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Regulation of metabolic health by essential dietary amino acids

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ABSTRACT

Although the beneficial effects of calorie restriction (CR) on health and aging were first observed a century ago, the specific macronutrients and molecular processes that mediate the effect of CR have been heavily debated. Recently, it has become clear that dietary protein plays a key role in regulating both metabolic health and longevity, and that both the quantity and quality - the specific amino acid composition - of dietary protein mediates metabolic health. Here, we discuss recent findings in model organisms ranging from yeast to mice and humans regarding the influence of dietary protein as well as specific amino acids on metabolic health, and the physiological and molecular mechanisms which may mediate these effects. We then discuss recent findings which suggest that the restriction of specific dietary amino acids may be a potent therapy to treat or prevent metabolic syndrome. Finally, we discuss the potential for dietary restriction of specific amino acids – or pharmaceuticals which harness these same mechanisms – to promote healthy aging.

1. Calorie and protein restriction promote metabolic health and longevity

Over 100 years ago, studies performed by Osborne and colleagues began to hint that restricting calorie intake might promote longevity (Osborne et al., 1917). Seminal work undertaken in 1935 demonstrated that this was indeed the case, as calorie restriction (CR) with adequate nutrition significantly extended the lifespan of rats (McCay et al., 1935). Since that time, CR has been investigated and shown to promote healthy aging in a wide range of organisms including yeast (*Saccharomyces cerevisiae*) (Lin et al., 2002), nematode worms (*Caenorhabditis elegans*) (Hosono et al., 1989), fruit flies (*Drosophila melanogaster*) (Bross et al., 2005), and mice (Weindruch and Walford, 1982). Dietary restriction has also been shown to increase lifespan in other species, including spiders (Austad, 1989), rotifers (Kirk, 2001; Gribble and Welch, 2013), water striders (Kaitala, 1991), dogs (Lawler et al., 2008) and cows (Pinney et al., 1972).

A major outstanding question is whether or not the dramatic benefits of CR on health and longevity in other mammals will also apply to humans. Studies of CR on the longevity of non-human primates suggest that CR does promote healthy aging. A study undertaken at the Wisconsin National Primate Research Center has found that adult onset CR in non-human primates lowers the frequency of age-related deaths, delays the onset of age-associated diseases (Colman et al., 2009), and reduces all-cause and age-related mortality (Colman et al., 2014). In contrast, a second colony of non-human primates studied at the National Institute for Health showed no improvement in survival outcomes following CR; however, once again beneficial effects of CR on health were observed, including reduced onset of cancer and diabetes (Mattison et al., 2012). Differences in diet and baseline intake of calories likely explains the disparities between the two studies, and analysis of the combined data from both studies suggest that CR promotes both health span and lifespan relative to *ad libitum* fed non-human primates (Mattison et al., 2017).

In humans, there have been several clinical trials of CR, as well as post-hoc analysis of individuals who self-impose CR (**reviewed** in Most et al., 2017). In the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE) randomized control trials, energy expenditure and fasting insulin were decreased, insulin sensitivity was increased, and a reduction in liver lipid deposition, oxidative stress, and other aging biomarkers were observed (Heilbronn et al., 2006; Il'yasova et al., 2018; Larson-Meyer et al., 2006). Non-obese humans from the Biosphere 2 experiments showed very similar responses to rodents on 20% CR, including decreases in insulin, cholesterol and triglycerides (Walford et al., 1992). Furthermore, in data from those who practice CR with optimal nutrition (CRON), CR reduces diseases of aging commonly associated with poor metabolic health, including type 2 diabetes, cardiovascular disease, cancer, stroke and vascular dementia (Most et al., 2017).

While it was originally hypothesized that CR increases longevity

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and improves metabolic health due to the reduction of calories, more recently it has been theorized that it is the concomitant reduction in protein that yields these beneficial effects (Fontana et al., 2008; Lee and Longo, 2016). Positive effects of a reduced protein diet on longevity were first reported in 1929 in brook trout (McCay et al., 1929), and it was later shown that male Sprague Dawley rats fed a diet in which 7.8% of calories were derived from protein lived almost 40% longer that rats fed a 20.4% protein diet (Ross, 1961). *Drosophila* fed a reduced yeast (protein) diet showed a similar lifespan increase to that of flies on CR, indicating that dietary protein may be a key mediator of the effects of a CR diet on longevity (Mair et al., 2005). Supporting this conclusion, a nutritional geometry study examining the effect of many different diets in *Drosophila* determined that a low protein:carbohydrate ratio promotes lifespan (Lee et al., 2008). Similar beneficial effects of reducing dietary protein on mice have also been observed, with Weindruch and colleagues finding that protein restriction (PR) increases the mean and maximal lifespan of female mice (Weindruch et al., 1986), and a very large study, utilizing a nutritional geometry approach to identify the optimal macronutrient ratio for mouse longevity, recently determined that male mice lived the longest on a low-protein, high-carbohydrate diet (Simpson et al., 2017; Solon-Biet et al., 2014).

While these results tilt strongly against traditional dietary advice for humans, a number of recent human studies have found that low protein intake is correlated with improved metabolic health and even increased longevity, and high protein intake is correlated with negative metabolic outcomes. A retrospective cohort analysis of humans between the ages of 50 and 65 found that a low protein diet (< 10% calories from protein) is associated with reduction in insulin-like growth factor 1 (IGF-1), cancer and mortality, whereas high protein intake was associated with an increase in diabetes at all ages above 50 (Levine et al., 2014). A similar beneficial effect of a low protein diet was observed in a large prospective cohort study, with people in the highest quartile of protein consumption having twice the diabetes risk of those in the lowest quartile (Sluijs et al., 2010). Most recently, a randomized control trial found that even short-term protein restriction could significantly reduce fat mass and improve blood glucose levels in humans (Fontana et al., 2016).

2. How does PR promote metabolic health?

Evidence that in humans low protein diets can reduce fat mass, improve blood glucose, reduce IGF-1 levels, and decrease the incidence of cancer without CR is extremely encouraging. As such, several hypotheses exist about the potential mechanisms underlying the beneficial effects of PR on metabolic health (Overview of studies in Table 1). Studies in rodents have consistently found that PR results in an increase in food intake whilst improving metabolic parameters, therefore it has been postulated that many benefits of PR result from increased energy expenditure (Laeger et al., 2014; Morrison et al., 2007; White et al., 2000). Here, we discuss potential molecules and pathways that may be crucial in mediating the beneficial effects of PR and the mechanisms by which they interact with each other.

2.1. FGF21

Recently, it has become clear that a key mediator of the effects of PR on metabolism, and in particular on insulin sensitivity and energy expenditure, is the hormone fibroblast growth factor 21 (FGF21) (Laeger et al., 2014). However, the effects of FGF21 on metabolism and metabolic health appear complex (Fig. 1). In rats fed a low protein diet, hepatic mRNA expression of *Fgf21* increases within 24 h of PR initiation, and circulating levels of FGF21 increase 10-fold after 4 days (Laeger et al., 2014). Similar increases in levels of FGF21 are seen in mice (Fontana et al., 2016; Solon-Biet et al., 2016) and in humans fed PR diets (Fontana et al., 2016; Laeger et al., 2014). Importantly, Laeger and colleagues have shown that mice lacking *Fgf21* do not exhibit alterations in food intake, energy expenditure, or weight gain when placed on a PR diet, demonstrating that FGF21 is a crucial mediator of the effects of PR (Laeger et al., 2014).

