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

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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. Introduction

Branched-chain amino acids (BCAA) are essential nutrients required in the human diet and have been linked with several important physiological effects, beyond being required for protein translation. Several reviews have discussed the potential metabolic benefits of BCAA including improved glucose homeostasis[1] and mitochondrial metabolism,[2–4] leading some to speculate that the strategic consumption of BCAA and essential amino acids (EAA) may provide some added benefit for diseased populations.[4] This narrative review will specifically describe studies that measured metabolic response to BCAA treatment including mitochondrial biogenic signaling (used to describe inductions in mRNA or protein content associated with increased mitochondrial content), mitochondrial biogenesis (used to describe an increase in mitochondrial content, mass, staining, abundance), and mitochondrial function (used to describe mitochondrial respiration/O_2 consumption) in metabolically consequential cell and tissue types such as skeletal muscle, cardiac muscle, and adipose.

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 rags (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, Nrf1, Esrra*, *Atp5g1, Cox5a*, *Cycs*, 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-1α) and transcription factor yin-yang 1 (YY1), namely the upregulation of PGC-1α (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 YY-1 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).[23] 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, 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-1α knockout mice given leucine showed diminished mTOR-mediated protein synthesis, suggesting PGC-1α 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).[22,39] Leucine appears to induce mitochondrial biogenesis through upregulation of PGC-1α[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-1α, and the sirtuins,[4,27–31,33,51–57] as well as peroxisome proliferator activated receptor beta (PPARβ/δ),[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[22,27–29,31,32,34,35,38,51,54,58–60] 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,[31,54] 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.[34,38] Unlike leucine treatment, treatment with isoleucine[59,61] or valine[39,59–61] 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 synthase (*Atp5o*), and *Cycs* (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, Nrf1*), 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 myocytes treated with leucine at either 100 or 500 µM for up to 48 h showing increases in PGC-1α 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 *Nrf1* gene expression.[29] Other reports

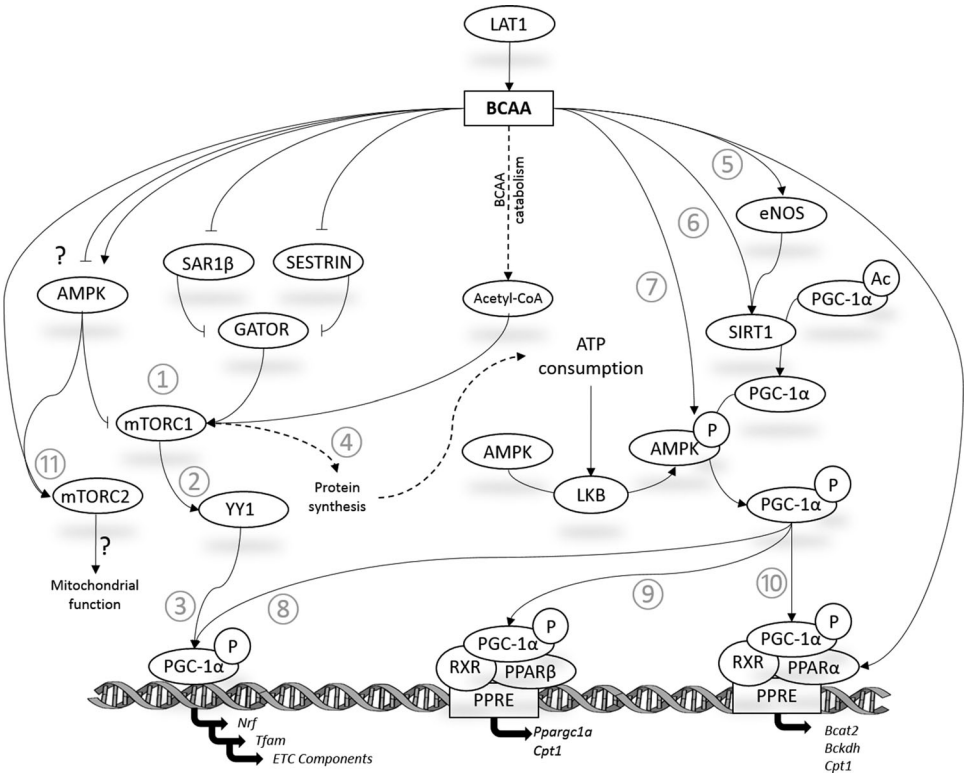

Figure 1. Mechanistic overview of the potential link between BCAA and mitochondrial biogenesis. ① BCAA stimulate protein synthesis through multiple mechanisms which activates mammalian/mechanistic target of rapamycin complex 1 (mTORC1), ② a known activator of yin-yang 1 (YY1) which works with peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) to ③ promote mitochondrial biogenesis. ④ Increased protein synthesis associated with mTOR activation increases ATP consumption and AMP-activated protein kinase (AMPK) activation. Along with activation of the ⑤ endothelial nitric oxide synthase (eNOS), ⑥ Sirtuin 1 (SIRT1), and ⑦ AMPK pathways, activated PGC-1α works in conjunction with PPARs (peroxisome proliferator-activated receptor alpha (PPARα) and peroxisome proliferator-activated receptor beta (PPARβ)) to stimulate ⑧ mitochondrial biogenesis, ⑨ drive its own (PGC-1α) expression, and ⑩ increase lipolytic and BCAA catabolic enzyme expression (including the mitochondrial catabolic enzymes associated with BCAA catabolism). ⑪ Leucine activation of mammalian/mechanistic target of rapamycin complex 2 (mTORC2) may also promote mitochondrial function. Other abbreviations: Bcat2, branched-chain amino acid transaminase 2; Bckdh, branched-chain alpha-keto acid dehydrogenase; Cpt1, carnitine palmitoyl transferase 1; GATOR, GAP activity towards Rags; LAT1, complex, large neutral amino acid transporter 1; Nrf, nuclear respiratory factor; Tfam, mitochondrial transcription factor A; and SAR1B, secretion associated Ras related GTPase 1B. "?" indicates these observations are still unclear and warrant additional study.

