) 48 h | ↑ Ppargc1α, Nrf1, Tfam, Cs, Atp5b, ↓ Cox5a mRNA expression; ↑ SIRT1 protein expression; ↑ mtDNA, ATP content | D’Antona 2010<sup>[33]</sup> |
| HL-1 cardiomyocytes | BCAA 2:1:1 (Leu:Val) 1 %wt:vol, 48 h | ↑ Ppargc1α, Nrf1, Tfam, Cox5a mRNA expression | Tedesco 2020<sup>[55]</sup> |
| HL-1 cardiomyocytes | n5 (BCAA mixture) 1 %wt:vol, 48 h | ↑ Ppargc1α, Nrf1, Tfam, Cox5a mRNA expression; ↑ COX IV, ↓ Cyt C protein expression; ↑ CS activity | Tedesco 2020<sup>[55]</sup> |
experimental models, such as male B6.Cg-Ay/J mouse soleus, aged C57BL/6J mouse quadriceps, septic C57BL/6J mouse gastrocnemius, ICR mouse quadriceps femoris, C57BL/6J mouse WAT, male Wistar rat soleus, male Wistar rat EDL, piglet Longissimus dorsi, piglet liver, and NW and growth-slowed piglet liver. For example, 1.5% leucine in drinking water for 4 months has been shown to increase expression levels of Ppara, Ucp3, and Nf1 in soleus muscle of Ay mice, as well as increase total energy expenditure (VO2).\(^\text{68}\) Leucine has shown similar effects in aged mice (19-month old), where leucine supplementation at 1250 mg kg\(^{-1}\) for 12 weeks increased quadriceps protein expression of pAMPK, PGC-1α, SIRT1, and PPARγ, while reducing FASN expression (a dose of 500 mg kg\(^{-1}\) was also examined but revealed no significant difference in any of the aforementioned protein vs control muscle)\(^{69}\). In septic mice, injection with leucine (1 g kg\(^{-1}\)) increased muscle mRNA expression of Ppargc1a after 4 days (but not 1 day) of treatment.\(^\text{70}\) And similar to their findings in an in vitro model of muscle, Institute of Cancer Research (ICR) mice supplemented with leucine at 500 mg kg\(^{-1}\) day\(^{-1}\) showed increased expression of mitochondrial biogenic-related targets (pAMPK, PGC-1α, NRF1, and TFAM expression, with unchanged SIRT1 expression) in quadriceps femoris muscle.\(^\text{58}\) Leucine supplementation at 135 mg kg\(^{-1}\) BW for 1–3 h also increased mitochondrial biogenetic signaling in male Wistar rats, evidenced by increased expression of Ppargc1a, Tfam, and Cyc in EDL, and increased Ppargc1a in soleus, which were harvested 3 h post gavage.\(^\text{22}\) Conversely, tumor-bearing Wistar rats given a 3% leucine-supplemented diet for 18 days displayed unaltered tibialis anterior Cs expression but elevated Cox5a expression.\(^\text{74}\) The same report also showed no effect of leucine supplementation on isolated muscle oxygen consumption in either the soleus or extensor digitorum longus.\(^\text{74}\) To examine the effects of individual BCAA supplementation on lipid metabolism in C57BL/6J mice, mice were given 3% either leucine, isoleucine, or valine, or a control diet for 5 weeks.\(^\text{71}\) In this experiment, leucine treatment significantly increased white adipose tissue mRNA expression of uncoupling protein 1 (Ucp1) and Ppargc1a but decreased expressions Sirt1 and Ppck1a, findings that were largely confirmed at the protein level (although Pgc-1α expression was not assessed, nor were the effect of isoleucine or valine as they did not influence blood lipid).\(^\text{71}\) In other animal models, piglets given either 0.25% leucine, 0.5% leucine, or an isocaloric control diet for 6-weeks displayed significantly increased expression of Ppargc1a, Tfam, Cycs, Atp5g, and mtDNA in muscle (longissimus dorsi), and increased expression of Ppargc1a, Tfam, Atp5g, and mtDNA in liver.\(^\text{70}\) The same group verified increases in PGC-1α and pAMPK expression and SDH activity in piglet longissimus dorsi following 7 weeks of 0.25% dietary supplementation with leucine (though similar effects were not observed following supplementation with 0.5% dietary leucine suggesting a dose-dependency).\(^\text{72}\) Using a 2 × 2 design, Su et al.\(^\text{73}\) also observed dietary leucine supplementation at 1.8% in both normal-weight and growth-slowed piglets increased hepatic mRNA expression of Ppargc1a, Nf1, Tfam, Ppara, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (protein); Ppargc1a, peroxisome proliferator-activated receptor gamma coactivator 1-beta (mRNA); Ppargc1a, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (protein); Ppara, peroxisome proliferator-activated receptor alpha (mRNA); Ppargc1a, peroxisome proliferator-activated receptor alpha (protein); Ppargc1a, peroxisome proliferator-activated receptor beta (protein); Sirt1, Sirtuin 1 (mRNA); Sirt1, Sirtuin 1 (protein); Sirt3, Sirtuin 3 (mRNA); Sirt3, Sirtuin 3 (protein), and Val, valine.