FGF21 was identified as a novel, liver produced fibroblast growth factor almost two decades ago (Nishimura et al., 2000), and came to the notice of metabolism researchers when it was discovered to be a potent stimulator of insulin-independent glucose uptake by 3T3-L1 mouse cells and primary human adipocytes (Kharitonenkov et al., 2005). Administration of FGF21 improves the metabolic profile of several different mouse models of diabetes, including *ob/ob, db/db* (Kharitonenkov et al., 2005) and diet-induced obese (DIO) mice (Xu et al., 2009). In addition, metabolic parameters were improved in obese, diabetic patients after one month of treatment with the FGF21 analog LY2405319 (Gaich et al., 2013), suggesting this is a fairly robust intervention. One of the primary effects of FGF21 is to promote hepatic insulin sensitivity; administration of FGF21 suppresses hepatic glucose production in *ob/ob* and *db/db* mice (Berglund et al., 2009) and DIO mice (Xu et al., 2009). Similar effects are observed following the infusion of FGF21 into the brains of obese rats (Sarruf et al., 2010). While the mechanism underlying the effects of FGF21 on hepatic glucose metabolism is not completely clear, FGF21's ability to promote hepatic insulin sensitivity requires suppression of the mechanistic Target Of Rapamycin Complex 1 (mTORC1) (Gong et al., 2016), a serine/threonine protein kinase which negatively regulates insulin sensitivity (Hsu et al., 2011; Rui et al., 2001; Yu et al., 2011). Several different FGF21 variants and agonists have now been tested as possible therapies for type 2 diabetes in mice and even in humans (Gaich et al., 2013; Sonoda et al., 2017).

In addition to improving hepatic insulin sensitivity directly, FGF21 has profound effects on energy balance and lipid metabolism. Energy expenditure is increased following administration of FGF21 to DIO mice (Coskun et al., 2008; Xu et al., 2009), significantly reducing the body weight and adipose mass. It is believed that this effect is due to the normal physiological role of FGF21 in adaptive thermogenesis; FGF21 mRNA is strongly induced in brown (BAT) and white adipose tissue (WAT) in response to cold exposure, and promotes the browning or beiging of WAT (Fisher et al., 2012). Cold-activation of beiging in subcutaneous WAT is abolished in adipose-specific *Fgf21* knockout mice, highlighting a critical role for adipose-produced FGF21 in the acute thermogenic response (Huang et al., 2017); although it is worth noting that FGF21-independent mechanisms can compensate for the lack of FGF21 during the long-term adaptation to cold (Keipert et al., 2017). One of the primary fuels for cold-induced thermogenesis is fatty acids, and FGF21 is also a potent mediator of fatty acid oxidation and lipid metabolism, increasing lipolysis in WAT (Inagaki et al., 2007) and substrate utilization in the liver (Badman et al., 2007). Fascinatingly, FGF21 is also induced by cold exposure in humans (Lee et al., 2014, 2013), and serum FGF21 levels correlate with BAT activity after acute cold exposure in males (Hanssen et al., 2015).

As mentioned above, knockout mouse studies have demonstrated that FGF21 is a key mediator of the metabolic effects of a PR diet. In mice, 14 days of PR is sufficient to increase energy expenditure, induce UCP1 expression in BAT and inguinal WAT, and induce morphological changes in WAT consistent with beiging; these changes are completely abrogated in *Fgf21*$^{-/-}$ mice (Laeger et al., 2016). Similarly, the effects of PR on energy expenditure and food intake are blocked in mice lacking *Ucp1* (Hill et al., 2017). The reduced metabolic effects of PR in *Fgf21*$^{-/-}$*and Ucp1*$^{-/-}$ mice suggests that activation of the FGF21-UCP1 axis may play a role in the beneficial effects of PR.

2.2. GCN2

FGF21 is regulated in part through the serine/threonine kinase general control nonderepressible 2 (GCN2), which is activated by binding to uncharged transfer ribonucleic acids (tRNAs) (Wek et al., 1989, 1995). Activated GCN2 phosphorylates eukaryotic initiation factor 2-α (eIF2α), which blocks translation of most messenger RNAs

Table 1
Recent studies examining the effects of protein restriction on metabolic health in rodents and humans. M = male, F = female, where not stated both sexes used. BAT = brown adipose tissue, BCAAs = branched chain amino acids, DIO = diet-induced obese, eIF2α = eukaryotic transcription factor 2α, FGF21 = fibroblast growth factor 21, IGF-1 = insulin-like growth factor 1, mTOR = mechanistic target of rapamycin, UCP1 = uncoupling protein 1, WAT = white adipose tissue.

Species/Strain/Sex	Dietary Protein (%)	Metabolic effect	Length of intervention	Study
Mice C57BL/6	5%	Increased food intake Increased adiposity Improved glucose tolerance Reduced circulating insulin Reduced mTOR activation	14 months	Solon-Biet et al. (2014)
Rats Sprague-Dawley (M)	9%	Increased food intake Increased liver expression and circulating FGF21 Increased liver eIF2α expression	14 days	Laeger et al. (2014)
Mice C57BL/6 (M)	4%	Increased food intake Increased energy expenditure Increased liver expression and circulating FGF21 Increased liver eIF2α expression	14 days	Laeger et al. (2014)
Human	5%	Increased circulating FGF21	28 days	Laeger et al. (2014)
Sprague Dawley rats (M)	10%	Increased food intake Increased fat mass Increased energy expenditure Reduced hepatic lipogenic expression Increased hepatic autophagy	14 days	Henagan et al. (2016)
Human (M)	7-9%	Decreased body weight Decreased fat mass Decreased fasting blood glucose Increased circulating FGF21 Decreased circulating BCAAs	43 days	Fontana et al. (2016)
Mice C57BL/6 J	5-7%	Increased food intake Reduced fat mass gain Weight loss Reduced lean mass Improved glucose and pyruvate tolerance Increased circulating FGF21 and adiponectin	12 weeks	Fontana et al. (2016)
Mice C57BL/6 (M)	4%	Increased food intake Reduced body weight and fat mass Increased energy expenditure Increased circulating and hepatic FGF21 expression Reduced hepatic lipogenesis expression Increased UCP1 expression (BAT and WAT) Increased UCP1 in BAT	27 weeks	Laeger et al. (2016)
Rats Obesity-prone Sprague Dawley (M)	0-5%	Decreased body and lean mass Increased energy expenditure Decreased plasma insulin, leptin and glucose Increased UCP-1, PGC1-α and FGF21 expression (BAT) Increased UCP-1 and FGF21 expression (muscle)	3 weeks	Pezeshki et al. (2016)
Mice C57BL/6NCrl (M)	5%	Increased energy intake Increased energy expenditure Reduced body, lean and fat mass Improved glucose metabolism Reduced circulating insulin, IGF-1 and leptin Increased circulating FGF21 Increased UCP1 expression (BAT and WAT)	16 weeks	Maida et al. (2016)
Human (M)	9%	Improved insulin sensitivity Decreased circulating insulin and glucose Increased serum FGF21	7 days	Maida et al. (2016)
Mice C57BL6/J	5%	Reduced body mass Low circulating insulin Increased liver expression and circulating FGF21 Increased *UCP1* expression (BAT)	14 months	Solon-Biet et al. (2016)
Mice C57BL/6 (M)	5%	Increased food intake Decreased body, lean and fat mass Increased energy expenditure Increased circulating and hepatic Fgf21 expression Reduced hepatic lipogenesis expression Increased UCP1 expression (BAT)	6 weeks	Hill et al. (2017)
Mice C57BL/6 J DIO (M)	5%	Increased energy intake Decreased body weight Decreased lean and fat mass Increased energy expenditure Improved glucose tolerance and insulin sensitivity Reduced hepatic lipogenesis expression Increased hepatic lipogenic expression	12 weeks	Cummings et al. (2018)
Rats Wistar fatty (M)	6%	Decreased fat and body weight Reduced circulating glucose Improved insulin resistance Increased plasma FGF21 Increased UCP1 expression (BAT)	24 weeks	Kitada et al. (2018)