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.[38] In addition to dose-dependence, Wu et al.[38] 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.[34] 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.[61]

Other studies have compared the effect of leucine versus its catabolite by products. For example, the leucine-catabolite β-hydroxy-β-methylbutyrate (HMB) was compared against leucine using cultured C2C12 myotubes and showed supplementation of 0.5 mM for Leu or 50 μM for HMB for 24 h significantly increased mitochondrial mass, mitochondrial respiration capacity, and mRNA expression of mitochondrial biogenesis-related genes.[32] And while both treatments increased mitochondrial function (oxygen consumption), only HMB increased *Ppargc1a* mRNA.[32] Zhong et al.[32] 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.[28] 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 μM or 500 μM, or HMB at 25 μM or 50 μM for 48 h and showed increased palmitate oxidation across all groups versus control cells.[60] The same report also showed treatment with HMB at 25 μM or 50 μM, or leucine or the leucine-derived alpha-ketoic 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.[60] The experiments by Stancliffe et al.[60] also 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.[62] 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 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.[63]

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*), *Cycs*, mitochondrial NADH dehydrogenase 1 (*mt-Nd1*), cytochrome c oxidase (*Cox1*), and *Atp5g*.[31] 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.[31] 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.[54] Leucine treatment for 3 days at low levels (50 μM) has also been shown to increase molecular regulators of mitochondrial biogenesis including pAMPK, PGC-1α, NRF1, TFAM, and SIRT1 expression in C2C12 myotubes.[58] And although not in skeletal muscle, 160 μM leucine treatment for 2 h increased mitochondrial size and fusion in primary mouse and rat cardiomyocytes.[64]

