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Lifelong restriction of dietary branched-chain amino acids has sex-specific benefits for frailty and life span in mice

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Protein-restricted diets promote health and longevity in many species. While the precise components of a protein-restricted diet that mediate the beneficial effects to longevity have not been defined, we recently showed that many metabolic effects of protein restriction can be attributed to reduced dietary levels of the branched-chain amino acids (BCAAs) leucine, isoleucine and valine. Here, we demonstrate that restricting dietary BCAAs increases the survival of two different progeroid mouse models, delays frailty and promotes the metabolic health of wild-type C57BL/6J mice when started in midlife, and leads to a 30% increase in life span and a reduction in frailty in male, but not female, wild-type mice when they undergo lifelong feeding. Our results demonstrate that restricting dietary BCAAs can increase health span and longevity in mice and suggest that reducing dietary BCAAs may hold potential as a translatable intervention to promote healthy aging.

Dietary interventions can robustly promote organismal health and longevity. The most well known of these is calorie restriction (CR), which extends the life span and health span of diverse species, including rodents[1,2]. While the role of reduced levels of specific dietary macronutrients in the benefits of a calorie-restricted diet is still under investigation, dietary macronutrient balance has a strong influence on longevity and health[3,4]. A recent meta-analysis of protein restriction (PR) in rodents found that while the reduction in calories, not protein, is responsible for the effects of a calorie-restricted diet on longevity, life span increases as dietary protein content decreases[5].

Many human diet plans call for high protein consumption, as protein promotes satiety, and benefits of dietary protein have been noted in short-term studies[6–8]. Decreased protein intake is also associated with frailty and sarcopenia[9,10]. However, a growing number of studies suggest that low-protein diets may be healthier and associated with longer life. A retrospective clinical trial found that lower protein consumption is associated with decreased mortality and decreased incidence of diabetes[11]; further, several prospective clinical trials have shown that high-protein diets are associated with insulin resistance, diabetes, obesity and mortality[12–14]. While long-term randomized clinical trials of PR in healthy humans have not yet been undertaken, a recent short-term randomized clinical trial of PR showed improved metabolic health, including reduced adiposity and improved insulin sensitivity[15].

Positive metabolic effects of low-protein diets are also observed in rodents, where PR promotes leanness, energy expenditure and improved glucose homeostasis[16–18,15], and PR promotes longevity in both *Drosophila* and rodents[5,19–22]. The precise amino acids (AAs) altered in a protein-restricted diet that mediate the beneficial effects of PR have not been defined. In *Drosophila*, methionine restriction extends life span, although levels of other essential AAs are also influential[23–25]. In rodents, adding back essential AAs to mice on a 40% calorie-restricted diet blunts the effect of CR on longevity, demonstrating that essential AAs have a powerful effect on life span[26]. In studies that restricted individual essential AAs, methionine restriction extends life span and improves health[27–29], and tryptophan restriction may increase maximum life span[30,31]. The role of other dietary AAs in mammalian life span has largely been unexplored.

Blood and dietary levels of the three BCAAs leucine, isoleucine and valine have been linked to insulin resistance and obesity in both humans and rodents in numerous studies over the last decade[15,18,32–35]. Rodent studies have shown that specifically restricting dietary BCAAs improves metabolic health and recapitulates the metabolic benefits of PR[15,18,36]. The effect of restricting dietary levels of BCAAs on the health span and longevity of mice has not been extensively investigated. While one study found a small, male-specific increase in median life span in mice that were provided BCAA-enriched drinking water[37], another study found that dietary supplementation with BCAAs leads to obesity and a reduction in median and maximal life span[38].

The BCAAs are potent agonists of the AA-sensitive mechanistic target of rapamycin complex 1 (mTORC1) protein kinase, a central regulator of metabolism and aging[39,40]. In rats, the negative effect of additional BCAAs on insulin sensitivity is accompanied by increased mTORC1 activity in skeletal muscle, and this