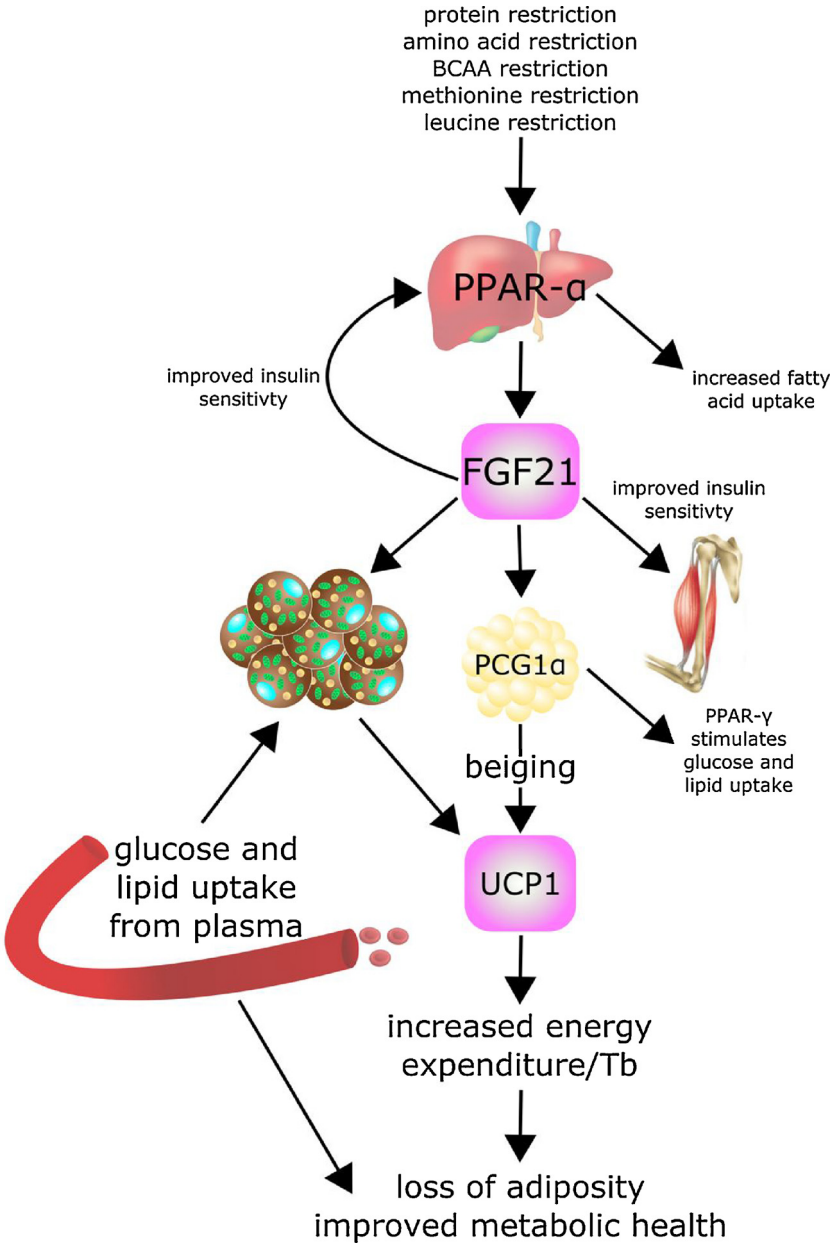

Fig. 1. FGF21 is a key mediator of the metabolic effects of protein and amino acid restriction. FGF21 = fibroblast growth factor 21, PCG1α = Peroxisome proliferator-activated receptor-γ coactivator-1α, PPAR-α = Peroxisome proliferator-activated receptor-α, UCP1 = uncoupling protein 1.

(mRNAs) (Dever et al., 1992). However, eIF2α induces the translation of activating transcription factor 4 (ATF4), which is a key effector of the integrated stress response (Harding et al., 2000; Vattem and Wek, 2004), and is involved in several cell signaling pathways including inflammation (Zhong et al., 2012), autophagy (B'chir et al., 2013), and responses to mitochondrial stress (Quirós et al., 2017). Among its many effects, ATF4 initiates translation of Sestrin2, which can inhibit mTORC1 through blocking lysosomal localization (Ye et al., 2015). GCN2 is highly conserved across eukaryotes and activation leads to several changes that act in opposition to mTOR including blocking translation, inducing autophagy and arresting growth (Lehman et al., 2015).

Mice lacking *Gcn2* initially have a delayed response to PR; however, after 14 days of PR feeding, changes in food intake, energy expenditure and body weight start to appear, likely in response to increased FGF21, the increase in which is similarly delayed (Laeger et al., 2016). Further work showed that although binding of ATF4 to the FGF21 promoter is blunted in *Gcn2* knockout mice during the acute response to PR, chronic PR eventually leads to the binding of wild-type levels of ATF4 (Laeger et al., 2016). This suggests that whilst GCN2 is necessary for the acute response to PR, pathways upstream of ATF4 may act as auxiliary activators to induce FGF21 stimulation in the absence of GCN2 (Laeger et al., 2016).

2.3. mTOR

Conserved across all eukaryotes, the mechanistic Target Of Rapamycin (mTOR) is a phosphatidylinositol 3-kinase (PI3K)-like serine/threonine protein kinase which can be found in two complexes, mTOR complex 1 (mTORC1) and complex 2 (mTORC2), which phosphorylate a diverse set of substrates to regulate numerous cellular and physiological processes. To coordinate the catabolic or anabolic direction of metabolic processes at a given time, mTORC1 integrates nutritional information, including the availability of glucose, amino acids

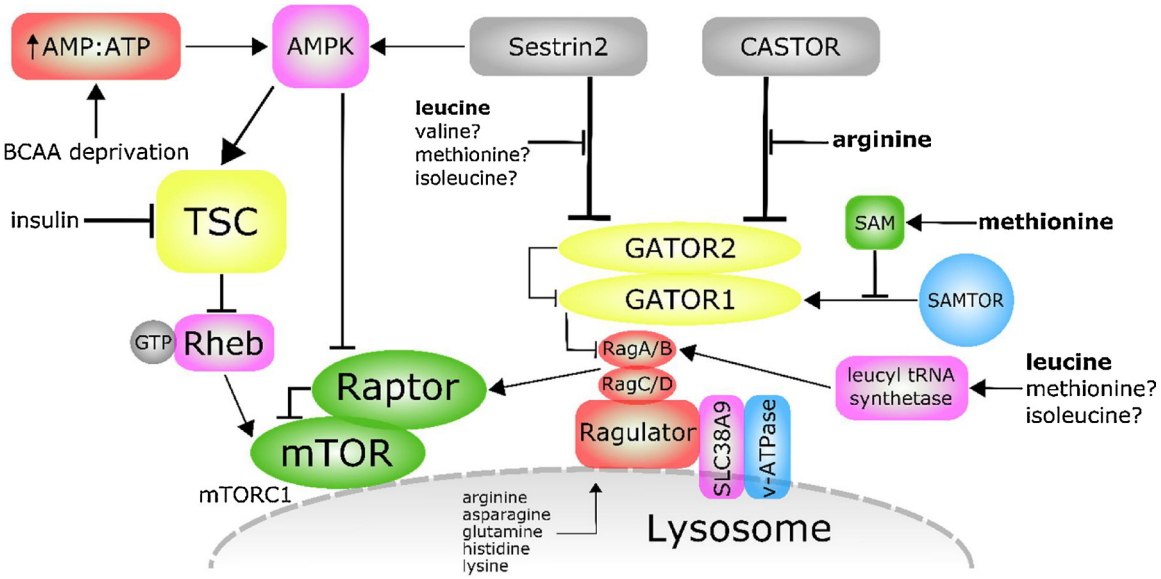

Fig. 2. Regulation of mTORC1 by amino acids. AMP = adenosine monophosphate, AMPK = AMP-activated protein kinase, ATP = adenosine triphosphate, mTORC1 = mechanistic target of rapamycin complex 1, Raptor = regulatory-associated protein of mTOR, Rheb = Ras homolog enriched in brain, SAM = S-adenosyl methionine, SAMTOR = S-adenosyl methionine/target of rapamycin, TSC = tuberous sclerosis complex.

and oxygen, to control processes such as protein synthesis and autophagy. In contrast, mTORC2 acts to regulate metabolism in response to many hormonal cues, including insulin and IGF-1. Inhibition of mTORC1, either genetically or pharmacologically, extends lifespan in organisms ranging from yeast to mice, whereas inhibition of mTORC2 results in insulin resistance and shorter lifespan in male mice (Kennedy and Lamming, 2016; Lamming et al., 2014).