Similar to leucine, cells treated with different ratios of BCAA mixtures for various durations also displayed increased mitochondrial biogenesis[33,55,56,59] and/or function[33,55,56,59] in C2C12 myotubes,[65] HL-1 cardiomyocytes,[33,55] mouse primary cardiomyocytes,[59] and HepG2 cells.[56] D'Antona et al.[33] 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.[29] 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.[66] 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*.[66] Another study using isolated mouse primary cardiomyocytes showed either leucine or valine, or their alpha-ketoic acid derivatives KIC or alpha-ketoisovalerate (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 for specific treatment concentrations).[59] Conversely, neither isoleucine or the alpha-ketoic acid derivative of isoleucine, α-keto-β-methylvalerate (KMV) altered PPARα expression or fatty acid oxidation.[59] 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*, *Cycs*, and *Cox4*, as well as increased protein expression of Cyt C and cytochrome c oxidase (COX IV).[55] 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.[55] D'Antona et al.[65] 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.[65] Tedesco et al.[56] also 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 using 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[22,30,31,58,68–73] and/or function[68,72] 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, 48 h	↑mitochondrial mass/respiration	Sun 2009[29]
C2C12 myocytes	Leu 0.5 mM, 48 h	↑*Sirt1*, *Ppargc1a*, *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[51]
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	↑*Ppargc1a*, *Tfam*, *Atp5o*, *Cycs*, ↔*Ndufa5* mRNA expression	Sato 2018[22]
C2C12 myotubes	Leu 0.5 mM, 1–48 h	↑*Ppargc1a, 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*, ↔*Ppargc1a*, *Ppara*, *Pparg*, ↓*Ucp3, Tfam* mRNA expression; ↔mitochondrial content	Wu 2019[38]
C2C12 myotubes	Leu 1.5 mM, 24 h	↑*Ppargc1a*, *Nrf1, Tfam, Sirt1, Ppara*, *Cpt1b, Pparg*, ↔*Ucp2, Ucp3* mRNA expression; ↔mitochondrial content	Wu 2019[38]
C2C12 myotubes	Leu 0.5 mM + PAM 0.75 mM, 24 h	↑*Sirt1, Nrf1, Tfam, Ppara*, *Cpt1b, Pparg*, ↔*Ppargc1a*, *Ucp2, Ucp3* mRNA expression; ↔mitochondrial content	Wu 2019[38]
C2C12 myotubes	Leu 1.5 mM + PAM 0.75 mM, 24 h	↑*Sirt1, Nrf1, Tfam, Ppara*, *Cpt1b, Pparg*, ↔*Ppargc1a* (rescued to control levels), *Ucp2, Ucp3* mRNA expression; ↑mitochondrial content	Wu 2019[38]
C2C12 myotubes	Leu 2 mM, 24 h	↑PGC-1α, NRF1, TFAM, Cyt C, PPARα, PPAR$\beta\delta$ protein expression; ↑mitochondrial content/respiration	Schnuck 2016[28]
C2C12 myotubes	Leu 0.5–2 mM, 24 h	↑*Ppargc1a*, *Sirt3*, *Cs*, ↔*Nrf1*, *Tfam* mRNA expression; ↑mitochondrial respiration	Johnson 2019[34]
C2C12 myotubes	Leu 0.5–2 mM + PAM 0.5 mM, 24 h	↔*Ppargc1a*, *Nrf1*, *Tfam*, *Sirt3*, *Cs* mRNA expression; ↑mitochondrial respiration	Johnson 2019[34]
C2C12 myotubes	Leu 2 mM, 24 h	↑*Ppargc1a*, *Nrf1*, *Tfam*, *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[58]
C2C12 myotubes	Leu 0.5 mM, 24 h	↑*Sirt1*, *Nrf1*, *Tfam*, ↔*Ppargc1a* mRNA expression; ↑mitochondrial content/respiration	Zhong 2018[32]
C2C12 myotubes	Leu 125 µM, 48 h	↑*Ppargc1a, 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	↑*Ppargc1a, Sirt1*, *Nrf1*, *Tfam* mRNA expression; ↑mitochondrial content/respiration	Zhong 2018[32]
C2C12 myotubes	HMB 6.25 µM, 24 h	↓*Ppargc1a*, *Tfam*, ↔*Nrf1*, *Sirt1*, *Sirt3* mRNA expression	Schnuck 2016[62]
C2C12 myotubes	HMB 12.5 µM, 24 h	↓*Ppargc1a*, *Tfam*, ↔*Nrf1*, *Sirt1*, *Sirt3* mRNA expression; ↔glucose/palmitate oxidation	Schnuck 2016[62]
C2C12 myotubes	HMB 25 µM, 24 h	↓*Ppargc1a, Sirt1, Nrf1*, *Tfam* mRNA expression; ↔PGC-1α, TFAM, SIRT1, SIRT3, ↓NRF1, PPARα, PPAR$\beta\delta$, pAMPK protein expression ↔glucose/palmitate oxidation	Schnuck 2016[62]
C2C12 myotubes	HMB 12.5 µM, 48 h	↑mitochondrial content	Stancliffe 2012[60]
C2C12 myotubes	HMB 25 µM, 48 h	↑*Ppargc1a, Nrf1*, *Ucp3* mRNA expression; ↑mitochondrial content/palmitate oxidation	Stancliffe 2012[60]
C2C12 myotubes	HMB 50 µM, 48 h	↑mitochondrial content/palmitate oxidation	Stancliffe 2012[60]
C2C12 myotubes	KIC 125 µM, 48 h	↑*Ppargc1a, Nrf1*, *Ucp3* mRNA expression; ↑mitochondrial content	Stancliffe 2012[60]
C2C12 myotubes	KIC 250 µM, 48 h	↑mitochondrial content/palmitate oxidation	Stancliffe 2012[60]
C2C12 myotubes	KIC 500 µM, 48 h	↑mitochondrial content/palmitate oxidation	Stancliffe 20[60]
C2C12 myotubes	Leu 0.5 mM with 10 nM sildenafil or icariin, 24–48 h	↑fat oxidation/mitochondrial content	Fu 2015[67]
Porcine skeletal muscle satellite cells	Leu 2 mM, 72 h	↑*Prkaa1, Tfam, Tfb1m, Cycs, mt-Nd1, Cox1, Atp5g* mRNA expression; ↑slow MyHC, SIRT1, pAMPK, PGC-1α, Cyt C protein expression; ↑MDH/SDH activity	Chen 2019[31]
Porcine skeletal muscle satellite cells	Leu 4 mM, 24 h	↑*Adipoq, Adipor1, Prkaa2, Ppargc1a, Nrf1, Atp5g, Tfam* 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[64]
Male Wistar rats (cardiomyocytes)	Leu 160 μM (media), 2 h	↑mitochondrial fusion/volume; ↓mitochondrial number	Morio 2021[64]
Mouse primary cardiomyocytes	Leu 0.208, 0.416, 0.832, 1.664 mM, 12 h	↑PPARα protein expression/fatty acid oxidation	Li 2020[59]
Mouse primary cardiomyocytes	KIC 0.208, 0.416, 0.832, 1.664 mM, 12 h	↑PPARα protein expression/fatty acid oxidation	Li 2020[59]
C2C12 myocytes	Ile 5 mM, 1 h	↔mitochondrial respiration	Suzuki 2020[61]
C2C12 myotubes	Ile 5 mM, 1 h	↔mitochondrial respiration	Suzuki 2020[61]
Mouse primary cardiomyocytes	Ile 0.107, 0.208, 0.416, 0.832 mM, 12 h	↔PPARα protein expression/fatty acid oxidation	Li 2020[59]
Mouse primary cardiomyocytes	KMV 0.107, 0.208, 0.416, 0.832 mM, 12 h	↔PPARα protein expression/fatty acid oxidation	Li 2020[59]
C2C12 myocytes	Val 5 mM, 1 h	↔mitochondrial respiration	Suzuki 2020[61]
C2C12 myotubes	Val 5 mM, 1 h	↔mitochondrial respiration	Suzuki 2020[61]
C2C12 myotubes	Val 125 μM, 48 h	↔mitochondrial content	Stancliffe 2012[60]
C2C12 myotubes	Val 250 μM, 48 h	↔mitochondrial content	Stancliffe 2012[60]
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[39]
Mouse primary cardiomyocytes	Val 0.117, 0.234, 0.418, 0.936 mM, 12 h	↑PPARα protein expression/fatty acid oxidation	Li 2020[59]
Mouse primary cardiomyocytes	KIV 0.117, 0.234, 0.418, 0.936 mM, 12 h	↑PPARα protein expression/fatty acid oxidation	Li 2020[59]
C2C12 myotubes	BCAA 2:1:1 (Leu:Ile:Val) 1 %wt:vol, 24 h	↑CS activity, oxygen consumption, ATP production	D'Antona 2016[65]
C2C12 myotubes	BCAA 2:1:1 (Leu:Ile:Val) 1 %wt:vol + Rvs 50 μM, 24 h	↑/rescued CS activity, oxygen consumption, ATP production	D'Antona 2016[65]
C2C12 myotubes	BCAA 2:1:1 (Leu:Ile:Val) 0.2 mM Leu content, 6 days	↔*Ppargc1a*, *Nrf1*, *Tfam*, *Cs, Atp5b,* ↓*Cox5a* mRNA expression; ↔mitochondrial content/basal and peak respiration	Rivera 2021[66]
C2C12 myotubes	BCAA 2:1:1 (Leu:Ile:Val) 2 mM Leu content, 6 days	↔*Ppargc1a*, *Nrf1*, *Tfam*, *Cs, Cox5a, Atp5b* mRNA expression; ↑mitochondrial content ↔basal and peak respiration	Rivera 2021[66]
C2C12 myotubes	BCAA 2:1:1 (Leu:Ile:Val) 20 mM Leu content, 6 days	↔*Ppargc1a*, *Nrf1*, *Tfam*, *Cs, Cox5a, Atp5b* mRNA expression; ↑mitochondrial content ↔basal ↓peak respiration	Rivera 2021[66]
C2C12 myotubes	BCAA 2:1:1 (Leu:Ile:Val) 0.2 mM Leu content, 6 days + IR	↔*Ppargc1a*, *Nrf1*, *Tfam*, *Cs, Atp5b,* ↓*Cox5a* mRNA expression; ↔mitochondrial content/basal and peak respiration	Rivera 2021[66]
C2C12 myotubes	BCAA 2:1:1 (Leu:Ile:Val) 2 mM Leu content, 6 days + IR	↔*Ppargc1a*, *Nrf1*, *Tfam*, *Cs, Atp5b,* ↓ *Cox5a* mRNA expression; ↔mitochondrial content/basal and peak respiration	Rivera 2021[66]
C2C12 myotubes	BCAA 2:1:1 (Leu:Ile:Val) 20 mM Leu content, 6 days + IR	↔*Nrf1*, *Tfam*, *Cs, Atp5b,* ↓ *Ppargc1a*, *Cox5a* mRNA expression; ↔mitochondrial content/basal and peak respiration	Rivera 2021[66]
HL-1 cardiomyocytes	BCAA 2:1:1 (Leu:Ile:Val,) 48 h	↑*Ppargc1a, Nrf1, Tfam, Cytc, Coxiv, Sirt1* mRNA expression; ↑SIRT1 protein expression; ↑mtDNA, ATP content	D'Antona 2010[33]
HL-1 cardiomyocytes	BCAA 2:1:1 (Leu:Ile:Val) 1 %wt:vol, 48 h	↑*Ppargc1a*, ↔*Nrf1, Tfam, Cytc, Coxiv* mRNA expression	Tedesco 2020[55]
HL-1 cardiomyocytes	α5 (BCAA mixture) 1 %wt:vol, 48 h	↑*Ppargc1a, Nrf1, Tfam, Cytc, Coxiv* mRNA expression; ↑COX IV, ↔Cyt C protein expression; ↑CS activity	Tedesco 2020[55]