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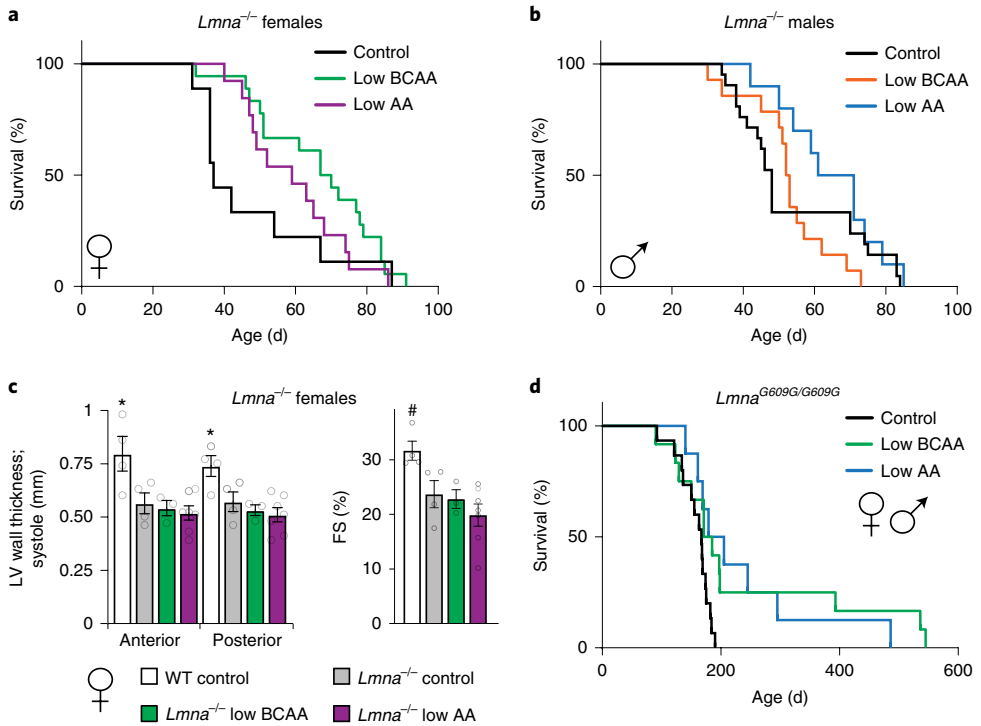


Fig. 1 | Branched-chain amino acid restriction extends the life span of progeroid mice. a,**b**, Kaplan–Meier plots of the survival of *Lmna*$^{-/-}$ females ($n=9$ (control), 18 (low BCAA) and 13 (low AA) biologically independent animals; **a**) and males ($n=20$ (control), 14 (low BCAA) and 10 (low AA) biologically independent animals; **b**) fed the indicated diets starting at weaning (Supplementary Tables 2 and 3). **c**, Echocardiography data of anterior and posterior left ventricular wall (LVAW and LVPW, respectively) thickness (mm) in systole and FS (%) in female wild-type (WT) and *Lmna*$^{-/-}$ mice fed the indicated diets from weaning to age 40–55 d; full echocardiography data is available in Supplementary Table 4 ($n=4$ (WT and *Lmna*$^{-/-}$ control), $n=3$ (low BCAA) and $n=7$ (low AA) biologically independent animals; two-sided Dunnett's test in comparison to *Lmna*$^{-/-}$ control values after one-way ANOVA; LVAWs: $^{*}P=0.0224$; LVPWs: $^{*}P=0.0406$; and FS: $^{\#}P=0.0724$). **d**, Kaplan–Meier plot of the survival of *Lmna*$^{G609G/G609G}$ mice fed the indicated diets starting at weaning ($n=15$ (control), 12 (low BCAA) and 8 (low AA) biologically independent animals; Supplementary Tables 3 and 5). Data are the mean ± s.e.m.

BCAA-induced insulin resistance is reversible by rapamycin, an acute inhibitor of mTORC1 (ref. [32]). In combination with human data suggesting that plasma BCAAs are associated with an increased risk of age-associated diseases[34], and evidence that mTORC1 signaling is a strong negative regulator of life span in mice and many model organisms[41–45] (and reviewed by Arriola Apelo et al.[46]), this suggests that dietary BCAAs may promote mortality and inhibit health span.

Here, we tested the hypothesis that reduced BCAA consumption would extend life span. Firstly, we find that restricting dietary BCAAs extends the life span of two progeroid mouse models. Further, a reduced BCAA diet improves the metabolic health and decreases frailty of wild-type C57BL/6J mice when feeding is begun in midlife and extends the life span of male, but not female, wild-type mice when started early in life. Finally, a reduced BCAA diet has sex-specific effects on the skeletal muscle transcriptome, with males showing alterations in multiple longevity-regulating pathways, including mTOR signaling. In conclusion, these results demonstrate that reducing dietary levels of BCAAs promotes metabolic health, reduces frailty and increases longevity in mice and should be investigated as a potential method for preventing and intervening in age-related disease.

Results

Branched-chain amino acid restriction extends the life span of progeroid mice. We first assessed the effect of BCAA restriction on the survival of lamin A/C-deficient mice (*Lmna*$^{-/-}$), originally described as a mouse model of Hutchinson–Gilford Progeria Syndrome (HGPS), but now considered a model of muscular dystrophy[47]. We utilized an AA-defined control diet based on a natural source 21% protein mouse diet, as well as a low BCAA diet and low AA diet, which have BCAAs or all dietary AAs, respectively, restricted by two-thirds (67%). All three of these AA-defined diets are isocaloric with identical levels of dietary fat (Supplementary Table 1).