| Table 1. (Continued). |
|------------------------|
| **Experimental model** | **Treatment** | **Main findings** | **Reference** |
| HL-1 cardiomyocytes | BCAA 2:1:1 (Leu:Ile:Val) 1%wt:vol 48 h + DOX 1uM, 16 h | ↑rescued CytC, Coxiv, →Ppargc1a, Nf1, Tfam mRNA expression | Tedesco 2020\(^\text{55}\) |
| HL-1 cardiomyocytes | a5 (BCAA mixture) 1%wt:vol 48 h + DOX 1μM, 16 h | ↑rescued Ppargc1a, Nf1, Tfam, Cyt, Coxiv mRNA expression; ↑COX IV, →Cyt C protein expression; ↑CS activity | Tedesco 2020\(^\text{55}\) |
| Mouse primary cardiomyocytes | BCAA 2:1:1 (Leu:Ile:Val) 0.208, 0.416, 0.832, 1.664 mM, 12 h | ↑Ppara, Acaaa2, Acaam, Cd36, Cpt1 mRNA expression; ↑PPARα protein expression; ↑PAM oxidation | Li 2020\(^\text{58}\) |
| HepG2 cells | BCAA 2:1:1 (Leu:Ile:Val) 1%wt:vol, 48 h | ↑Ppargc1a, Cpt1, Sirt1, eNOS, →Tfam mRNA expression; ↑SIRT1, PGC-1α, Cyt C, Cox IV protein expression | Tedesco 2018\(^\text{56}\) |
| HepG2 cells | BCAA 2:1:1 (Leu:Ile:Val) 1%wt:vol + 100 mM ETOH, 48 h | ↑rescued Ppargc1a, Cpt1, Sirt1, eNOS, →Tfam mRNA expression; ↑SIRT1, PGC-1α, Cyt C, Cox IV protein expression | Tedesco 2018\(^\text{56}\) |
and Atp5b, as well as increased mtDNA content. Moreover, finishing pigs given a protein-restricted diet exhibited decreased adipose mRNA expression of Sirt1 and Prkaa, which were restored to control (adequate protein levels) in groups provided additional BCAA at varied ratios.[75] The protein-restricted diet also exhibited decreased adipose protein expression of PGC-1α compared to the control group, and concurrent supplementation with BCAA restored or increased PGC-1α protein levels beyond the control diet.[75] Similarly, pSIRT1 and pAMPK expression were restored to control levels in BCAA-supplemented pigs.[79] The same group also examined the effect of a reduced-protein diet supplemented with varied ratios of BCAA (the most optimal of which was 2.2:1, leucine:isoleucine:valine) given as 2.5% feed for 43 days in the skeletal muscle of finishing pigs.[76] The report showed increased protein levels of p-AMPKα, p-SIRT1, and PGC-1α, as well as reduced phospho-acetyl CoA carboxylase (p-ACC) and PPARY expression in longissimus dorsi for 43 days.[76] In addition, increased Prkaa, Cpt1, and Ucp3 expression were observed.[76] Moreover, another similar study using longissimus dorsi muscle isolated from finishing pigs given a protein-restricted diet (12% crude protein) supplemented with BCAA at various ratios showed increased Opa1, Mfn2, and Ppargc1α, which was also associated with increased mTORC1 activation.[77] Conversely, some evidence exists for reduced Ppargc1α expression in rats following the addition of dietary leucine at 3.2% to either a low (7%) or high-fat (60%) diet for 42 days.[78] Specifically, gastrocnemius muscle displayed unchanged Ppargc1α expression following leucine supplementation in low-fat-fed rats, while leucine supplementation reduced Ppargc1α expression in high-fat-fed rats (but increased Pparg expression in both low- and high-fat-fed rats).[78] Moreover, a report by Duan et al.[79] examined the effect of either leucine or its two catabolites HMB or KIC on metabolic gene expression in longissimus dorsi and soleus muscles from Landrace pigs given diets with either additional leucine (+1.25%), KIC (+1.25%), or HMB (+0.62%). Leucine had no effect on Ppargc1α, Nrf1, Nrf2, Ucp3, Prkaa, or Sirt1 in soleus muscle, but down regulated Nrf1 (but left all other targets unaltered) in longissimus dorsi. Similar to leucine, KIC only reduced the expression of Nrf1 in longissimus dorsi, while Ppargc1a, Nrf2, Ucp3, Prkaa, and Sirt1 remained unchanged.[79] KIC did however upregulate the expression of Ppargc1α and Nrf1 and downregulated the expression of Prkaa in soleus muscle.[79] Moreover, HMB had no effect on these transcripts in longissimus dorsi, but increased Prkaa and Sirt1 (but not Ppargc1α, Nrf1, Nrf2, or Ucp3) expression in soleus muscle.[79] Lastly, Duan et al.[79] measured pAMPK expression in both muscle types and found leucine decreased AMPK activity in longissimus dorsi but increased its activity in soleus. In longissimus dorsi, neither HMB nor KIC had an effect on AMPK activity, however the two had divergent effects on AMPK activation in soleus muscle (with KIC decreasing the phosphorylation of AMPK and HMB increasing its phosphorylation).[79] Thus, these findings suggest that the effects of leucine and its catabolites on signaling related to mitochondrial biogenesis in skeletal muscle might be dependent on fiber type.[79]