(*Continued*)

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*, *Nrf1*, *Tfam* mRNA expression	Tedesco 2020[55]
HL-1 cardiomyocytes	α5 (BCAA mixture) 1 %wt:vol, 48 h + DOX 1 μM, 16 h	↑/rescued *Ppargc1a, Nrf1, Tfam, Cytc, Coxiv* mRNA expression; ↑COX IV, ↔Cyt C protein expression; ↑CS activity	Tedesco 2020[55]
Mouse primary cardiomyocytes	BCAA 2:1:1 (Leu:Ile:Val) 0.208, 0.416, 0.832, 1.664 mM, 12 h	↑*Ppara*, *Acaa2*, *Acadm*, *Cd36*, *Cpt1* mRNA expression; ↑PPARα protein expression; ↑PAM oxidation	Li 2020[59]
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[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[56]

Acaa2, acetyl-CoA acyltransferase 2 (mRNA); *Prkaa*, AMP-activated protein kinase alpha or AMPKα (mRNA); *Prkaa1*, AMP-activated protein kinase alpha 1 or AMPKα1 (mRNA); *Prkaa2*, AMP-activated protein kinase alpha 2 or AMPKα2 (mRNA); AMPK, AMP-activated protein kinase (protein); ATP synthase (mRNA transcripts from *Atp5b* gene, *Atp5g1, Atp5g*, and *Atp5o* genes); BCAA, branched-chain amino acid; fatty acid translocase (*Cd36* or *Fat*); 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); *Cycs*, cytochrome c (mRNA); Cyt C, cytochrome c (protein); ERRα, estrogen related receptor alpha (protein); *Esrra*, estrogen related receptor alpha (mRNA); HMB, β-hydroxy-β-methylbutyrate; isocitrate dehydrogenase (mRNA transcripts from *Idh3a* gene); Ile, isoleucine; IR, insulin resistance; KIC, α-ketoisocaproic acid; KIV, α-ketoisovalerate; KMV, α-keto-β-methylvalerate; Leu, leucine; *Acadm*, medium-chain acyl-CoA dehydrogenase (mRNA); *mt-Nd1*, mitochondrial NADH dehydrogenase 1 (mRNA); *Tfam*, mitochondrial transcription factor A (mRNA); TFAM, mitochondrial transcription factor A (protein); MyHC, myosin heavy chain (protein); *Nrf1*, nuclear respiratory factor 1 (mRNA); NRF1/2, nuclear respiratory factor 1/2 (protein); NADH dehydrogenase (mRNA transcripts from *Nufda1* gene); NADH dehydrogenase (mRNA transcripts from *Nufda9* gene); NADH dehydrogenase (mRNA transcripts from *Ndufb8* gene); *Ppargc1a*, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (mRNA); *Ppargc1b*, peroxisome proliferator-activated receptor gamma coactivator 1-beta (mRNA); PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (protein); *Ppara*, peroxisome proliferator-activated receptor alpha (mRNA); PPARα, peroxisome proliferator-activated receptor alpha (protein); *Ppard*, peroxisome proliferator-activated receptor beta (mRNA); PPARβ, 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.