We fed *Lmna*$^{-/-}$ mice control, low BCAA or low AA diets starting at weaning and determined their survival (Fig. 1a,b and Supplementary Table 2). As the shape of the survival curves for low BCAA-fed and low AA-fed mice suggested that the hazard ratio changed during the life span, we analyzed this data using both a log-rank test, which is most powerful in the case of proportional hazards, and the Wilcoxon test, which is more sensitive to early deaths. We observed that low BCAA-fed *Lmna*$^{-/-}$ females lived longer ($P=0.0794$, log-rank test; $P=0.0098$, Wilcoxon test), with an 86% increase in median life span (Fig. 1a and Supplementary Table 3). In contrast, low BCAA-fed *Lmna*$^{-/-}$ males did not live longer (Fig. 1b and Supplementary Table 3). Similarly, low AA-fed *Lmna*$^{-/-}$ female mice lived longer ($P=0.366$, log-rank test; $P=0.0355$ Wilcoxon test), with a 59% increase in median life span, while low AA-fed *Lmna*$^{-/-}$ male mice did not live longer despite a 38% increase in median life span (Fig. 1a,b and Supplementary Table 3). The survival of the longest-lived quartile (hereafter, maximum life span), evaluated using the Wang–Allison test[48] as the proportion of mice still alive at the age of 75% mortality in the combined life span distribution, was not extended by either diet (Supplementary Table 3).

We performed echocardiography on female *Lmna*$^{-/-}$ mice to detect diet-induced differences in heart structure and function.

While *Lmna*$^{-/-}$ mice had clear cardiac deficits, no diet treatment corrected the thinning walls or decreased fractional shortening (FS) characteristic of dilated cardiomyopathy (Fig. 1c and Supplementary Table 4).

We next utilized a mouse model of HGPS, *Lmna*$^{G609G/G609G}$, in which the mouse *Lmna* gene has the same mutation found in most human cases of HGPS[49,50]. For this and all subsequent experiments, we utilized the same AA-defined isocaloric control and low AA diets as above, and an advanced low BCAA diet with the same percentage of calories derived from AAs as the control diet; this was achieved by proportionally increasing the nonessential AAs (Supplementary Table 1). We examined the survival of *Lmna*$^{G609G/G609G}$ mice fed these diets starting at weaning. We observed an increase in the median life span of low BCAA-fed and low AA-fed mice (7% and 15%, respectively; $P=0.0274$ and 0.0144, log-rank test; Fig. 1d and Supplementary Tables 3 and 5) and a significant increase in maximum life span (125%; $P<0.01$ (low BCAA); 69%, $P<0.05$ (low AA); Supplementary Table 3).

A diet low in branched-chain amino acids improves the metabolic health of aged mice. These results suggested that BCAA restriction might be geroprotective in wild-type mice. To test this starting in midlife, we randomized 16-month-old male and female C57BL/6J. Nia mice from the National Institute on Aging (NIA) Aged Mouse Colony to the control or low BCAA diet. We followed these animals longitudinally, with periodic assessments of metabolic health, frailty and physical performance and determined their survival (Fig. 2a).

Low BCAA-fed female mice weighed less than control-fed animals throughout their life (Fig. 2b), due principally to weight gain by control-fed mice. In contrast, low BCAA-fed female mice did not gain fat mass with age, and therefore remained leaner than age-matched control-fed females despite a small reduction in lean mass (Fig. 2c,d and Extended Data Fig. 1a). This decreased adiposity was not due to CR, as low BCAA-fed females consumed more, not less, food than aged-matched control-fed females relative to their body weight (Fig. 2e and Extended Data Fig. 1b).

This seeming paradox in energy balance could result from altered energy expenditure; we found that low BCAA-fed females had increased energy expenditure at 20 and 25 months of age (Fig. 2f and Extended Data Fig. 1c). Although the control and low BCAA diets had an identical percentage of calories derived from AAs, carbohydrates and fats, the respiratory exchange ratio (RER) was higher in low BCAA-fed females (Extended Data Fig. 1d), suggesting a higher reliance on carbohydrates. These animals showed no difference in activity levels (Extended Data Fig. 1e).

Low BCAA-fed middle-aged females were more glucose tolerant than control-fed mice as early as 3 weeks after starting the diet and remained more glucose tolerant with increasing age (Fig. 2g and Extended Data Fig. 1f). However, there was no difference in insulin tolerance between control-fed and low BCAA-fed females (Extended Data Fig. 1g). Overall, low BCAA-fed female mice had improved metabolic health relative to control-fed mice.