Like leucine, mixtures of BCAA containing leucine have been consistently shown to increase mitochondrial biogenic signaling[33,55,57,75–77,80] and/or function[55,80] in C57BL/6 mouse gastrocnemius,[57] C57BL/6 mouse heart,[57] male middle-aged mice-hybrid B6.129S2 soleus,[33] tibialis,[33] diaphragm,[33] and heart,[33] mice heart,[51] male rat liver,[80] and finishing pig adipose/muscle.[75–77] However, these effects may be dosage- or exercise-dependent.[33,57] In mice, Abedpoor et al.[57] showed BCAA supplementation increased expressional indicators of mitochondrial biogenesis in both skeletal and cardiac muscle of C57BL/6 mice. The report specifically showed that mice supplemented with 500 μL BCAA at 20 mg mL−1, 5 days weekly for 8 weeks displayed elevated Prpargc1α expression versus control mice in both gastrocnemius and cardiac muscle, as was Tjam, Cytochrome C Oxidase Subunit 41I (Cox4I1), and Atp5b expression.[57] D’Antona et al.[33] showed in aged mice that BCAA-treatment as 1.5 mg g−1 BW for 3 months improved expressional profiles in heart, diaphragm, soleus, and tibialis muscle, which included increased expression of Prpargc1α, Nrf1, Tjam, and Sirt1. Similar changes were induced by exercise, and the combination of exercise with BCAA supplementation increased gene expression of each target significantly higher than training or BCAA treatments alone.[33] Moreover, visual analysis of mitochondrial content within skeletal and cardiac muscle via electron microscopy revealed significantly increased mitochondrial content in both tissue types versus respective controls (with the additive effect of exercise also observed).[33] In a comparable study, mice received either control or BCAA at 1.5 mg g−1 BW, both with and without rosuvastatin, within the drinking water for 1 month.[65] CS activity was rescued in BCAA with rosuvastatin versus rosuvastatin alone in both gastrocnemius and tibialis muscles, as was mitochondrial content within the gastrocnemius.[65] Tedesco et al.[56] also assessed the resucing effect of BCAA on ethanol-mediated liver dysfunction in rats. Livers of Wistar rats given BCAA supplementation at 1.5 mg g−1 BW with ethanol exhibited restored or significantly increased mitochondrial content (mtDNA) and number (confirmed via electron microscopy), Prpargc1α, Nrf1, Tjam, and Sirt1 mRNA expression, along with restored COX IV and Cyt C protein content.[56] The same group also showed that a 5 BCAA mixture at 1.5 mg g−1 BW can partially or completely restore the mRNA levels of Prpargc1a, Tjam, Cysc, and Cox4 in doxorubicin-damaged mouse cardiac muscle.[55] Additionally, BCAA-treated mice displayed rescued protein expression of COX IV, which corresponded with elevated CS activity and oxygen consumption.[55] Also consistent with these results, rats with carbon tetrachloride-induced cirrhosis exhibited increased liver ATP content and Cpt1a mRNA expression in rats given BCAA at 10 g kg−1 BW for 6 weeks.[86] The same report also showed rats treated with BCAA and l-carnitine for 6 weeks demonstrated significantly increased Tjam, Acacb, Cpt1a, Ndufs8, and Sildh expression.[86] Moreover, in Dahl salt-sensitive (DS) rats, BCAA supplementation at 1.5 mg g−1 BW for 21 weeks restored sodium-mediated suppression of Prpargc1a, NADH dehydrogenase 1 alpha subcomplex subunit 9 (Ndufa9), succinate dehydrogenase b (Sdhb), and cytochrome c oxidase (Cox1/mt-CoI) in skeletal muscle but not cardiac muscle.[83] However, mice that received unloading followed by leucine at 2.3 g kg−1 BW for 28 days displayed unchanged pAMPK and PGC-1α gastrocnemius protein expression, regardless of reloading procedures.[82]

In lean or obese Zucker rats given either low or high protein diets, Prpargc1α expression increased in skeletal muscle of high protein groups in lean mice, but not obese.[83] Conversely,
the same report found reduced Ppargc1a expression in the adipose of leucine-supplemented mice, which occurred regardless of adiposity.[83] Similarly, adipose (dorsal subcutaneous adipose (DSA) and abdominal subcutaneous adipose (ASA)) tissue of pigs given BCAA was assessed for response to BCAA supplementation during protein restriction, and divergent findings were observed between the two tissue responses.[84] For example, DSA showed reduced Ppargc1a, Cycs, uncoupling protein 2 (Ucp2), Ucp3, Nrf1, Tfam, and Sirt1 mRNA expression, as well as decreased Prkaa and pAMPK protein expression, while ASA tissue displayed either increased or restored Ppargc1a, Cycs, Ucp2, Ucp3, Nrf1, Tfam, and Sirt1, and Prkaa mRNA expression with increased pAMPK expression.[84]

Lastly, while the effect of BCAA in humans is under-investigated, one study examined the effects of whey protein isolate supplementation in conjunction with carbohydrate supplementation (CHO + WPI) versus carbohydrate alone (CHO), using six endurance-trained male athletes that received carbohydrates with or without whey protein isolate in a cross-over fashion.[85] Subjects were supplemented for 16-days and underwent an exercise performance test. Muscle biopsies were taken and mRNA expression of Ppargc1a and AMPK-a2 mRNA (Prkaa2) was assessed. Results showed Prkaa2 and Ppargc1a levels were higher in the CHO + WPI group compared to the CHO supplemented group during and after the 6-h recovery phase, suggesting WPI supplementation enhanced mitochondrial biogenic signaling following exercise.[85] Taken together, similar to findings using in vitro models, these data suggest leucine and/or combinations of BCAA may induce signaling associated with increased mitochondrial biogenesis in vivo, but the effects are likely dependent on BCAA dose, duration of treatment, the composition of feed, presence of obesity/exercise, and tissue type. Table 2 summarizes much of the current evidence examining the effect of BCAA on mitochondrial biogenesis using in vitro experimental models.

1.2. Complexity of BCAA in Experimental Physiology and Disease

In addition to discussing the effect of BCAA on common signaling pathways, it is important to address some of the experimental challenges and inconsistencies in findings, as well as the potential implications beyond fundamental findings in various experimental models. First, experimentally, dietary components such as BCAA are often studied in proof-of-concept models during which the variable of interest is studied in isolation (often as a single independent variable in one setting, cell line, tissue type, or under well-controlled and well-defined dietary availability). While this is ideal for isolating the effect of individual variables/treatments on various outcomes, the isolation fails to incorporate the importance of other variables (which often vary considerably across populations). For example, cell culture models may study BCAA using a control media void of BCAA, or conversely, supplement media containing adequate BCAA with additional BCAA. While each approach has validity, both have clear limitations and the comparison between the two sets of findings could have obvious differences. Similar obstacles in rodent and human feeding studies in vivo exist, which is significant as the influence of other variables (e.g., dietary lipid[86–88] or obesity[89]) are known to influence the effects of BCAA.