experimental models, such as male B6.Cg-Ay/J mouse soleus,[68] aged C57BL/6J mouse quadriceps,[69] septic C57BL/6J mouse gastrocnemius,[70] ICR mouse quadriceps femoris,[58] C57BL/6J mouse WAT,[71] male Wistar rat soleus,[22] male Wistar rat EDL,[22] piglet *Longissimus dorsi*,[31,72] piglet liver,[30] and NW and growth-slowed piglet liver.[73] For example, 1.5% leucine in drinking water for 4 months has been shown to increase expression levels of *Ppara, Ucp3,* and *Nrf1* in soleus muscle of Ay mice, as well as increase total energy expenditure (VO_2).[68] Leucine has shown similar effects in aged mice (19-month old), where leucine supplementation at 1250 mg kg^{-1} for 12 weeks increased quadricep 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.[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.[58] Leucine supplementation at 135 mg kg^{-1} BW for 1–3 h also increased mitochondrial biogenic signaling in male Wistar rats, evidenced by increased expression of *Ppargc1a, Tfam, Atp5o*, and *Cycs* in EDL, and increased *Ppargc1a* in soleus, which were harvested 3 h post gavage.[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.[74] The same report also showed no effect of leucine supplementation on isolated muscle oxygen consumption in either the soleus or extensor digitorum longus.[74] To examine the effects of individual BCAA supplementation on lipid metabolism in C57BL/6J mice, mice were given 3% either leucine, isoleucine, valine, or a control diet for 5 weeks.[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 *Prkaa1*, 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 lipids).[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.[30] 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).[72] Using a 2 × 2 design, Su et al.[73] also observed dietary leucine supplementation at 1.8% in both normal-weight and growth-slowed piglets increased hepatic mRNA expression of *Ppargc1a, Nrf1, Tfam,*