The effects of the low BCAA diet were similar in males; low BCAA-fed males weighed less, principally due to weight and fat mass gained by control-fed mice (Fig. 2h,i), although low BCAA-fed males initially lost a small amount of lean mass (Extended Data Fig. 1h). Low BCAA-fed males mice were overall leaner than control-fed males, although the body composition of the two groups was more similar when last measured at 24 months of age, as control-fed males began to lose adipose mass (Fig. 2j). As with females, low BCAA-fed males were not calorically restricted, with an increase in food consumption relative to body weight that did not reach statistical significance ($P=0.12$; Fig. 2k and Extended Data Fig. 1i). The increased caloric intake of low BCAA-fed males was balanced by increased energy expenditure, which was significantly higher at 20 months of age, and higher, but not significantly so ($P=0.148$), at 25 months of age without increased activity (Fig. 2l and Extended Data Fig. 1j). However, in contrast to females, RER was not significantly increased; there was also no difference in activity levels (Extended Data Fig. 1k–l).

Low BCAA-fed middle-aged males rapidly became more glucose tolerant than control-fed animals and remained more glucose tolerant as they aged (Fig. 2m and Extended Data Fig. 1m). In contrast to low BCAA-fed females, low BCAA-fed males had improved insulin sensitivity relative to control-fed males, but this did not reach statistical significance ($P=0.095$; Extended Data Fig. 1n).

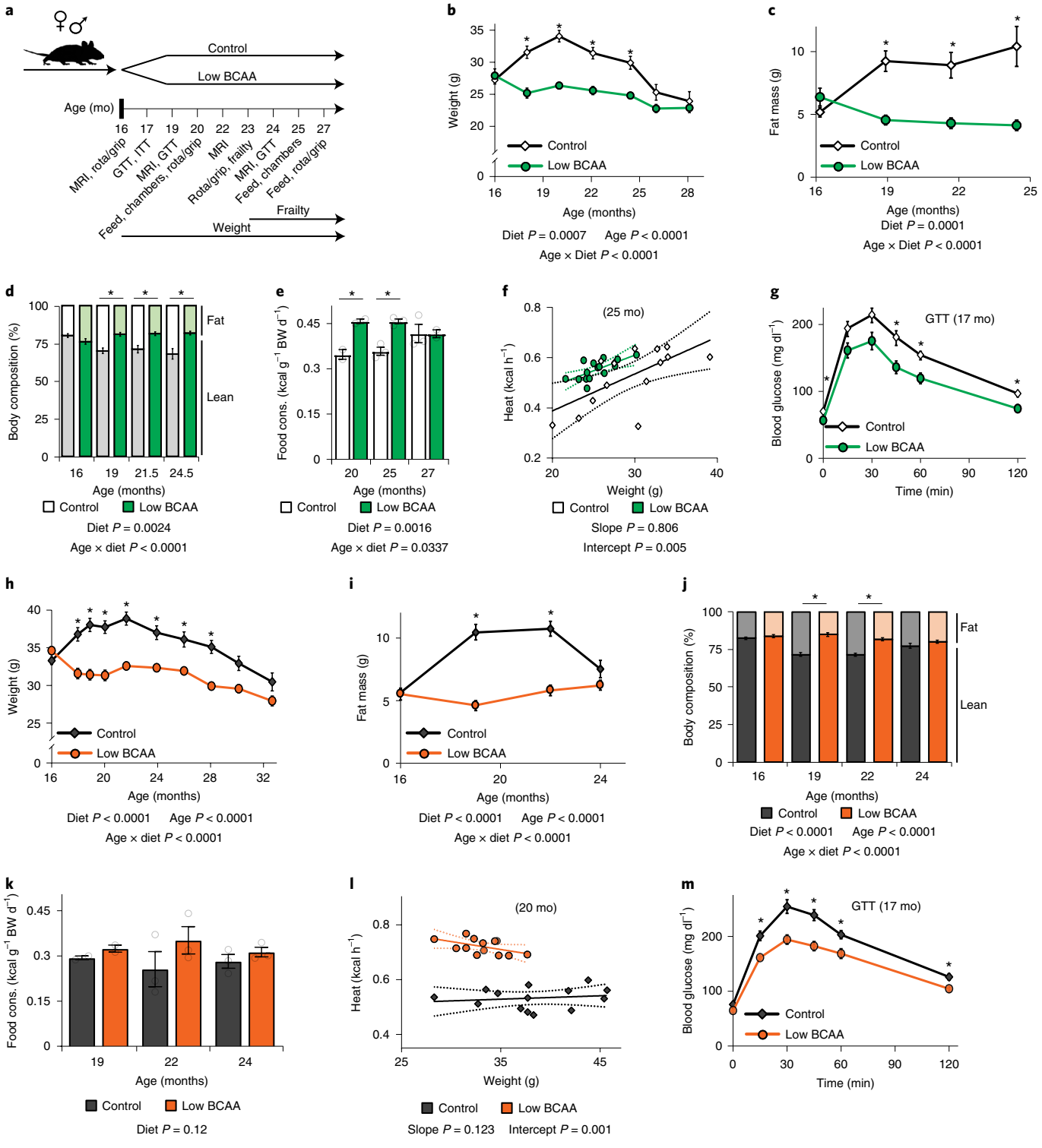
A low BCAA diet fed to middle-aged mice reduces frailty but does not extend life span. Mice and humans become increasingly frail with age, and starting at approximately 23 months of age, we utilized a recently developed mouse frailty index to assess the impact of a low BCAA diet on frailty[51,52]. As expected, we observed increased frailty with age in control-fed mice of both sexes (Fig. 3a,b). Overall, frailty scores were significantly lower in the