As amino acids are agonists of mTORC1, it is natural to conclude that reduced protein diets would reduce mTORC1 activity. Indeed, the weight of evidence of suggests that this is indeed the case. *Drosophila* on a low-protein, high-sugar diet have decreased TOR signaling (Sun et al., 2012), and Sprague-Dawley rats consuming a low protein, ketogenic diet have decreased mTORC1 signaling in the liver and hippocampus (McDaniel et al., 2011). Low protein intake is associated with reduced mTOR activity in the livers of mice (Solon-Biet et al., 2014), and mice fed a low protein diet have reduced mTOR activity in heart, skeletal muscle, and WAT (Lamming et al., 2015).

Amino acid activation of mTORC1 is a complex process that has only recently begun to be unraveled (Fig. 2) (Goberdhan et al., 2016; Wolfson and Sabatini, 2017). In response to amino acids, mTORC1 is recruited to the lysosome by the Rag family of small GTPases (Sancak et al., 2010). The Rag GTPases are regulated in turn by the Ragulator complex, which has guanine nucleotide exchange factor (GEF) activity towards RagA and RagB (Bar-Peled et al., 2012). The Ragulator and v-ATPase interact with a low affinity amino acid transporter in the lysosome membrane (SLC38A9), which is required for mTORC1 activation by amino acids glutamine, arginine and leucine (Jung et al., 2015; Rebsamen et al., 2015; Wang et al., 2015). Recent work has found that arginine acts to stimulate the efflux of many of essential amino acids, including leucine, from the lysosome into the cytoplasm; sensing of leucine and other amino acids then occurs in the cytosol (Wyant et al., 2017). This stands in contrast to the previous "inside-out" model, in which the sensing of amino acids occurs inside the lysosome (Zoncu et al., 2011).

The Rag GTPases are also regulated by the GATOR complex, which is composed of two subcomplexes termed GATOR1 and GATOR2; GATOR1 is a GTPase-activating protein (GAP) for RagA and RagB, and acts to inhibit the lysosomal recruitment of mTORC1 in response to amino acids. In contrast, GATOR2 negatively regulates GATOR1 activity (Bar-Peled et al., 2013), and appears to be a major site of regulation by amino acids. Thus far, two inhibitors of GATOR2 have been described, Sestrin2 and CASTOR1. As shown in Fig. 2, leucine and arginine disrupt the interaction between GATOR2 and Sestrin2 (leucine) and GATOR2 and CASTOR1 (arginine), thereby allowing lysosomal recruitment of mTORC1 (Chantranupong et al., 2016; Wolfson et al., 2016). In addition to inhibiting mTORC1 via this pathway, Sestrin2 also activates AMPK, which negatively regulates mTORC1 via the phosphorylation of the mTORC1 subunit RAPTOR and the Tuberous Sclerosis Complex (TSC) (Gwinn et al., 2008; Inoki et al., 2002). Recruitment of GATOR1 to the lysosome requires the KICSTOR complex, which is composed by KPTN, ITFG2, C12orf66, and SZT2 (Wolfson et al., 2017). It is not yet known if the KICSTOR complex plays a role in amino acid sensing upstream of mTORC1.

mTORC1 activity is also regulated by folliculin (FLCN), a tumor suppressor, associated with Birt-Hogg-Dubé (BHD) syndrome. FLCN and its binding partners folliculin-interacting proteins 1 and 2 (FNIP1/2) were recently discovered to positively regulate mTORC1 activity by acting as a GAP for RagC and RagD hetreodimers (Tsun et al., 2013). FLCN itself is recruited to the lysosome when amino acids are limiting, and also interacts with RagA (Petit et al., 2013). Suppression of *FCLN* reduces lysosomal leucine levels, which results in a decrease in mTORC1 activity. The reduction in lysosomal leucine may be due to FCLNs role in supression of Proton-Assisted Amino Acid Transporter 1 (PAT1), a lysosomal transporter of amino acids (Wu et al., 2016). In addition to amino acids, FLCN is also involved of the sensing of other environmental cues. At least in some cell types, FLCN is found in primary cilia, and links mTORC1 activity to flow stress through the regulation of liver kinase B1 (LKB1) and AMPK (Zhong et al., 2016). FLCN has recently been shown to regulate cytoplasmic retention of Transcription Factor Binding To IGHM Enhancer 3 (TFE3) by mTOR in response to amino acid signaling through RagC and RagD (Wada et al., 2016).

Activation of mTORC1 requires not only localization to the lysosome, but also interaction with GTP-bound Rheb, a small GTPase which is, like mTORC1, localized to the lysosome when amino acids are present (Fawal et al., 2015). However, in the absence of insulin/IGF-1 signaling, Rheb is kept in an inactive state by TSC, which acts as a GAP for Rheb; phosphorylation of TSC by Akt causes TSC to depart from the lysosome, permitting Rheb-GTP to activate mTORC1 (Menon et al., 2014). mTORC1 activation thus requires not only the availability of

amino acids, but also a permissive hormonal state for anabolism, as signaled by insulin/IGF-1.

The relationship between mTORC1 and insulin sensitivity is complex; while genetic mouse models of altered liver mTORC1 activity generally have normal glucose tolerance (Lamming et al., 2012; Sengupta et al., 2010), mTORC1 may play a role in the regulation of fasting glucose levels (Caron et al., 2017). Furthermore, it is widely accepted that hyperactive mTORC1 signaling in the liver contributes to insulin resistance via feedback inhibition of insulin receptor substrate (Saxton and Sabatini, 2017). Conversely, the activity of mTORC1 in skeletal muscle and adipose tissue may actually promote glucose tolerance – and in the context of adipose tissue, leanness as well (Bentzinger et al., 2008; Polak et al., 2008). The contribution of mTORC1 in different tissues to the effect of PR on glycemic control remains to be determined.

mTORC1 also plays an important role in regulating energy expenditure through the regulation of BAT as well being involved in the beiging of WAT. The loss of mTORC1 in adipocytes completely blocks BAT expansion in response to cold exposure and reduces mitochondrial biogenesis and oxidative metabolism (Labbé et al., 2016). Activation of mTORC1 by cold exposure was also associated with an increase in Akt (Labbé et al., 2016), which plays a critical role in the response to cold exposure by promoting glucose uptake (Albert et al., 2016).

This is confounding, as PR decreases the expression of mTOR and ribosomal protein S6 kinase 1 (S6K1), an important mTORC1 substrate (Ma et al., 2015; Xiao et al., 2011), but increases BAT activation in rodents (Elsukova et al., 2012; Selman et al., 2005), and increased energy expenditure through mitochondrial uncoupling is thought to have a positive effect on lifespan (Speakman et al., 2004). During protein restriction, energy expenditure is also increased via other mechanisms in response to FGF21, including the beiging of WAT. However, mTORC1 also play an important role in beiging; treatment with rapamycin or adipocyte-specific deletion of *Raptor* blocks the ability of β-adrenergic signaling to induce beiging in response to cold (Tran et al., 2016). The exact mechanism by which PR induces energy expenditure and the role of mTORC1 in this response thus remains to be determined.

2.4. Cross talk between metabolic pathways

The interaction between these nutrient sensing and energy regulating pathways is complex (Fig. 3). Amino acid availability regulates mTORC1 and GCN2 directly. Amino acid depletion activates GCN2, inhibiting eIF2α and thus stimulating the translation of ATF4. It was recently shown that ATF4 promotes the transcription of Sestrin2, thus inhibiting mTORC1 by preventing its lysosomal localization (Ye et al., 2015) and also by activating AMPK (Budanov and Karin, 2008). Cross-talk between FGF21 and mTORC1 has been well-documented, and each is believed to regulate the other, with some differences between cell types. In adipocytes, administration of FGF21 activates both mTORC1 and its downstream target S6K1 via mitogen-activated protein kinase (MAPK), with the presence of mTORC1 and S6K1 being essential for the ability of FGF21 to induce *Ucp1* and stimulate glucose uptake (Minard et al., 2016). However, administration of FGF21 to mice promotes hepatic insulin sensitivity by suppressing mTORC1 activity in the liver; conversely, mice lacking *Fgf21* have increased liver mTORC1 activity (Gong et al., 2016).