Another perplexing issue that may accompany the experimental obstacles described above is the observation that leucine seems to have simultaneously opposing actions on cell energetics (specifically, the activation of both AMPK and mTORC1). Experimentally, the effect of leucine on AMPK activation has been inconsistent. Some trials have shown leucine[27,31,72] increases pAMPK expression, which has been shown to be dependent on SIRT1 activity or correlated with SIRT1 expression.[27,31] Increased AMPK activation by leucine has also been associated with elevated LKB phosphorylation, as well as the upregulation of Prkaa1 expression.[31,72] This activation of AMPK seems at odds with the well-established effect of leucine activating mTORC1 (which in general is known for regulating protein synthesis and opposing AMPK). The two are often compartmentalized as being diametrically opposed, though this is a point that is elegantly and thoroughly discussed in the context of exercise (please see ref.[17] for a thoughtful review). Importantly, of the reports that identified upregulation of pAMPK via leucine treatment,[27,31,72] none simultaneously evaluated mTORC1 activity. And of the reports summarized herein that investigated leucine or BCAA (as whey protein hydrolysate or concentrate) on the effects of leucine or BCAA on both pAMPK and mTORC1 activity simultaneously, neither showed an effect of either treatment on the activity of either target.[82,90] Thus, to our knowledge, the simultaneous activation of AMPK and mTORC1 by leucine or BCAA treatment has yet to be experimentally observed. And although not the focus of this review, leucine or BCAA activation of mTORC1 have been associated with either unchanged[14,91] or decreased pAMPK expression.[92,93] Thus, the observation that BCAA increase pAMPK expression could be a product of differing experimental composition, and may still occur mutually exclusive from mTORC1 activation (as the activation of both simultaneously has yet to be observed experimentally). It could be that an increase in protein synthesis leads to altered cell energetics possibly contributing to AMPK activation, but again, this speculation is not supported by experimental observation. It could also be that differences in pAMPK activation in skeletal muscle are in part dependent on muscle fiber type.[79] Limited evidence has shown mTORC2 can be activated by amino acids,[94] and is directly activated by AMPK in response to energetic stress,[18] and given mTORC2 can also contribute to mitochondrial biogenesis, it could be that leucine may act in part through a yet to be determined mTORC2 mechanism that involves AMPK (although this hypothesis is highly speculative and requires further investigation). Again, there is evidence both supporting and refuting the diametric opposition of mTORC1 and AMPK.[17] Additionally, it should be noted that commonly used rapamycin inhibitor studies may not differentiate between which mTOR complex is involved, which is a limitation of the use of the inhibitor only in mechanistic studies. Another similar issue is determining the true dependence of mitochondrial biogenesis on mTOR signaling (in general) and vice versa. Because silencing or chemical inhibition of mTORC1 downregulates protein synthesis, reduced mitochondrial content associated with mTORC1 silencing/inhibition might simply reflect the inability of the cells to undergo the protein translation required to maintain mitochondrial proteins.[21] Similarly, the silencing of proteins such as PGC-1α may depress
Table 2. Current evidence on the effect of BCAA on various indicators of mitochondrial biogenesis and related metabolism in metabolically consequential cell types/tissues using in vivo experimental models.

| Experimental model | Treatment | Main findings | Reference |
|--------------------|-----------|---------------|-----------|
| Male B6 Cg-Ajifj mice (soleus) | Leu 1.5% drinking water, 4 months | ↑Ppara, Ucp3, Cat, Nrf1, ↔Nrf2 mRNA expression; ↑VO2 max | Guo 2010 [46] |
| Aged C57BL/6 mice (quadriceps) | Leu 500 mg kg⁻¹ BW (gavage), 12 weeks | ↔pAMPK, PGC-1α, SIRT1, PPARG, FASN expression | Liao 202 [69] |
| Aged C57BL/6 mice (quadriceps) | Leu 1250 mg kg⁻¹ BW (gavage), 12 weeks | ↑pAMPK, PGC-1α, SIRT1, PPARG, ↓FASN expression | Liao 202 [69] |
| Septic C57BL/6 mice (gastrocnemius) | Leu 100 mg kg⁻¹ BW (injection) | ↑Parg1a mRNA expression | Hou 202 [70] |
| Septic C57BL/6 mice (gastrocnemius) | Leu 100 mg kg⁻¹ + glutamine 500 mg kg⁻¹ BW (injection) | ↔Parg1a mRNA expression | Hou 202 [70] |
| ICR mice (quadriceps femoris) | Leu 500 mg kg⁻¹, 5 weeks | ↑PGC-1α, NRF1, TFAM, ↔SIRT1 protein expression | Jin 2022 [58] |
| C57BL/6 mice (WAT) | Leu 3% diet, 5 weeks | ↑Parg1a, Ucp1, ↓Sirt1, Prkaa mRNA expression; ↑UCP1, ↓AMPK, SIRT1 protein expression | Zhou 202 [71] |
| C57BL/6 mice (liver) | Leu 3% diet, 5 weeks | ↓Prkaa, ↓Sirt1, ↓Ppara, Cpi1 mRNA expression; ↑AMPK, ↓SIRT1 protein expression | Zhou 202 [71] |
| Male C57BL/6 mice (unloaded) | Leu 2.3 g kg⁻¹ BW day⁻¹, 28 days | ↔PGC-1α, pAMPK protein expression | Petrocelli 202 [42] |
| Male C57BL/6 mice (unloaded and reloaded) | Leu 2.3 g kg⁻¹ BW day⁻¹, 28 days | ↔PGC-1α, pAMPK protein expression | Petrocelli 202 [42] |
| Male SD rats (muscle) | Leu 3.2%wt wt low-fat feed (7%) feed, 42 days | ↑Parg1a mRNA expression | Baum 2016 [78] |
| Male SD rats (muscle) | Leu 3.2%wt wt high-fat feed (60%) feed, 42 days | ↑Parg1a mRNA expression | Baum 2016 [78] |
| Male Wistar rats (soleus) | Leu 135 mg kg⁻¹ BW (gavage), 1–3 h | ↑Parg1a, Tfam, Atp5a, Cycs, ↔Ndufa5 mRNA expression | Sato 2018 [22] |
| Male Wistar rats (EDL) | Leu 135 mg kg⁻¹ BW (gavage), 1–3 h | ↑Parg1a, ↓Tfam, Atp5a, Ndufa5, Cycs mRNA expression | Sato 2018 [22] |
| Tumor-bearing male Wistar rats (soleus) | Leu 3% diet, 18 days | ↔oxygen consumption | Viana 2021 [74] |
| Tumor-bearing male Wistar rats (EDL) | Leu 3% diet, 18 days | ↔oxygen consumption | Viana 2021 [74] |
| Tumor-bearing male Wistar rats (tibialis anterior) | Leu 3% diet, 18 days | ↑Cox5a, ↔C3 mRNA expression | Viana 2021 [74] |
| Piglets (Longissimus dorsi) | Leu 0.25% diet, 6 weeks | ↑Parg1a, Nrf2, Tfam, Cycs, Atp5g mRNA expression; ↑mtDNA content | Chen 2019 [10] |
| Piglets (liver) | Leu 0.25% diet, 6 weeks | ↑Parg1a, Nrf2, Tfam, Cycs, Atp5g mRNA expression; ↑mtDNA content | Chen 2019 [10] |
| Piglets (Longissimus dorsi) | Leu 0.5% diet, 6 weeks | ↔Parg1c, Nrf2, Tfam, Cycs, Atp5g mRNA expression; ↔mtDNA content | Chen 2019 [10] |
| Piglets (liver) | Leu 0.5% diet, 6 weeks | ↓Nrf2, ↔Parg1c, Tfam, Cycs, Atp5g mRNA expression; ↔mtDNA content | Chen 2019 [10] |
| Piglets (Longissimus dorsi) | Leu 0.25% diet, 7 weeks | ↑Prkaa2 mRNA expression; ↑pAMPK, PGC-1α protein expression; ↑SDH activity | Chen 2021 [72] |
| Piglets (Longissimus dorsi) | Leu 0.5% diet, 7 weeks | ↔Prkaa2 mRNA expression; ↔pAMPK, PGC-1α protein expression; ↔SDH activity | Chen 2021 [72] |
| NW and growth-slowed piglets (liver) | Leu 1.8% diet, 21 days | ↑Parg1a, Nrf1, Tfam, Sirt1, Atp5b, ↔Cox1, Coxiv, Coxv, Ndufa1, Ndufa13, Nuf2, Atp5g mRNA expression; ↔PPARG-1α protein expression; ↑mtDNA content | Su 2017 [73] |
| Landrace pigs (Longissimus dorsi) | +Leu 1.25% diet, 45 days | ↔Parg1a, Nrf1, Nrf2, Ucp3, Prkaa, Sirt1 mRNA expression; ↓pAMPK protein expression | Duan 2018 [79] |
| Landrace pigs (Soleus) | +Leu 1.25% diet, 45 days | ↔Parg1a, Nrf1, Nrf2, Ucp3, Prkaa, Sirt1 mRNA expression; ↓pAMPK protein expression | Duan 2018 [79] |
Table 2. (Continued).