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.[75] 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 PPARγ 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 *Ppargc1a*, which was also associated with increased mTORC1 activation.[77] Conversely, some evidence exists for reduced *Ppargc1a* 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 *Ppargc1a* expression following leucine supplementation in low-fat-fed rats, while leucine supplementation reduced *Ppargc1a* 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 *Ppargc1a, 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 *Ppargc1a* and Nrf1 and downregulate 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 *Ppargc1a, 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,[55] male rat liver,[80] and finishing pig adipose/muscle.[75–77] However, these effects may be dosage-,[57] diet-,[81] and/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 *Ppargc1a* expression versus control mice in both gastrocnemius and cardiac muscle, as was *Tfam*, Cytochrome C Oxidase Subunit 4I1 (*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 *Ppargc1a*, *Nrf1*, *Tfam*, 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 rescuing 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 mitochondria content (mtDNA) and number (confirmed via electron microscopy), *Ppargc1a*, *Nrf1*, *Tfam*, and *Sirt1* mRNA expression, along with restored COX IV and Cyt C protein content.[56] The same group also showed that α5 BCAA mixture at 1.5 mg g^{-1} BW can partially or completely restore the mRNA levels of *Ppargc1a*, *Tfam*, *Cycs*, 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.[80] The same report also showed rats treated with BCAA and l-carnitine for 6 weeks demonstrated significantly increased *Tfam*, *Acadsb*, *Cpt1a*, *Ndufb8*, and *Sdhd* expression.[80] Moreover, in Dahl salt-sensitive (DS) rats, BCAA supplementation at 1.5 mg g^{-1} BW for 21 weeks restored sodium-mediated suppression of *Ppargc1a*, NADH dehydrogenase 1 alpha subcomplex subunit 9 (*Ndufa9*), succinate dehydrogenase b (*Sdhb*), and cytochrome c oxidase (*Cox1/mt-Co1*) in skeletal muscle but not cardiac muscle.[81] 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, *Ppargc1a* 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 underinvestigated, 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-α2 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,[27,31,72,79] 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-Ay/J mice (soleus)	Leu 1.5% drinking water, 4 months	↑*Ppara, Ucp3, Crat, Nrf1,* ↔*Nrf2* mRNA expression; ↑VO_2 max	Guo 2010[68]
Aged C57BL/6J mice (quadricep)	Leu 500 mg kg^{-1} BW (gavage), 12 weeks	↔pAMPK, PGC-1α, SIRT1, PPARγ, FASN expression	Liao 2021[69]
Aged C57BL/6J mice (quadricep)	Leu 1250 mg kg^{-1} BW (gavage), 12 weeks	↑pAMPK, PGC-1α, SIRT1, PPARγ, ↓FASN expression	Liao 2021[69]
Septic C57BL/6J mice (gastrocnemius)	Leu 100 mg kg^{-1} BW (injection)	↑*Ppargc1a* mRNA expression	Hou 2021[70]
Septic C57BL/6J mice (gastrocnemius)	Leu 100 mg kg^{-1} + glutamine 500 mg kg^{-1} BW (injection)	↔*Ppargc1a* mRNA expression	Hou 2021[70]
ICR mice (quadriceps femoris)	Leu 500 mg kg^{-1}, 5 weeks	↑*PGC-1α*, NRF1, TFAM, ↔SIRT1 protein expression	Jin 2022[58]
C57BL/6J mice (WAT)	Leu 3% diet, 5 weeks	↑*Ppargc1a, Ucp1*, ↓*Sirt1, Prkaal* mRNA expression; ↑UCP1, ↓AMPK, SIRT1 protein expression	Zhou 2021[71]
C57BL/6J mice (liver)	Leu 3% diet, 5 weeks	↑*Prkaal*, ↓*Sirt1, Ppara, Cpt1* mRNA expression; ↑AMPK, ↓SIRT1 protein expression	Zhou 2021[71]
Male C57BL/6 mice (unloaded)	Leu 2.3 g kg^{-1} BW day^{-1}, 28 days	↔PGC-1α, pAMPK protein expression	Petrocelli 2021[82]
Male C57BL/6 mice (unloaded and reloaded)	Leu 2.3 g kg^{-1} BW day^{-1}, 28 days	↔PGC-1α, pAMPK protein expression	Petrocelli 2021[82]
Male SD rats (muscle)	Leu 3.2 %wt:wt low-fat feed (7%) feed, 42 days	↓*Ppargc1a* mRNA expression	Baum 2016[78]
Male SD rats (muscle)	Leu 3.2 %wt:wt high-fat feed (60%) feed, 42 days	↓*Ppargc1a* mRNA expression	Baum 2016[78]
Male Wistar rats (soleus)	Leu 135 mg kg^{-1} BW (gavage), 1–3 h	↑*Ppargc1a, Tfam, Atp5o, Cycs,* ↔*Ndufa5* mRNA expression	Sato 2018[22]
Male Wistar rats (EDL)	Leu 135 mg kg^{-1} BW (gavage), 1–3 h	↑*Ppargc1a*, ↔*Tfam, Atp5o, 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*, ↔*Cs* mRNA expression	Viana 2021[74]
Piglets (Longissimus dorsi)	Leu 0.25% diet, 6 weeks	↑*Ppargc1a, Nrf2, Tfam, Cytc, Atp5g* mRNA expression; ↑mtDNA content	Chen 2019[30]
Piglets (liver)	Leu 0.25% diet, 6 weeks	↑*Ppargc1a, Nrf2, Tfam, Cytc, Atp5g* mRNA expression; ↑mtDNA content	Chen 2019[30]
Piglets (Longissimus dorsi)	Leu 0.5% diet, 6 weeks	↔*Ppargc1a, Nrf2, Tfam, Cytc, Atp5g* mRNA expression; ↔mtDNA content	Chen 2019[30]
Piglets (liver)	Leu 0.5% diet, 6 weeks	↑*Nrf2*, ↔*Ppargc1a, Tfam, Cytc, Atp5g* mRNA expression; ↔mtDNA content	Chen 2019[30]
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	↑*Ppargc1a, Nrf1, Tfam*, *Sirt1*, *Atp5b,* ↔*Cox1, Coxiv, Coxv, Nufda1, Nufda13, Nufd1, Atp5g1* mRNA expression; ↔PGC-1α protein expression; ↑mtDNA content	Su 2017[73]
Landrace pigs (Longissimus dorsi)	+Leu 1.25% diet, 45 days	↔*Ppargc1a, Nrf2, Ucp3, Prkaa, Sirt1,* ↓*Nrf1* mRNA expression; ↓pAMPK protein expression	Duan 2018[79]
Landrace pigs (Soleus)	+Leu 1.25% diet, 45 days	↔*Ppargc1a, Nrf1, Nrf2, Ucp3, Prkaa, Sirt1* mRNA expression; ↑pAMPK protein expression	Duan 2018[79]

(*Continued*)

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*, ↔ *Nrf2, Ucp3, Sirt1*, ↓ *Prkaa* mRNA expression; ↓pAMPK protein expression	Duan 2018[79]
Landrace pigs (Longissimus dorsi)	+HMB 0.62% diet, 45 days	↔*Ppargc1a, Nrf1, Nrf2, Prkaa, Ucp3*, *Sirt1* mRNA expression; ↔pAMPK protein expression	Duan 2018[79]
Landrace pigs (soleus)	+HMB 0.62% diet, 45 days	↑*Prkaa*, ↔*Ppargc1a, Nrf1, Nrf2, 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 (gastrocnemius)	BCAA 60 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 20 mg mL^{-1}, (5 days $week^{-1}$), 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]
C57BL/6 mice (cardiac)	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 60 mg mL^{-1}, (5 days $week^{-1}$) + exercise, 8 weeks	↓*Ppargc1a, Tfam, Sirt1, Cox4i1, Atp5a1*, ↔*Atp5b* mRNA expression	Abedpoor 2018[57]
Male middle-aged mice-Hybrid B6.129S2 (soleus)	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]
Male middle-aged mice-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-Hybrid B6.129S2 (tibialis)	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-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-Hybrid B6.129S2 (diaphragm)	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-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-Hybrid B6.129S2 (heart)	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]
Male middle-aged mice-Hybrid B6.129S2 (heart)	BCAA 1.5 mg g^{-1} BW drinking water, 3 months + training, 1 month	↑*Ppargc1a, Nrf1, Tfam, Sirt1* mRNA expression; ↑mitochondrial content	D'Antona 2010[33]
C57BL/6JN Mice (gastrocnemius)	BCAA 1.5 mg g^{-1} BW drinking water, 1 month	↔CS activity/mitochondrial content	D'Antona 2016[65]
C57BL/6JN 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 2016[65]
C57BL/6JN Mice (tibialis)	BCAA 1.5 mg g^{-1} BW drinking water, 1 month	↔CS activity/mitochondrial content	D'Antona 2016[65]

(*Continued*)

Table 2. (Continued).