In turn, mTORC1 plays an important role in the regulation of FGF21. *Fgf21* transcription in hepatocytes is controlled physiologically by insulin and glucagon, which act to induce ATF4 by stimulating the activity of the PI3K/AKT/mTORC1 and cAMP/PKA signaling pathways, respectively (Alonge et al., 2017). This appears to be true *in vivo* as well, as mice lacking hepatic *Tsc1*, which have constitutively active mTORC1 signaling, have increased hepatic expression of *Fgf21* and increased circulating levels of FGF21 (Cornu et al., 2014). Constitutive activation of mTORC1 signaling in skeletal muscle, via deletion of *Tsc1* in skeletal muscle (Guridi et al., 2015) or by constitutively active 4E-BP1 (Tsai et al., 2015) leads to increased circulating levels of FGF21 as a direct result of increased muscle expression of *Fgf21*.

3. Amino acids act as metabolic health regulators

While both human and rodent studies demonstrate the metabolic benefits of a low protein diet, a long-standing question has been whether all types of dietary protein are equivalent. For instance, are plant-derived proteins healthier than animal proteins? Protein intake studies in humans have suggested that whilst high intake of animal proteins has a negative effect on metabolic health, this may not be the case for vegetable protein intake (Azemati et al., 2017; Sluijs et al., 2010). Indeed, clinical trials of vegan diets have found that they promote metabolic health, although the lower levels of proteins consumed by many vegans is a confounding factor (Barnard et al., 2009, 2006; Lee et al., 2016). While there are many differences between animal-derived protein and plant-based protein, one suggestion is that the beneficial metabolic effects of a vegan diet are driven by decreased levels of the sulfur containing amino acids, cysteine and methionine, which are particularly low in vegetable sources (Sosulski and Imafidon, 1990). More recently, work has highlighted the possibility that dietary branched-chain amino acids (BCAAs) may be critical regulators of metabolic health. Below, we discuss emerging evidence that the specific amino acid composition of the diet is a critical regulator of metabolic health.

3.1. Methionine

For the past quarter of a century, reduced consumption of methionine has been theorized to be a key driver of the response to CR, as methionine restriction (MR) extends the lifespan of Fischer 344 rats (Orentreich et al., 1993). While the mechanism of this effect is unclear, MR increases blood glutathione levels, which could be indicative of improved resistance to oxidative stress (Richie et al., 1994). Restricting methionine by approximately 70% is sufficient to increase lifespan in mice, as well as mirroring some of the metabolic effects of CR, including decreased blood levels of insulin and IGF-1 (Miller et al., 2005). The naturally low levels of methionine in vegan foods has led to the suggestion that MR may be a more feasible lifespan-extending strategy than CR (McCarty et al., 2009). However, recent research suggests that at least in mice, a relatively large 70–80% reduction of dietary methionine levels is optimal for achieving the metabolic benefits of MR (Forney et al., 2017).

From a metabolic standpoint, MR has many beneficial effects in both rodents and humans; preventing weight gain and fat accretion, and improving control of blood glucose (Brown-Borg and Buffenstein, 2017; Cummings and Lamming, 2017; Miller et al., 2005; Plaisance et al., 2011). Many of these factors have been linked to FGF21, levels of which are upregulated by MR in both young and aged mice (Lees et al., 2014; Perrone et al., 2012). FGF21 is also thought to mediate the increased energy expenditure seen in response to MR, potentially by stimulating the beiging of WAT to a more metabolically active adipose that resembles BAT (Douris et al., 2015).

Alterations in methionine intake appear to be strongly linked to the other sulfur-containing amino acid, cysteine. In addition to its role in protein translation, cysteine is a precursor for important cellular regulators such as glutathione (GSH), taurine, and hydrogen sulfide (H_2S). Supplementation of cysteine is able to reverse the effects of MR on mouse and rat adiposity and energy intake; however cysteine supplementation alone does not appear to cause negative effects to metabolic health (Elshorbagy et al., 2011; Wanders et al., 2016). Further work indicated that cysteine supplementation reversed most of the genetic and metabolic changes induced by MR in the inguinal adipose tissue depots, and some of the changes in the liver, in addition to enhancing transcription of inflammation and carcinogenic genes. Moreover, metabolite levels returned to control fed levels in the liver, serum, muscle and fat depots of MR cysteine fed rats (Perrone et al., 2012). Additional

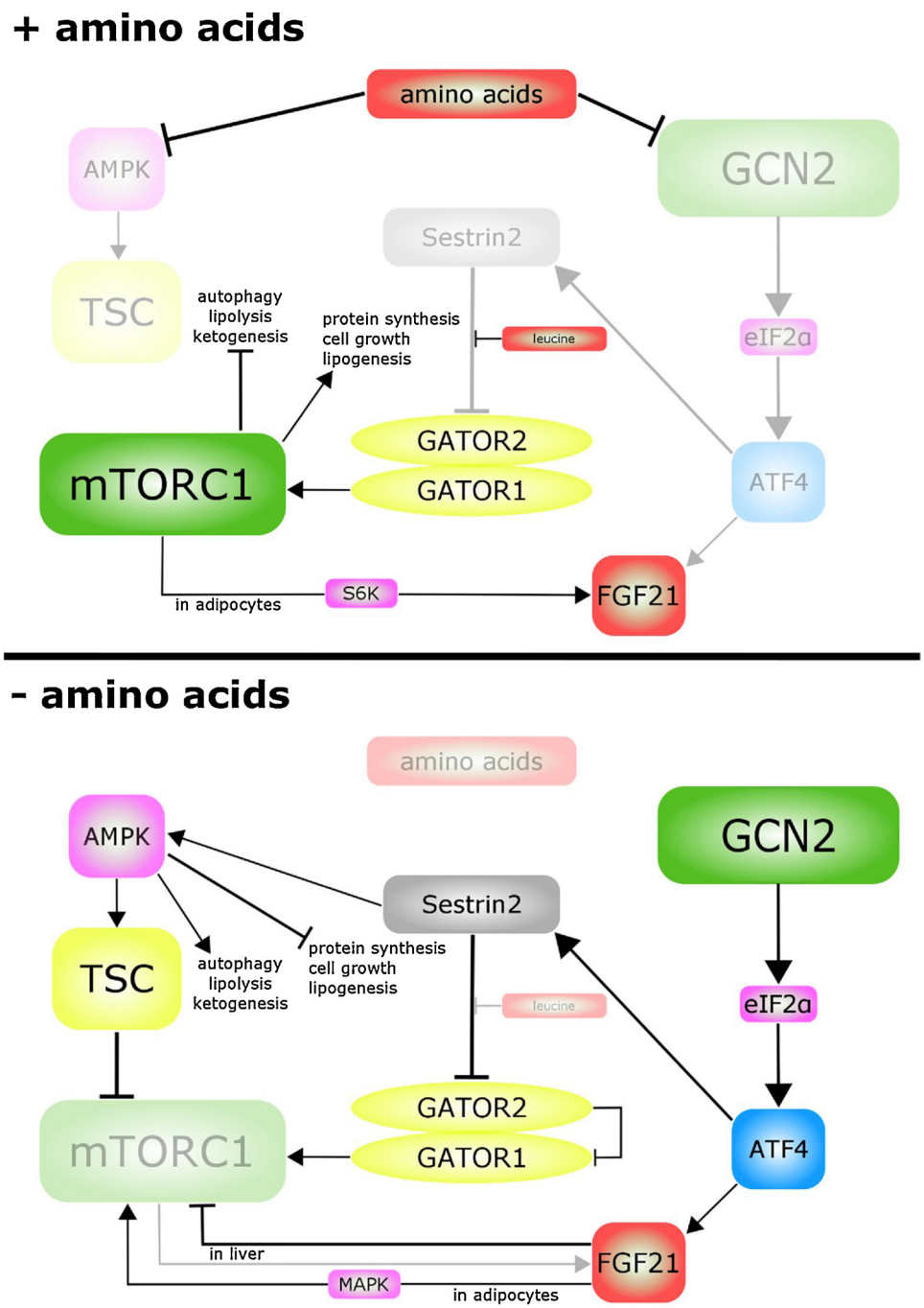

Fig. 3. Cross-talk between the mTORC1, GCN2 and FGF21 signaling pathways in the presence and absence of amino acids. AMPK = AMP-activated protein kinase, ATF4 = activating transcription factor 4, eIF2α = eukaryotic initiation factor 2, FGF21 = fibroblast-growth factor 21, GCN2 = general control nonderepressible 2, mTORC1 = mechanistic target of rapamycin complex 1, TSC = tuberous sclerosis complex.