| Experimental model       | Treatment                                                                 | Main findings                                                                 | Reference        |
|--------------------------|---------------------------------------------------------------------------|-------------------------------------------------------------------------------|------------------|
| Landrace pigs (Longissimus dorsi) | +KIC 1.25% diet, 45 days                                                  | ↑Ucp3, ↔Ppargc1a, Nrf2, Prkaa, Sirt1, ↓Nrf1 mRNA expression; ↓pAMPK protein expression | Duan 2018[79]    |
| Landrace pigs (Soleus)   | +KIC 1.25% diet, 45 days                                                  | ↑Ppargc1a, Nrf1, ↔Ucp3, Sirt1, ↓Prkaa mRNA expression; ↓pAMPK protein expression | Duan 2018[79]    |
| Male middle-aged pigs    | +HMB 0.62% diet, 45 days                                                  | ↔Ppargc1a, Nrf1, Prkaa, Ucp3, Sirt1 mRNA expression; ↓pAMPK protein expression | Duan 2018[79]    |
| C57BL/6 mice (gastrocnemius) | BCAA 20 mg mL⁻¹, (5 days week⁻¹), 8 weeks                                | ↑Ppargc1a, Tfam, Sirt1, Cox4i1, Atp5a1, Atp5b mRNA expression                 | Abedpoor 2018[57] |
| C57BL/6 mice (gastrocnemius) | BCAA 60 mg mL⁻¹, (5 days week⁻¹), 8 weeks                                | ↑Atp5b, ↔Ppargc1a, Tfam, Sirt1, Cox4i1, Atp5a1 mRNA expression                | Abedpoor 2018[57] |
| C57BL/6 mice (gastrocnemius) | BCAA 20 mg mL⁻¹, (5 days week⁻¹) + exercise, 8 weeks                     | ↑Ppargc1a, Tfam, Sirt1, Cox4i1, Atp5b, ↔Atp5a1 mRNA expression               | Abedpoor 2018[57] |
| C57BL/6 Mice (cardiac)   | BCAA 20 mg mL⁻¹, (5 days week⁻¹) + exercise, 8 weeks                     | ↑Ppargc1a, Tfam, Sirt1, Cox4i1, Atp5a1, ↔Atp5b mRNA expression               | Abedpoor 2018[57] |
| C57BL/6 mice (cardiac)   | BCAA 60 mg mL⁻¹, (5 days week⁻¹), 8 weeks                                | ↓Atp5a1, Atp5b, ↔Ppargc1a, Tfam, Sirt1, Cox4i1 mRNA expression                | Abedpoor 2018[57] |
| Male middle-aged mice    | BCAA 1.5 mg g⁻¹ BW drinking water, 3 months                              | ↑Ppargc1a, Nrf1, Tfam, ↔Sirt1 mRNA expression; ↑mitochondrial content/Cs activity | D’Antona 2010[33] |
| Hybrid B6.129S2 (soleus) | BCAA 1.5 mg g⁻¹ BW drinking water, 3 months + training, 1 month         | ↑Ppargc1a, Nrf1, Tfam, Sirt1 mRNA expression; ↑mitochondrial content/Cs activity | D’Antona 2010[33] |
| Male middle-aged mice    | BCAA 1.5 mg g⁻¹ BW drinking water, 3 months                              | ↑Ppargc1a, Nrf1, Tfam, ↔Sirt1 mRNA expression; ↓CS activity                  | D’Antona 2010[33] |
| Hybrid B6.129S2 (tibialis) | BCAA 1.5 mg g⁻¹ BW drinking water, 3 months + training, 1 month         | ↑Ppargc1a, Nrf1, Tfam, Sirt1 mRNA expression;↑CS activity                    | D’Antona 2010[33] |
| Male middle-aged mice    | BCAA 1.5 mg g⁻¹ BW drinking water, 3 months                              | ↑Ppargc1a, Nrf1, Tfam, Sirt1 mRNA expression; 1CS activity                  | D’Antona 2010[33] |
| Hybrid B6.129S2 (tibialis) | BCAA 1.5 mg g⁻¹ BW drinking water, 3 months                              | ↑Ppargc1a, Nrf1, Tfam, Sirt1 mRNA expression; 1CS activity                  | D’Antona 2010[33] |
| Male middle-aged mice    | BCAA 1.5 mg g⁻¹ BW drinking water, 3 months                              | ↑Ppargc1a, Nrf1, Tfam, Sirt1 mRNA expression; CS activity                    | D’Antona 2010[33] |
| Hybrid B6.129S2 (diaphragm) | BCAA 1.5 mg g⁻¹ BW drinking water, 3 months + training, 1 month         | ↑Ppargc1a, Nrf1, Tfam, Sirt1 mRNA expression; CS activity                    | D’Antona 2010[33] |
| Male middle-aged mice    | BCAA 1.5 mg g⁻¹ BW drinking water, 3 months                              | ↑Ppargc1a, Nrf1, Tfam, Sirt1 mRNA expression; 1CS activity                  | D’Antona 2010[33] |
| Hybrid B6.129S2 (heart)  | BCAA 1.5 mg g⁻¹ BW drinking water, 3 months                              | ↑Ppargc1a, Nrf1, Tfam, Sirt1 mRNA expression; CS activity                    | D’Antona 2010[33] |
| Male middle-aged mice    | BCAA 1.5 mg g⁻¹ BW drinking water, 3 months                              | ↑Ppargc1a, Nrf1, Tfam, Sirt1 mRNA expression; CS activity                    | D’Antona 2010[33] |
| Hybrid B6.129S2 (heart)  | BCAA 1.5 mg g⁻¹ BW drinking water, 3 months                              | ↔CS activity/mitochondrial content                                           | D’Antona 2010[33] |
| C57BL/6J N Mice (gastrocnemius) | BCAA 1.5 mg g⁻¹ BW drinking water, 1 month                               | ↔CS activity/mitochondrial content                                           | D’Antona 2010[33] |
| C57BL/6J N Mice (gastrocnemius) | BCAA 1.5 mg g⁻¹ BW drinking water, 1 month + Rvs 20 mg kg⁻¹ day⁻¹      | ↑/rescued CS activity/mitochondrial content                                 | D’Antona 2010[33] |
| C57BL/6J N Mice (tibialis) | BCAA 1.5 mg g⁻¹ BW drinking water, 1 month                               | ↔CS activity/mitochondrial content                                           | D’Antona 2010[33] |