Experimental model	Treatment	Main findings	Reference
C57BL/6JN Mice (tibialis)	BCAA 1.5 mg g^{-1} BW drinking water, 1 month + Rvs20 mg kg^{-1} day^{-1}	↑/rescued CS activity	D'Antona 2016[65]
Wistar rats (liver)	BCAA 1.5 mg g^{-1} BW liquid diet, 8 weeks	↔*Ppargc1a, Nrf1, Tfam, Sirt1* mRNA expression; ↔COX IV, Cyt C, SIRT1 protein expression; ↔mitochondrial morphology/density, mtDNA content, CS activity	Tedesco 2018[56]
Wistar rats (liver)	BCAA 1.5 mg g^{-1} BW liquid diet + ethanol, 8 weeks	↑/rescued *Ppargc1a, Nrf1, Tfam, Sirt1* mRNA expression; ↑/rescued COX IV, Cyt C, ↔SIRT1 protein expression; ↑/rescued mitochondrial morphology/density, mtDNA content, CS activity	Tedesco 2018[56]
Mice (cardiac)	α5 (BCAA mix) 1.5 mg g^{-1} BW drinking water, 10 days	↑*Ppargc1a*, *Cytc*, *Coxiv*, ↔*Nrf1*, *Tfam* mRNA expression; ↑COX IV, ↔Cyt C expression; ↑CS activity, oxygen consumption	Tedesco 2020[55]
Mice (cardiac)	α5 (BCAA mix) BCAA 1.5 mg g^{-1} BW drinking water, 10 days + DOX 20 mg kg^{-1} day^{-1} (i.p. injection) 3 days	↑/rescued *Ppargc1a*, *Cytc*, *Coxiv*, ↔*Nrf1*, *Tfam* mRNA expression; ↑/rescued COX IV, ↔Cyt C protein expression; ↑/rescued CS activity/oxygen consumption	Tedesco 2020[55]
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 DS rats (skeletal)	BCAA 1.5 mg g^{-1} BW drinking water + HS diet, 21 weeks	↑/rescued *Ppargc1a, Ndufa9, Sdhb, Cox1*, ↔*Cytb*, *Cox4* mRNA expression	Tanada 2015[81]
Male DS rats (cardiac)	BCAA 1.5 mg g^{-1} BW drinking water + LS diet, 21 weeks	↓*Cytb*, ↔*Ppargc1a, Ndufa9, Sdhb, Cox1*, *Cox4* mRNA expression	Tanada 2015[81]
Male DS rats (cardiac)	BCAA 1.5 mg g^{-1} BW drinking water + HS 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*, ↔*Acadsb*, *Tfam*, *Sdhd, Nrufb8* mRNA expression; ↑ATP content	Tamai 2021[80]
Male rats (liver)	BCAA 10 g kg^{-1} BW day^{-1} + l-carn 40 mg kg^{-1} day^{-1}, 6 weeks	↑*Cpt1a*, *Acadsb*, *Tfam*, *Sdhd*, ↔*Nrufb8* 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, pAPMK protein expression	Zhang 2021[75]
Finishing pigs (adipose)	Protein-restricted feed with 2.5% BCAA 2:2:1 (Leu:Ile:Val) 43 days	↑*Sirt1*, ↔*Prkaa, Ucp3* mRNA expression; ↑PGC-1α, pSIRT1, pAPMK protein expression	Zhang 2021[75]
Finishing pigs (adipose)	Protein-restricted feed with 2.5% BCAA 2:1:2 (Leu:Ile:Val) 43 days	↑*Sirt1, Prkaa, Ucp3* mRNA expression; ↑PGC-1α, pSIRT1, pAPMK protein expression	Zhang 2021[75]
Finishing pigs (longissimus dorsi)	Protein-restricted feed with 2.5% BCAA 2:1:1 (Leu:Ile:Val) 43 days	↔*Sirt1, Prkaa, Ucp3* mRNA expression; ↑PGC-1α, pSIRT1, ↔pAPMK protein expression	Zhang 2021[76]
Finishing pigs (longissimus dorsi)	Protein-restricted feed with 2.5% BCAA 2:2:1 (Leu:Ile:Val) 43 days	↑*Sirt1, Prkaa, Ucp3* mRNA expression; ↑PGC-1α, pSIRT1, pAPMK protein expression	Zhang 2021[76]
Finishing pigs (longissimus dorsi)	Protein-restricted feed with 2.5% BCAA 2:1:2 (Leu:Ile:Val) 43 days	↔*Sirt1, Prkaa, Ucp3* mRNA expression; ↑PGC-1α, ↔pSIRT1, pAPMK protein expression	Zhang 2021[76]
Finishing pigs (longissimus dorsi)	Protein-restricted feed with 2.5% BCAA 2:1:1 (Leu:Ile:Val) 43 days	↑*Ppargc1a*, ↔*Opa1, Mtf2* mRNA expression	Zhang 2[77]
Finishing pigs (longissimus dorsi)	Protein-restricted feed with 2.5% BCAA 2:2:1 (Leu:Ile:Val) 43 days	↑*Ppargc1a*, *Opa1, Mtf2* mRNA expression	Zhang 2022[77]
Finishing pigs (longissimus dorsi)	Protein-restricted feed with 2.5% BCAA 2:1:2 (Leu:Ile:Val) 43 days	↑*Opa1, Mtf2*, ↔*Ppargc1a* mRNA expression	Zhang 202[77]
Pigs (DSA)	BCAA supplementation (adequate) with protein-restriction	↓*Ppargc1a, Ucp2, Ucp3, Nrf1, Tfam, Prkaa, Atp6*, ↔*Cytc*, *Sirt1* mRNA expression; ↓pAMPK protein expression	Li 2016[84]
Pigs (DSA)	2XBCAA supplementation (adequate) with protein-restriction	↓*Ppargc1a, Cytc, Ucp2, Ucp3, Nrf1, Tfam, Sirt1, Atp6, Prkaa* mRNA expression; ↓pAMPK protein expression	Li 2016[84]
Pigs (ASA)	BCAA supplementation (adequate) with protein-restriction	↑/rescued *Ppargc1a, Cytc, Ucp3, Tfam, Sirt1, Prkaa*, ↔*Nrf1*, *Ucp2*, *Atp6* mRNA expression; ↔pAMPK protein expression	Li 2016[84]
Pigs (ASA)	2XBCAA supplementation (adequate) with protein-restriction	↑/rescued *Ppargc1a, Cytc, Ucp2, Ucp3, Nrf1, Tfam, Sirt1, Atp6, Prkaa* mRNA expression; ↑/rescued pAMPK protein expression	Li 2016[84]
Lean Zucker rats (skeletal)	High protein (40%), 12 weeks	↑*Ppargc1a*, ↔*Sirt1* mRNA expression	French 2017[83]