Fig. 2 | A low BCAA diet promotes the metabolic health of aged mice. a, Female and male C57BL/6J.Nia mice were fed control or low BCAA diets beginning at 16 months (mo) of age (schematic relevant to Figs. 2 and 3 and Extended Data Fig. 1). MRI, magnetic resonance imaging. **b**–**g**, Female C57BL/6J.Nia mice were fed the indicated diets beginning at 16 mo. **b**, The weight of the mice was tracked starting at 16 mo (*n* varies by month; maximum $n=20$ (control) and 19 (low BCAA) biologically independent animals; $^*P<0.05$ (*P* values by age: 18 mo, $P<0.0001$; 20 mo, $P<0.0001$; 22 mo, $P=0.0016$; 24.5 mo, $P=0.0222$)). **c**,**d**, The fat mass (**c**) and body composition (**d**) of a subset of mice was tracked (*n* varies by month; maximum $n=10$ biologically independent animals for both groups; $^*P<0.05$ (*P* values for **c** by month of age: 19 mo, $P=0.0007$; 21.5 mo, $P=0.0052$; 24.5 mo, $P=0.016$; *P* values for **d** by age: 19 mo, $P=0.0005$; 21.5 mo, $P=0.0134$; 24.5 mo, $P=0.0193$)). **e**, Food consumption over time (maximum $n=3$ independent cages for both groups; $^*P<0.05$, *P* values by month of age: 20 mo, $P=0.0141$; 25 mo, $P=0.0082$). BW, body weight. **f**, Energy expenditure (heat) was assessed using metabolic chambers at 25 mo ($n=15$ (control) and 15 (low BCAA) biologically independent animals). **g**, Glucose tolerance test (GTT) after 3 weeks of diet feeding ($n=19$ (control) and 20 (low BCAA) biologically independent animals; $^*P<0.05$; *P* values by time: 0 min, 0.0369; 45 min, 0.0118; 60 min, 0.0066; 120 min, 0.0005). **h**–**m**, Male C57BL/6J.Nia mice were fed the indicated diets beginning at 16 mo. **h**, The weight of the mice was tracked starting at 16 mo (*n* varies by month; maximum $n=20$ biologically independent animals for both groups; $^*P<0.05$ (*P* values by month of age: 18 mo, 0.0005; 19–21.5 mo, $P<0.0001$; 24 mo, $P=0.0023$; 26 mo, $P=0.0073$; 28 mo, $P=0.0251$)). **i**,**j**, The fat mass (**i**) and body composition (**j**) of a subset of mice was tracked (*n* varies by month; maximum $n=20$ biologically independent animals for both groups; $^*P<0.05$ (*P* values for **i** by month of age: 19–22 mo, $P<0.0001$; *P* values for **j** by month of age: 19–22 mo, $P<0.0001$)). **k**, Food consumption in mice over time (maximum $n=3$ independent cages for both groups). **l**, Energy expenditure (heat) was assessed using metabolic chambers at 20 mo ($n=14$ (control) and 13 (low BCAA) biologically independent animals). **m**, GTT after 3 weeks of diet feeding ($n=20$ biologically independent animals for both groups; $^*P<0.05$, *P* values by time: 15 min, 0.0034; 30 min, 0.0019; 45 min, 0.0006; 60 min, 0.0279; 120 min, 0.0469). Statistics for the overall effects of diet, age and the interaction represent the *P* value from a mixed-effects model (restricted maximum likelihood (REML)) or two-way repeated-measures ANOVA, multiple comparisons by two-sided Sidak's post hoc test (**b**–**e** and **h**–**k**). Energy expenditure data were analysed by linear regression of energy expenditure by body weight (ANCOVA) (**f** and **l**). Two-way repeated-measures ANOVA with multiple comparisons using two-sided Sidak's post hoc test was also performed (**g** and **m**). Data are the mean ± s.e.m.

low BCAA diet groups, in both males and females (Fig. 3a,b). The low BCAA diet did not negatively or positively impact grip strength or rotarod performance in either sex (Fig. 3c–f). Detailed frailty data can be found in Supplementary Tables 6 and 7.