work in the liver of rats indicated that cysteine supplementation reversed the decrease in mitochondrial reactive oxygen species (ROS), however cysteine supplementation with and without MR decreased mTORC1 activity, suggesting an alternative role for cysteine in mTOR signaling (Gomez et al., 2015). Addition of cysteine also reduced the increase in energy expenditure and serum FGF21 levels, and the reduction of fasting insulin and plasma glutathione in MR mice and reversed activation of eIF2α and protein kinase R–like endoplasmic reticulum (ER) kinase (PERK) (Wanders et al., 2016). This result supports evidence that glutathione depletion may mediate the beneficial phenotypes seen with MR via a GCN2-independent, PERK/eIF2α/ATF-dependent mechanism (Laeger et al., 2016; Wanders et al., 2016).

Recent work on sulfur amino acid restricted (SAAR) rats revealed that MR combined with cysteine deprivation for 12 weeks can halve hepatic protein synthesis rates relative to controls, despite a 40% increase in food intake. The restricted mice, though appearing healthy and maintaining normal habits, weighed half that of the controls, which indicated that an increase in energy expenditure, plus reduced protein synthesis were contributing to this phenotype. Furthermore, liver expression levels of eIF2α and phosphorylated eIF2α, which is activated by GCN2 and results in mTORC1 inhibition through activation of ATF4 (Fig. 3), were increased in the SAAR mice, indicating that low levels of free hepatic methionine and cysteine repress the mTORC1 pathway (Nichenametla et al., 2018).

FGF21, which is strongly induced by MR (Wanders et al., 2016), is proposed to be one of the major effectors of MR on metabolic health.

Indeed, in *Fgf21*$^{-/-}$ mice, MR induced increases in energy expenditure and thermogenic activation of WAT and BAT are lost, demonstrating the importance of FGF21 for the MR phenotype (Wanders et al., 2017). However, while investigating an alternative short-term methionine deprivation (MD) regimen, in which DIO mice were fed an amino acid defined diet containing no methionine, we found that while MD increased energy expenditure in both male and female mice, plasma levels of FGF21 and WAT expression of *Ucp1* were increased exclusively in male mice. This indicates that at least in females, the increased energy expenditure induced by short-term MD is independent of the FGF21-UCP1 axis (Yu et al., 2018). The effects of MD may therefore be mediated via sex-specific mechanisms, or via an entirely FGF21-UCP1 independent mechanism in mice of both sexes, as was recently shown to be the case for cold-induced thermogenesis (Keipert et al., 2017). Female DIO mice on a MD diet likewise showed equivalent improvements to male mice with respect to glucose tolerance and hepatic insulin sensitivity, supporting an FGF21-independent mechanism for these phenotypes (Yu et al., 2018). UCP1 has been shown to be required for increased energy expenditure on a MR diet, but not for the effects of MR on insulin sensitivity (Wanders et al., 2015).

Recently, a methionine derived metabolite, S-adenosyl-L-methionine (SAM) has been identified as a potent regulator of mTORC1 activity. A newly identified protein, which has been named S-adenosyl methionine/target of rapamycin (SAMTOR), directly binds to SAM; SAMTOR interacts with GATOR1 to inhibit mTORC1 signaling, and in the presence of SAM this interaction is disrupted (Gu et al., 2017). In model organisms both diet and genetic changes to SAM have been investigated, and currently the results of these studies appear conflicted. During the aging process, systemic SAM increases in *Drosophila*, and lifespan can be extended by increasing SAM catabolism (Obata and Miura, 2015). However, in mice, cognitive performance was improved with a SAM supplemented folate deficient diet (Montgomery et al., 2014) and in long-lived Snell dwarf mice levels of SAM were significantly higher than in normal mice (Vitvitsky et al., 2013). In yeast, overexpression of SAM synthetase increases lifespan, which may be a result of increased consumption of adenosine triphosphate (ATP) and methionine and activation of AMPK (Ogawa et al., 2016).

3.2. Branched Chain Amino Acids

The BCAAs have been of interest to metabolic researchers since at least the 1970′s, when it was first observed that leucine, isoleucine, and valine levels were elevated in the blood of obese humans (Felig et al., 1974). Since that time, it has become clear that the BCAAs are increased in obese adult humans relative to their lean counterparts (Newgard et al., 2009), and are also positively correlated with body mass index (BMI) in children and adolescents (McCormack et al., 2013). Circulating BCAAs are a positive predictor of diabetes in normoglycemic individuals (Wang et al., 2011). In both rodents and humans, research suggests that levels of circulating BCAAs are dependent on protein intake (Fontana et al., 2016; Noguchi et al., 2006; Solon-Biet et al., 2014), and are thus decreased in both rodents and humans on a PR diet.

In addition to this correlative data suggesting that BCAAs have a negative effect on metabolic health, supplementation of a Western diet with additional BCAAs further impairs insulin sensitivity in both rats and mice (Cummings et al., 2018; Newgard et al., 2009). Conversely, acute dietary deprivation of either leucine, isoleucine, or valine improves insulin sensitivity in mice (Xiao et al., 2014). Finally, we have recently shown that consumption of a diet with reduced levels of the BCAAs improves glucose tolerance in both lean and diet-induced obese mice (Cummings et al., 2018; Fontana et al., 2016), and a reduced BCAA diet also improves the insulin sensitivity of hyperphagic Zucker Fatty rats (White et al., 2016).

Although this evidence strongly indicates that BCAAs have an overall negative effect on metabolic health, several studies have identified positive effects of BCAA supplementation, particularly when started at old age in humans (Solerte et al., 2008a, 2008b) and rats (Pansarasa et al., 2008). BCAAs are widely sold as dietary supplements, particularly for athletes, and BCAA supplementation has been explored in both human and animal models as a treatment for sarcopenia (D'Antona et al., 2010; Pansarasa et al., 2008). Additionally, the catabolite of leucine, β-Hydroxy β-methylbutyrate, which decreases in rats with age (Shreeram et al., 2016), has been shown to stimulate genes associated with muscle repair in aged mice (Munroe et al., 2017). Similarly, the acute effects of leucine supplementation have been seen in exercised rats, where leucine fed animals have greater stimulation of skeletal muscle protein synthesis (Anthony et al., 1999). Leucine supplementation of aged humans for six months proved ineffective at increasing muscle mass, but also had no negative effects on glycemic control (Leenders et al., 2011). The differences in responses seen in BCAA studies may be due to differential requirements for BCAAs in catabolic (trauma) and anabolic (obesity) situations as well as the changing nutritional needs of mammals with age (Bifari and Nisoli, 2017). On balance, the evidence suggests that dietary BCAA consumption or supplementation is detrimental in obese individuals eating an unhealthy diet.