(Continued)
Table 2. (Continued).

| Experimental model | Treatment | Main findings | Reference |
|---------------------|-----------|---------------|-----------|
| CS7BL/6J Mice (tibialis) | BCAA 1.5 mg g⁻¹ BW drinking water, 1 month + Rvs20 mg kg⁻¹ day⁻¹ | ↑rescued CS activity | D’Antono 2016[81] |
| Wistar rats (liver) | BCAA 1.5 mg g⁻¹ BW liquid diet, 8 weeks | ↑Ppargc1a, Ndufa9, Sdhb, Cytb, Cox1, Cox4 mRNA expression; ↑ATP content | Tanada 2015[80] |
| Male DS rats (skeletal) | BCAA 1.5 mg g⁻¹ BW drinking water + LS diet, 21 weeks | ↑Ppargc1a, Ndufa9, Sdhb, Cytb, Cox1, Cox4 mRNA expression | Tanada 2015[81] |
| Male rats (liver) | BCAA 10 g kg⁻¹ BW day⁻¹, 6 weeks | ↑Cpt1a, ↔Acad6, Tjam, Sdhh, Nufb8 mRNA expression; ↑ATP content | Tamai 2021[80] |
| Finishing pigs (adipose) | Protein-restricted feed with 2.5% BCAA 2:1:1 (Leu/Ile/Val) 43 days (adipose) | ↑Sirt1, UCP3, ↔Prkaa mRNA expression; ↑PGC-1α, pSIRT1, pAMPK protein expression | Zhang 2021[75] |
| Pigs (DSA) | BCAA supplementation (adequate) with protein-restriction | ↑Ppargc1a, Ucp2, Ucp3, Nrf1, Tfam, Prkaa, Atp6, ↔Cytb, Sirt1 mRNA expression; ↑pAMPK protein expression | Li 2016[84] |
| Pigs (ASA) | BCAA supplementation (adequate) with protein-restriction | ↑Ppargc1a, Ucp2, Ucp3, Nrf1, Tfam, Sirt1, Prkaa, Atp6 mRNA expression; ↔pAMPK protein expression | Li 2016[84] |
| Pigs (ASA) | BCAA supplementation (adequate) with protein-restriction | ↑Ppargc1a, Ucp2, Ucp3, Nrf1, Tfam, Sirt1, Prkaa, Atp6 mRNA expression; ↑pAMPK protein expression | Li 2016[84] |
| Lean Zucker rats (skeletal) | High protein (40%), 12 weeks | ↑Ppargc1a, ↔Sirt1 mRNA expression | French 2017[83] |

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leucine-stimulated mTORC1 activation and protein synthesis in some experimental models, though it could be that reduced ATP generation is responsible for reduced protein synthesis (instead of PGC-1α having direct interplay with mTORC1 signaling). Globally, it seems reasonable to suggest that intact protein synthesis capacity is likely necessary to reveal the true effects of a stimulus on mitochondrial biogenesis (and in that way, mTORC1 signaling is likely required). However, this conclusion should be coupled to the notion that the potential role of mTORC1 in mitochondrial respiration may be in-part dependent on tissue/cell type. For example, Raptor knockout in 3T3L1 adipocytes reduced lipid content, and WAT-specific Raptorknockdown increased mitochondrial respiration which was associated with heightened Ucp1 expression. Ultimately the authors concluded the role of mTORC1 is dependent on tissue-specific effectors. It is also worthy to note lipogenic signaling including Pparγ (a known target of mTORC1) was depressed in WAT of knockout mice, thus greater leanness could have been in-part attributable to reduced lipogenesis.