Circulating levels of monocyte chemoattractant protein (MCP) 1 increase with age and frailty in both rodents and humans[53]. We found a significant interaction between sex and diet on the levels of MCP-1 in aged mice, with a significant increase in MCP-1 in low BCAA-fed male mice despite the decrease in frailty (Fig. 3g). Levels of insulin-like growth factor 1 (IGF-1) are associated with aging and mortality and are decreased by geroprotective interventions, including PR, CR, methionine restriction and rapamycin[54–57]. We did not detect a significant change in the levels of IGF-1 in either male or female mice fed a low BCAA diet (Fig. 3h).

Although a low BCAA diet improved metabolic health and promoted robustness in both sexes, there was no significant effect on the overall survival of either sex when started at midlife (Fig. 4a,b and Supplementary Tables 3 and 8). However, we observed an excess of early mortality among female mice that switched to the low BCAA diet, with 26% dying before 717 d (the life span of the last



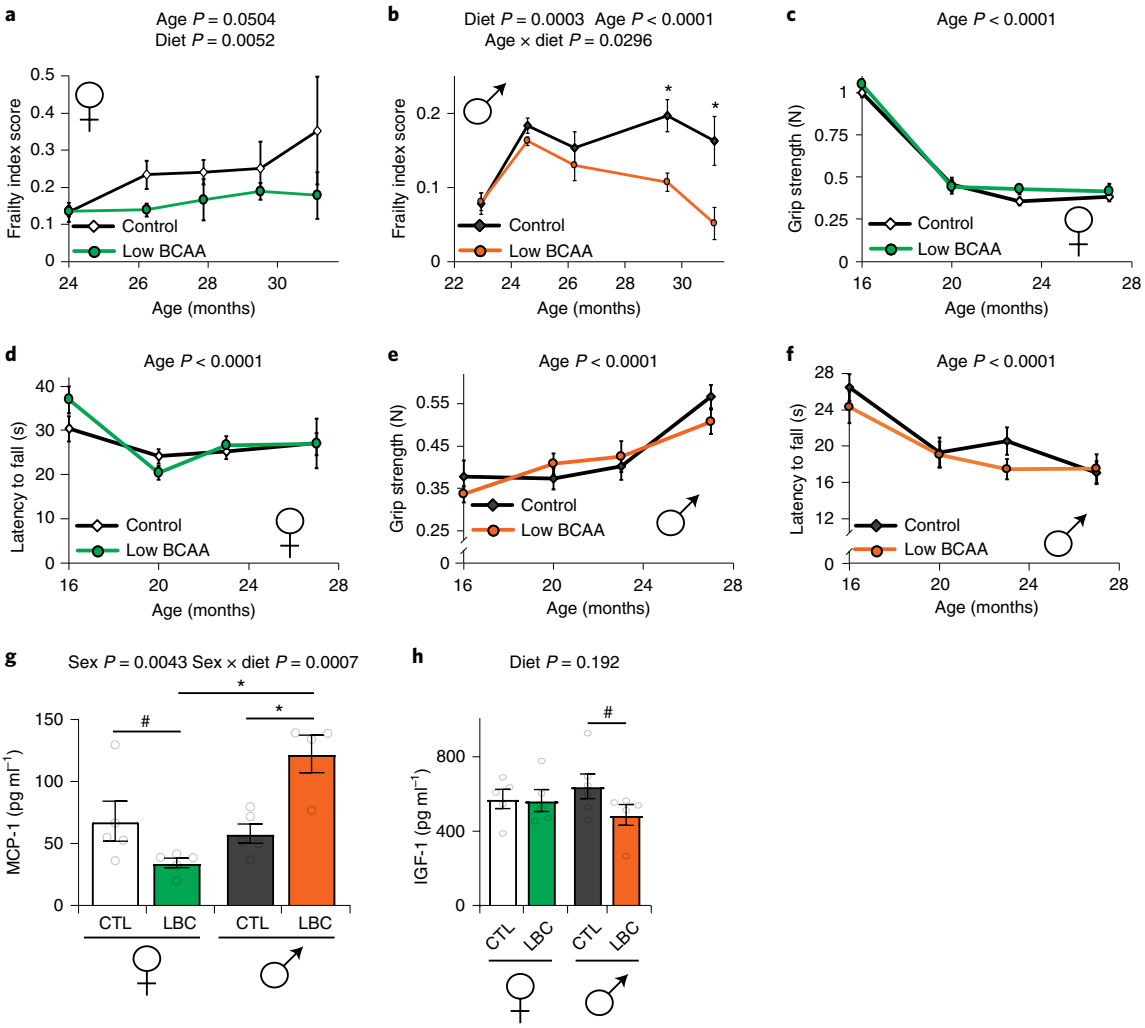


Fig. 3 | A low BCAA diet promotes healthy aging. C57BL/6J.Nia mice were fed the indicated diets beginning at 16 months of age. **a**,**b**, Frailty was assessed longitudinally in female (*n* varies by month; maximum $n=7$ (control) and 8 (low BCAA) biologically independent animals; **a**) and male (**b**) mice starting at 23–24 months of age (*n* varies by month; maximum $n=10$ biologically independent animals for both groups; $^{*}P<0.05$, adjusted *P* values by month of age: 29.5 mo, 0.0075; 31 mo, 0.003); average frailty by index measured can be found in Supplementary Tables 6 and 7, respectively. **c**,**d**, Grip strength (**c**) and rotarod performance (**d**) in females was assessed longitudinally. **e**,**f**, Grip strength (**e**) and rotarod performance (**f**) in males was assessed longitudinally. **c**–**f**, *n* varies by month; maximum $n=20$ biologically independent animals for all groups. **a**–**f**, Statistics for the overall effects of diet, age and the interaction represent the *P* value from a mixed-effects model (REML) or two-way ANOVA; $^{*}P$ values reported in **b** represent a two-sided Sidak's post hoc test. **g**,**h**, Levels of MCP-1 (**g**) and IGF-1 (**h**) in serum was determined by enzyme-linked immunosorbent