The effect of dietary BCAAs on longevity and robustness remains unclear. Low levels of circulating BCAAs are seen in the long lived dwarf Ames mice (Wijeyesekera et al., 2012), and blood levels of the BCAAs inversely correlate with the longevity of C57BL/6 J mice (Solon-Biet et al., 2014). However, supplementation with BCAAs increases the chronological lifespan of yeast (Alvers et al., 2009) and *C. elegans* (Mansfeld et al., 2015), while BCAA supplementation begun at 9 months of age increases the average, but not maximum lifespan of male mice (D'Antona et al., 2010). The effect of specifically restricting dietary BCAAs on mammalian health and longevity remains to be determined.

The physiological and molecular mechanisms by which the BCAAs regulate metabolic health are complex. The BCAAs, particularly leucine, are well described as potent agonists of mTORC1, and leucine restriction reduces mTORC1 activity *in vivo* (Lees et al., 2017). However, while leucine deprivation promotes metabolic health in wild-type mice through improved glucose homeostasis and reduced fat mass (Lees et al., 2017), *Gcn2*$^{-/-}$ mice deprived of leucine for one week exhibit liver steatosis and increased triglycerides, and the beneficial effects of leucine deprivation were all but abolished, suggesting that these effects are primarily mediated by GCN2 (Guo and Cavener, 2007). Xiao and colleagues have shown that short-term leucine deprivation improves hepatic insulin sensitivity via sequential activation of GCN2 and inhibition of mTORC1 signaling (Xiao et al., 2011).

At the physiological level, a direct comparison between 80% restriction of both leucine and methionine in ten month old C57BL/6 J male mice indicated that both decreased body fat and body mass, however MR had a greater effect on body mass (Lees et al., 2017). Although both interventions increased food intake, WAT lipid cycling, whole body glucose metabolism, and hepatic insulin sensitivity, MR had a greater positive impact on glucose and lipid homeostasis (Lees et al., 2017). These results suggest that BCAAs such as leucine and other amino acids influence metabolic health through both distinct and overlapping mechanisms. In addition, recent work has found that a subset of neurons in mediobasal hypothalamus can rapidly respond to physiological changes in extracellular leucine concentration (Heeley et al., 2018). Leucine was shown to activate both POMC and NPY/AGRP human and mice hypothalamic neurons. Interestingly, this mechanisms was not modulated by either K_{ATP} channels or mTOR but by activation and inhibition of Ca^{2+} channels (Heeley et al., 2018). This may suggest a completely unique method by which leucine can exert its effect compared to other BCAAs, which may explain some of the idiosyncratic results seen with deprivation and supplementation with this amino acid.

Though amino acids themselves can act as signaling molecules, their catabolites can also induce metabolic changes; for example the

catabolite, 3-hydroxy-isobutyrate (3-HIB), of the BCAA valine has recently been implicated in insulin resistance (Jang et al., 2016). 3-HIB increases the uptake of fatty acids into the muscle through the transcriptional activator peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α) promoting accumulation of lipids in muscle and insulin resistance in mice. Interestingly, in the diabetic *db/db* mouse model and in diabetic humans 3-HIB is increased in the muscle (Jang et al., 2016). This study is important in recognizing that the flux of BCAA catabolites, rather than the BCAAs themselves, may be key regulators of the metabolic response to PR and amino acid restriction.

To explore the role of BCAA catabolism in metabolic health, mice were created that lack the branched chain amino acid transaminase 2 (*Bcat2*) gene. This resulted in decreased body weight and adiposity despite increased food consumption, improved glucose and insulin tolerance and increased energy expenditure even though circulating BCAAs were increased (She et al., 2007). Conversely, transgenic expression of *Bcat2* in hepatocytes, where it is not normally expressed, reduced hepatic levels of BCAAs and attenuated mTOR signaling in high fat fed mice, and impaired blood glucose tolerance, suggesting that BCAAs regulate blood glucose during diet-induced obesity (Ananieva et al., 2017).

In addition to “canonical” leucine activation by mTORC1 via Sestrin2 (Fig. 2), it was recently shown that leucyl-tRNA synthetase, which can sense intracellular levels of leucine, can activate mTOR in both yeast (Bonfils et al., 2012) and humans (Han et al., 2012). Aminoacyl-tRNA synthetases are the enzymes that catalyze the reaction between an amino acid and its cognate tRNA, and are able to discriminate between specific amino acids. The leucyl-tRNA synthetase in particular may act to regulate mTORC1 signaling; the leucyl-tRNA synthetase may function as a GAP for RagD (Han et al., 2012), and can regulate the ability of RagA and RagB to recruit mTORC1 to the lysosome through the leucylation of specific lysine residues on RagA and RagB (He et al., 2018).

3.3. Tryptophan

In addition to methionine and the BCAAs, the amino acid tryptophan has been the most thoroughly studied in terms of metabolic health. Most investigations have concluded that tryptophan restriction is beneficial for longevity. Treatment of *S. cerevisiae*, *C. elegans* and *Drosophila* with ibuprofen increases longevity through destabilization of a tryptophan transporter, which inhibits uptake of tryptophan (He et al., 2014). In mammals, tryptophan restriction promotes longevity; in rats it has been shown to delay tumor onset and increase mean and maximal lifespan (De Marte and Enesco, 1986; Ooka et al., 1988; Segall and Timiras, 1976). Conversely, activation of tryptophan catabolism has been implicated in the progression of Alzheimer’s (Anderson and Ojala, 2010; Gulaj et al., 2010) and Parkinson’s disease (Ogawa et al., 1992; Widner et al., 2002). However, other studies in mammalian animal models have suggested that tryptophan may be beneficial. In growth-retarded rats, tryptophan supplementation was able to rescue the phenotype and return rats to normal size within 6–22 months and increased lifespan (Segall, 1977); however, this may be due to correction of early growth retardation.

Taken together, these results suggest that even single amino acid supplementation or deprivation can strongly alter metabolic phenotypes, and that these processes are mediated through a myriad of downstream pathways. In addition to activation by specific amino acids, the catabolites of amino acid degradation, or the absence of amino acids can also regulate metabolic processes. Furthermore, amino acids can act as metabolic substrates and be degraded into the tricarboxylic acid (TCA) cycle during periods of low carbohydrate availability. The potent metabolic effects of restricting methionine, tryptophan or the BCAAs may have clinical relevance for the prevention of metabolic diseases (Table 2).

4. Amino acid restriction as an intervention in obesity and diabetes

In humans, plasma amino acid concentrations are biomarkers for several diseases (Roth and Druml, 2011). A comparison of obese versus lean humans indicated that serum levels of BCAAs, aromatic amino acids, glutamate and alanine were increased (Newgard et al., 2009) and further work indicated that increased plasma levels of BCAAs are correlated with development of insulin resistance and diabetes in humans (Newgard, 2012). Furthermore, BCAAs can predict the development of diabetes 12 years later in normoglycemic individuals (Wang et al., 2011). Weight loss diets have been shown to consistently reduce levels of plasma amino acids in humans including BCAAs (Zheng et al., 2016). It has also been suggested that visceral white adipose tissue may be involved in the accumulation of BCAAs in obese humans and mice (Lackey et al., 2013)

While it has been known for almost a decade that supplementing BCAAs to rats fed a high fat diet results in insulin resistance, possibly due to hyperactivation of mTORC1 in skeletal muscle (Newgard et al., 2009), it has only recently become apparent that normal dietary levels of the BCAAs play a key role in maintaining an obese, insulin resistant state. We recently demonstrated that specifically reducing dietary levels of the BCAAs by 67% in DIO mice caused a rapid reduction in weight due to fat mass loss, even as the mice continued to eat an otherwise high fat, high sugar Western diet (Cummings et al., 2018). Glucose tolerance and insulin sensitivity were also rapidly restored. This phenomena is thought to be mediated at least in part by increased energy expenditure, which may be initiated by transient activation of FGF21 in response to reduced levels of the BCAAs (Cummings et al., 2018). This provides evidence that by altering the precise macronutrient composition of diets can improve health parameters, without calorie restriction.