Another limitation of available evidence is the use of physiologically unattainable concentrations by some experiments, which may produce findings that have limited generalizability to other models. Some in vitro experiments used physiologically attainable concentrations, but in vitro reports still lack generalizability due to the isolated nature of the amino acids and the lack of fluctuations in amino acid concentrations as seen with pre- and post-prandial states. Other studies have used proof-of-concept concentrations that are not physiologically attainable. Thus, it is important to consider the wide continuum of concentrations used in in vitro experiments, and how they relate to physiologically attainable levels in animals and humans. Standard cell culture media contains high levels of BCAA (leucine &800 μM, isoleucine &800 μM, and valine &400 μM), while venous sampling of lean/insulin sensitive, obese/insulin sensitive, and obese/insulin resistant, revealed increasing levels of each BCAA with severity of insulin resistance, but still far below that used for in vitro experiments. Specifically, venous leucine was reported as &107, &113, and &151 μM, isoleucine reported as &53, &56, and &75 μM, and valine concentrations were reported as &211, &228, and &274 μM in each group, respectively. Similar concentrations to those reported above are observed in plasma lean and high-fat-fed mice. For that reason, existing evidence should be interpreted with these limitations in mind and future research should better attempt to consider these experimental knowledge gaps.

Lastly, it is important to note the emergence of the relationship of BCAA across several metabolic diseases. Circulating BCAA have been positively associated with obesity, insulin resistance, metabolic syndrome, and several other diseases. Importantly, the current knowledge of the relationships between BCAA on insulin resistance, cardiac dysfunction, and liver disease has been previously reviewed in detail by others. In general, the relationship between elevated circulating BCAA and such diseases is often attributed to an inability to degrade BCAA metabolism, often associated with downregulation or inhibition of BCAA catabolic enzymes. Though decreased catabolic capacity has been observed in several studies, the accumulation of BCAA is far more complicated. For example, it has also been suggested that circulating BCAA may be promoted in-part by de novo biosynthesis via the microbiome which could contribute to accumulation from dysregulated BCAA metabolism.

Mechanistically in insulin resistance, excess BCAA may cause an over stimulation of IRS-1 leading to depressed insulin sensitivity. The production of select catabolites such as

### Table 2. (Continued).

| Experimental model | Treatment | Main findings | Reference |
|--------------------|-----------|---------------|-----------|
| Obese Zucker rats (skeletal) | High protein (40%), 12 weeks | ↓Pparg1α, Sirt1 mRNA expression | French 2017(ref1) |
| Lean Zucker rats (adipose) | High protein (40%), 12 weeks | ↓Pparg1α, Sirt1 mRNA expression | French 2017(ref1) |
| Obese Zucker rats (adipose) | High protein (40%), 12 weeks | ↓Pparg1α, Sirt1 mRNA expression | French 2017(ref1) |
| Lean Zucker rats (liver) | High protein (40%), 12 weeks | ↓Pparg1α, Sirt1 mRNA expression | French 2017(ref1) |
| Obese Zucker rats (liver) | High protein (40%), 12 weeks | ↓Pparg1α, Sirt1 mRNA expression | French 2017(ref1) |
| Endurance-trained male athletes | WPI (additional 1.2 g kg⁻¹ BW) + CHO, 16 days | ↑Pparg1α, Prkka2 mRNA expression | Hill 2013(ref1) |

AMPKα, AMP-activated protein kinase alpha (Prkaa mRNA); AMPKα1, AMP-activated protein kinase alpha 1 (Prkaa1 mRNA); AMPKα2, AMP-activated protein kinase alpha 2 (Prkaa2 mRNA); AMPK, AMP-activated protein kinase (protein); ATP synthase (mRNA transcripts from Atpl6 gene, Atpl5g1, Atpl5g, and Atpl5g6 genes); BW, body weight; BCAA, branched-chain amino acid; cytochrome c oxidase (mRNA transcripts from Cox1, Cox4, Cox5a, Cox5b, and Cox7c genes); COX IV, cytochrome c oxidase subunit 4 (protein); Cpt1, carnitine palmitoyl transferase 1 (mRNA); Cs, citrate synthase (mRNA); CS, citrate synthase (protein); Cysc, cysteine c (mRNA); Ct, citrate synthase (protein); ERRα, estrogen related receptor alpha (protein); Esra, estrogen related receptor alpha (mRNA); HMB, β-hydroxy-β-methylbutyrate; ICR, Institute of Cancer Research; isocitrate dehydrogenase (mRNA transcripts from Idh3a gene); IC, α-ketocisocapric acid; Leu, leucine; mt-Atf4, mitochondrial NADH dehydrogenase 1 (mRNA); Tfam, mitochondrial transcription factor A (mRNA); TFAM, mitochondrial transcription factor A (protein); Nrf1, nuclear respiratory factor 1 (mRNA); Nufda1, nuclear respiratory factor 1/2 (protein); NADH dehydrogenase (mRNA transcripts from Ndufa1 gene); NADH dehydrogenase (mRNA transcripts from Ndufa9 gene); NADH dehydrogenase (mRNA transcripts from Ndufb8 gene); Pparγ1a, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (mRNA); Pparγ1b, peroxisome proliferator-activated receptor gamma coactivator 1-beta (mRNA); PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (protein); Prkaa2, peroxisome proliferator-activated receptor alpha (mRNA); Pparα, peroxisome proliferator-activated receptor alpha (mRNA); Pparβ, peroxisome proliferator-activated receptor beta (protein); Sirt1, Sirt1 (mRNA); Sirt1, Sirtuin 1 (protein); Sirt3, Sirtuin 3 (protein); WAT, white adipose tissue; WPI, whey protein isolate.
the valine catabolite 3-hydroxyisobutyrate (3-HIB) may further promote insulin resistance by enhancing lipid accumulation.[98] Additionally, the accumulation of BCAA leads to incomplete metabolism of fatty acids and resultant acyl-co-A or accumulated acyl-carnitine accumulation ultimately contributing to dysfunctional mitochondrial.[101] In cardiac dysfunction, BCAA accumulation could inhibit mitochondrial function, increase ROS production, and promote mTORC1 activation leading to hypertrophy.[105] In liver disease (such as liver cirrhosis), circulating BCAA may be reduced as a result of heightened BCAA metabolism to synthesize glutamine; a situation that is further complicated by insulin resistance.[101] Thus, another important consideration is the potential impact of existing disease which may alter the effect of BCAA on cell/tissue physiology.