(*Continued*)

Table 2. (Continued).

Experimental model	Treatment	Main findings	Reference
Obese Zucker rats (skeletal)	High protein (40%), 12 weeks	↔*Ppargc1a, Sirt1* mRNA expression	French 2017[83]
Lean Zucker rats (adipose)	High protein (40%), 12 weeks	↓*Ppargc1a*, ↔*Sirt1* mRNA expression	French 2017[83]
Obese Zucker rats (adipose)	High protein (40%), 12 weeks	↓*Ppargc1a*, ↔*Sirt1* mRNA expression	French 2017[83]
Lean Zucker rats (liver)	High protein (40%), 12 weeks	↔*Ppargc1a, Sirt1* mRNA expression	French 2017[83]
Obese Zucker rats (liver)	High protein (40%), 12 weeks	↔*Ppargc1a, Sirt1* mRNA expression	French 2017[83]
Endurance-trained male athletes	WPI (additional 1.2 g kg^{-1} BW) + CHO, 16 days	↑*Ppargc1a*, *Prkaa2* mRNA expression	Hill 2013[85]

AMPKα, AMP-activated protein kinase alpha (*Prkaa*, mRNA); AMPKα1, AMP-activated protein kinase alpha 1 or (*Prkaa1*, mRNA); AMPKα2, AMP-activated protein kinase alpha 2 (*Prkaa2*, mRNA); AMPK, AMP-activated protein kinase (protein), ATP synthase (mRNA transcripts from *Atp5b* gene, *Atp5g1, Atp5g*, and *Atp5o* 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); *Cycs*, cytochrome c (mRNA); *Cytb*, cytochrome b (mRNA); Cyt C, cytochrome c (protein); ERRα, estrogen related receptor alpha (protein); *Esrra*, estrogen related receptor alpha (mRNA); HMB, β-hydroxy-β-methylbutyrate; ICR, Institute of Cancer Research; isocitrate dehydrogenase (mRNA transcripts from *Idh3a* gene); KIC, α-ketoisocaproic acid; Leu, leucine; *mt-Nd1*, mitochondrial NADH dehydrogenase 1 (mRNA); *Tfam*, mitochondrial transcription factor A (mRNA); TFAM, mitochondrial transcription factor A (protein); *Nrf1*, nuclear respiratory factor 1 (mRNA); NRF1/2, nuclear respiratory factor 1/2 (protein); NADH dehydrogenase (mRNA transcripts from *Nufda1* gene); NADH dehydrogenase (mRNA transcripts from *Nufda9* gene); NADH dehydrogenase (mRNA transcripts from *Ndufb8* gene); *Ppargc1a*, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (mRNA); *Ppargc1b*, peroxisome proliferator-activated receptor gamma coactivator 1-beta (mRNA); PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (protein); *Ppara*, peroxisome proliferator-activated receptor alpha (mRNA); PPARα, peroxisome proliferator-activated receptor alpha (protein); *Ppard*, peroxisome proliferator-activated receptor beta (mRNA); PPARβ, peroxisome proliferator-activated receptor beta (protein); *Sirt1*, Sirtuin 1 (mRNA); SIRT1, Sirtuin 1 (protein); *Sirt3*, Sirtuin 3 (mRNA); SIRT3, Sirtuin 3 (protein); WAT, white adipose tissue; WPI, whey protein isolate.

leucine-stimulated mTORC1 activation and protein synthesis in some experimental models,[26] 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 Raptor knockdown increased mitochondrial respiration which was associated with heightened *Ucp1* expression.[24] Ultimately the authors concluded the role of mTORC1 is dependent on tissue-specific effectors.[24] It is also worthy to note lipogenic signaling including *Pparg* (a known target of mTORC1[95]) was depressed in WAT of knockout mice, thus greater leanness could have been in-part attributable to reduced lipogenesis.[24]

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.[96] Similar concentrations to those reported above are observed in plasma lean and high-fat-fed mice.[97] 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,[87,98–101] cardiac dysfunction,[102] and liver disease[103] 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 biogenesis via the microbiome which could contribute to accumulation from dysregulated BCAA metabolism.[104]

Mechanistically in insulin resistance, excess BCAA may cause an over stimulation of IRS-1 leading to depressed insulin sensitivity.[101] The production of select catabolites such as

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.[103] 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 biogenic 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.

Data Availability Statement

Research data are not shared.

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