assay (ELISA; 22-mo females and 25-mo males; $n=5$ (females and control males) and 4 (low BCAA males) biologically independent animals; statistics for the overall effects of diet, age, sex and the interaction represent the *P* value from a two-way ANOVA with multiple comparisons by two-sided Sidak's post hoc test; $^{*}P<0.05$, $^{\#}P<0.16$, adjusted *P* values in **g**: control (CTL) female versus low BCAA (LBC) female, 0.1016; CTL male versus LBC male, 0.0034; LBC female versus LBC male, 0.0002; adjusted *P* value in **h**: CTL male versus LBC male, 0.1525. Data are the mean ± s.e.m.

mouse in the low BCAA group before the survival curves crossed) compared with only 8% of the female mice that switched to a control diet. Similar initial increases in mortality have been observed in methionine-restricted mice[27] and tryptophan-restricted rats[30], as well as calorie-restricted mice[58].

While cause of death analysis is difficult in mice, we noted the presence or absence of observable cancers by gross necropsy. We observed cancer in 68% of the control-fed females; in contrast, we observed cancer in only 33% of low BCAA-fed females (Fig. 4c). There was no observed decrease of cancer in low BCAA-fed males (Fig. 4d).

Low BCAA diet feeding throughout life extends male life span. Dietary interventions, including CR, have a more pronounced effect on longevity when begun early in life[59]. As the survival of progeroid mice increased when low BCAA and low AA diets were initiated at weaning, we examined how lifelong feeding of these diets impacts the health span and longevity of wild-type mice (Extended Data Fig. 2a).

Low BCAA-fed females weighed significantly less than control-fed mice throughout the majority of their life (Fig. 5a). While the body composition of these groups was initially similar, as the mice aged, low BCAA-fed females became leaner than their control-fed counterparts due to reduced fat mass gain (Fig. 5b and Extended Data Fig. 2b,c). In contrast to females that were fed a low BCAA diet starting in midlife, the food consumption, RER, activity and energy expenditure of low BCAA-fed females after 4 months of diet feeding were similar to those of control-fed mice (Extended Data Fig. 2d–h). We observed that a low BCAA diet improved glucose tolerance in females at multiple ages (Extended Data Fig. 2i),