Collectively, it appears leucine and/or BCAA mixtures can activate signaling associated with mitochondrial biogenesis, though as mentioned above, these effects may be conditional on the experimental environment such as cell culture model/treatment concentrations, animal model/tissue/and husbandry conditions, and existing disease state. Additionally, given the association of BCAA accumulation with several diseases, it is also unclear if BCAA-mediated upregulation of the mitochondrial biogenetic program is potentially favorable (such as that associated with exercise), or if the change in metabolic programming occurs as a compensation mechanism only (increased BCAA catabolism requires increased mitochondrial enzymes BCAT2 and BCKDH). Thus, increased mitochondrial biogenic signaling could be activated to facilitate increased BCAA degradation. Given the link between BCAA accumulation and various diseases, the accumulation of BCAA during these diseases and potential compensatory upregulation of mitochondrial signaling is worthy of study.

1.3. Concluding Remarks

As outlined above, several studies have shown BCAA (either individual or as a mixture) may promote signaling associated with increased mitochondrial biogenesis, though several limitations of existing evidence exist as noted above. We would also like to acknowledge several studies included findings that were not summarized within this review for the sake of brevity; however such outcomes are related and meaningful (such as endogenous antioxidant enzyme response to BCAA treatment) but are outside the scope of the current report. Taken together however, it does appear a body of evidence has emerged demonstrating the ability of BCAA to stimulate mitochondrial biogenesis. Yet, the sparse data in humans both with and without various diseases associated with elevated circulating BCAA, as well as the difficulty of controlling variables associated with feeding studies leaves the relevance of these findings to human physiological unclear in the context of improved health and/or disease prevention or management. Future well-controlled diet studies will be needed to assess if BCAA consumption is associated with increased mitochondrial biogenesis and improved metabolic outcomes in healthy and/or diseased populations.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

J.S.H. and R.A.V. reviewed and authored summaries of the reviewed literature. C.N.R. reviewed the manuscript and designed/produced the supporting mechanistic figure. R.A.V. was responsible for the conception and oversight of the review. All authors read and approved the final manuscript.

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Research data are not shared.

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[1] H. S. Brunetta, C. Q. de Camargo, E. A. Nunes, Amino Acids 2018, 50, 1663.
[2] N. P. Gannon, R. A. Vaughan, Amino Acids 2015, 48, 321.
[3] L. Zhang, F. Li, Q. Guo, Y. Duan, W. Wang, Y. Zhong, Y. Yang, Y. Yin, Nutrients 2020, 12, 1299.
[4] C. Ruocco, A. Segala, A. Valerio, E. Nisoli, Curr. Opin. Clin. Nutr. Metab. Care 2021, 24, 88.
[5] J. C. Anthony, T. G. Anthony, S. R. Kimball, L. S. Jefferson, J. Nutr. 2001, 131, 856S.
[6] J. L. Areata, J. A. Hawley, J. M. Ye, M. H. Chan, V. G. Coffey, Nutr. Res. 2014, 34, 1000.
[7] D. R. Bolster, T. C. Vary, S. R. Kimball, L. S. Jefferson, J. Nutr. 2004, 134, 1704.
[8] S. L. Casperson, M. Sheffield-Moore, S. J. Hewlings, D. Paddon-Jones, Clin. Nutr. 2012, 31, 512.
[9] Y. Chen, S. Sood, K. McIntire, R. Roth, R. Rabkin, Am. J. Physiol. Endocrinol. Metab. 2011, 301, E873.
[10] L. Deldicque, C. S. Canedo, S. Horman, I. De Potter, L. Bertrand, L. Hue, M. Francaux, Amino Acids 2008, 35, 147.
[11] M. Du, Q. W. Shen, M. J. Zhu, S. P. Ford, J. Anim. Sci. 2007, 85, 919.
[12] E. L. Glynn, C. S. Fry, M. J. Drummond, K. L. Timmerman, S. Dhanani, E. Volpi, B. B. Rasmussen, J. Nutr. 2010, 140, 1970.
[13] S. M. Son, S. J. Park, H. Lee, F. Siddiqi, J. E. Lee, F. M. Menzies, D. C. Rubinsztein, Cell Metab. 2019, 29, 192.
[14] A. Suryawan, A. J. Compri, W. A. Orellana, F. A. Wilson, H. V. Nguyen, T. A. Davis, Am. J. Physiol. Endocrinol. Metab. 2008, 295, E868.
[15] Y. Yin, K. Yao, Z. Liu, M. Gong, Z. Ruan, D. Dang, B. Tan, Z. Lui, G. Wu, Amino Acids 2010, 39, 1477.
[16] Q. Yin, J. M. Brameld, T. Parr, A. J. Murton, Amino Acids 2020, 52, 477.
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