In both humans and mice, MR promotes leanness and improves metabolic health, however, long term adherence in humans to such diets is poor. We recently established a short term MD regimen that improves the metabolic health of DIO mice through rapidly reducing adiposity and improving glycemic control. This did not occur through reduced calorie intake, but through increased energy expenditure, which mirrors responses we previously saw to BCAA restriction in obese mice. Intriguingly, this effect appears to be metabolically distinct from the effects of MR, as the effects of MR are dependent upon FGF21; while male mice on a MD diet showed strong induction of FGF21 and evidence of WAT beiging (induction of *Ucp1*), female mice on a MD diet had similar improvements in glucose homeostasis and adiposity without engaging the FGF21-UCP1 axis (Yu et al., 2018).

5. Conclusions

As obesity and diabetes become increasingly common, the necessity for new interventions that improve metabolic health is essential (Centers for Disease Control and Prevention, 2014; Fothergill et al., 2016). In particular, as diets based on reduced calorie intake have poor long-term adherence, recent findings suggesting that diets based on altered macronutrient content in humans and mice have greater long-term compliance may point to strategies based on these diets as a step in the right direction. Recent work demonstrates that dietary protein, and indeed specific dietary amino acids, are powerful mediators of metabolic health. In particular, dietary restriction of the amino acids including leucine, isoleucine, methionine, tryptophan, and valine promote metabolic improvements in rodents; further studies will need to take place to determine the effect of restriction these dietary amino acids on human health.

Though it is clear that reducing amino acid intake has benefits on metabolic health parameters such as weight, adiposity, and insulin sensitivity, there is still no consensus on how this benefit is conferred. To understand these mechanisms we need a deeper molecular understanding of the downstream effects of amino acid deprivation and

Table 2
Recent studies of the effect of amino acid restricted diets on the metabolic health of humans and rodents. M = male, F = female, where not stated both sexes used, FGF21 = fibroblast growth factor 21, UCP1 = uncoupling protein 1, eIF2α = eukaryotic transcription factor 2α, BCAAs = branched chain amino acids, BAT = brown adipose tissue, WAT = white adipose tissue, DIO = diet induced obesity, S6K1 = S6 protein kinase 1, Met = methionine, Val = valine, Ile = isoleucine.

Species/Strain/Sex	Restricted amino acid	Level of intake	Metabolic effect	Length of intervention	Study
Mice CB6F1 (F)	Met	23-35%	Decreased circulating IGF-I, insulin and glucose Increased resistance to liver stress	Longevity study	Miller et al. (2005)
Mice C57BL/6 J (M)	Leu	0%	Increased oxygen consumption	7 days	Cheng et al. (2010)
Mice C57BL/6 J (M)	Leu	0%	Improved glucose tolerance Improved hepatic insulin sensitivity Decreases mTOR/S6K1 signaling Activates GCN2	7 days	Xiao et al. (2011)
Mice C57BL/6 J (M)	Leu/Val/Ile	0%	Improved insulin sensitivity	1 day	Xiao et al. (2014)
Mice C57BL/6 J (M)	Val	0%	Improved glucose and insulin sensitivity Decreased hepatic mTOR activation Increased hepatic GCN2 activation Increased hepatic AMPK activation	7 days	Xiao et al. (2014)
Mice C57BL/6 J (M)	Ile	0%	Improved glucose and insulin sensitivity Decreased hepatic mTOR activation Increased hepatic AMPK activation	7 days	Xiao et al. (2014)
Mice C57BL/6 J (M)	Met	20%	Improved insulin sensitivity Suppresses hepatic glucose production Increased hepatic expression of FGF21 Increased circulating FGF21	8 weeks	Stone et al. (2014)
Mice C57BL/6 J (M)	Met	20%	Increased food intake Reduced body weight Increased physical activity Improved hepatic insulin sensitivity Remodeling of WAT metabolism Decreased hepatic lipogenic gene expression Increased circulating and hepatic expression of FGF21	8 weeks	Lees et al. (2014)
Rats Zucker-fatty (M)	BCAAs	55%	Improved skeletal muscle glucose disposal Improves skeletal muscle insulin sensitivity	15 weeks	White et al. (2016)
Mice C57BL/6 J	Leu	33%	No body mass change Increased adiposity Improved glucose tolerance	13 weeks	Fontana et al. (2016)
Mice C57BL/6 J	BCAAs	33%	Increased food intake Improved glucose and pyruvate tolerance Decreased fasting blood glucose and insulin secretion	13 weeks	Fontana et al. (2016)
Mice C57BL/6 J (M)	Met	15%	Increased in energy intake Increased energy expenditure Lower accumulation of body weight Lower accumulation of fat mass Reduction in adipocyte size	10 weeks	Wanders et al. (2017)
Mice C57BL/6 J (M)	Met	20%	Increased food intake Decreased body and fat mass Improved whole body glucose metabolism Decreased fasting blood glucose and insulin Elevated lipid cycling in WAT Reduced hepatic lipogenic gene expression Elevated fasting serum FGF21	8 weeks	Lees et al. (2017)
Mice C57BL/6 J (M)	Leu	20%	Increased food intake Decreased body and fat mass Improved whole body glucose metabolism Decreased fasting insulin Elevated lipid cycling in WAT	8 weeks	Lees et al. (2017)
Mice C57BL/6 J	Met	0%	Increased food intake Weight and adiposity loss Improved insulin sensitivity Improved pyruvate tolerance	5 weeks	Yu et al. (2018)
Mice C57BL/6 J (DIO)	Met	0%	Increased food intake (females) Increased energy expenditure Restores body weight Reduced adiposity Normalized glucose tolerance Improved insulin sensitivity Induces FGF21 (males)	5 weeks	Yu et al. (2018)
Mice C57BL/6 J (DIO)	BCAAs	33%	Rapid weight loss Loss of fat and lean mass Loss of dermis WAT Improved glucose and insulin sensitivity Increases energy expenditure Decreased liver droplet size Transiently increases FGF21 Decreased hepatic lipogenic gene expression	14 weeks	Cummings et al. (2018)

supplementation, which may allow the development of novel therapies for obesity and diabetes. While many of the effects of a PR diet and amino acid restriction may be mediated by GCN2 and mTOR, there is little consensus on how this is achieved. In *Gcn2*$^{-/-}$ mice, strong activation of mTORC1 is seen in the liver after injection with arginase, suggesting that GCN2 is a powerful mTOR inhibitor (Nikonorova et al., 2018). However, other studies have suggested that PR mediated inactivation of mTOR is regulated by eIF2, but not ATF4, which may indicate that different amino acids modulate mTOR through distinct cell signaling pathways (Averous et al., 2016; Nikonorova et al., 2018). New potential avenues for research include investigating the sexually dimorphic molecular effects of methionine restriction (Yu et al., 2018), and identifying the molecular basis for the different effects of methionine and leucine restriction (Lees et al., 2017). Finally, the ability of aminoacyl-tRNA synthetases to aminoacylate lysines of diverse substrates suggest that this may be a novel mechanism by which the abundance of individual amino acids may regulate diverse metabolic processes (He et al., 2018).

There are still many questions to answer with regards to the regulation of metabolic health via amino acid deprivation. Development of clinical therapies involving these mechanisms will require a greater understanding of the sexual dimorphism between responses to amino acid restriction, as different mechanisms may be induced in different sexes. In addition, though there have been many studies analyzing the effects of amino acid changes on the liver, muscle, BAT and WAT, it is important to understand how these interventions affect other tissues, for example how they may affect signaling in areas of the brain, such as the hypothalamus, which is involved in hunger signaling and energy homeostasis. Furthermore, though restriction of BCAAs generally appears beneficial, studies in aged mammals suggest that lifelong restriction may not be optimal, and that as animals age and nutritional requirements change, diets may need to be tailored for maintenance of bone and muscle. This may shed some light on potential uses of increasing beiging of WAT to increase energy expenditure and weight loss. Developing our knowledge of the mechanisms behind these effects is imperative for developing novel therapeutic approaches to diabetes and obesity.

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