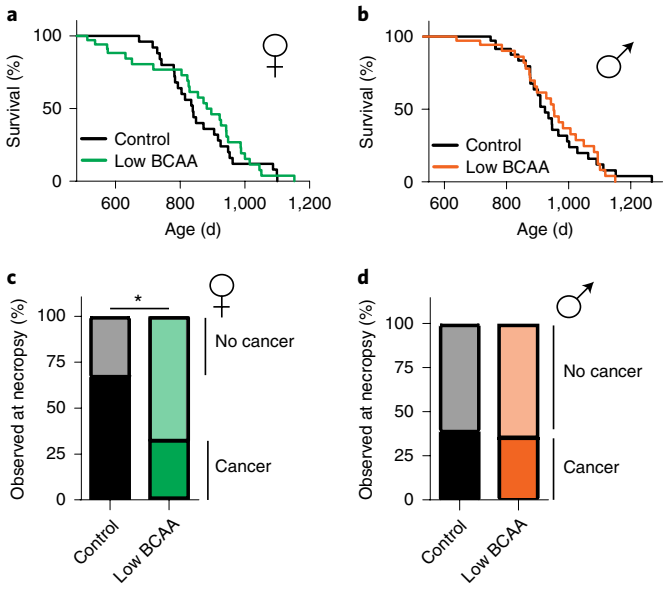


Fig. 4 | A low BCAA diet does not extend the life span of middle-aged mice. a,**b**, Kaplan–Meier plots showing the survival of female ($n=34$ biologically independent animals for both groups; **a**) and male (**b**) C57BL/6J.Nia mice fed the indicated diets starting at 16 months of age ($n=35$ biologically independent animals for both groups; Supplementary Table 8). **c**, The percentage of female mice with and without cancer observed at necropsy from the life span in **a** ($n=17$ (control cancer), 8 (low BCAA cancer), 8 (control non-cancer) and 17 (low BCAA non-cancer) biologically independent animals). **d**, The percentage of males with and without cancer observed at necropsy from the life span in **b** ($n=10$ (control cancer), 9 (low BCAA cancer), 15 (control non-cancer) and 16 (low BCAA non-cancer) biologically independent animals). *P* values were obtained from a two-sided Fisher's exact test (**c** and **d**); $^*P=0.0254$.

with no change in systemic insulin sensitivity as assessed by insulin tolerance test (ITT; Extended Data Fig. 2j).

During the first year, we performed assays to determine the effect of a low BCAA diet on physiological development, muscle strength and neuromuscular coordination. We found no change in either rotarod performance or grip strength in female mice on a low BCAA diet (Extended Data Fig. 2k,l). We collected blood at 16 months of age and quantified insulin and fibroblast growth factor 21 (FGF21); we observed no effect of diet on the levels of these hormones (Extended Data Fig. 2m,n). Starting at 16 months of age, we switched to noninvasive frailty assessment; we observed no statistically significant differences between control-fed and low BCAA-fed females (Fig. 5c and Supplementary Table 9). The overall life span of low BCAA-fed female mice was indistinguishable from that of control female mice (Fig. 5d and Supplementary Tables 3 and 10). A small cohort of female mice that were fed a low AA diet in parallel showed decreased survival, with a 22% reduction in median life span, although not statistically significant (Fig. 5d and Supplementary Table 3).

Lifelong low BCAA diet-fed males also weighed less than control-fed males for the majority of their life (Fig. 5e). In contrast to females, low BCAA-fed males rapidly became leaner due to reduced accretion of fat mass, but lean mass gain was also substantially impaired, and by 15 months of age the body composition of low BCAA-fed males was similar to that of control-fed males (Fig. 5f and Extended Data Fig. 3a,b). Low BCAA-fed males did not consume more food, alter activity level or increase energy expenditure, but RER was increased (Extended Data Fig. 3c–g). Glucose tolerance was robustly and consistently improved, with no change in systemic insulin sensitivity as assessed by ITT (Extended Data Fig. 3h,i).

As in females, a low BCAA diet did not impair rotarod performance or grip strength during the first year of life (Extended Data Fig. 3j,k). At 16 months of age, low BCAA-fed males showed decreased fasting insulin (Extended Data Fig. 3l) and increased levels of FGF21 (Extended Data Fig. 3m), but these effects were not statistically significant. We observed that low BCAA-fed males had reduced frailty relative to control-fed males after 25 months of age (Fig. 5g and Supplementary Table 11). In agreement with this significant difference in frailty, we observed that lifelong consumption of a low BCAA diet significantly increased life span, with a 31.8% increase in median life span relative to control-fed males and a 12.3% increase in maximum life span ($P=0.0251$; Fig. 5h and Supplementary Tables 3 and 10). A parallel cohort of low AA-fed males also lived significantly longer, with a 34.9% increase in median life span and an 18.2% increase in maximum life span ($P=0.0066$), and were indistinguishable from low BCAA-fed males (Fig. 5h and Supplementary Table 3). The longest-lived low BCAA-fed male lived to 1,456 d, just short of 4 years (Fig. 5h and Supplementary Table 10), exceeding the life span of the longest-lived survivor in several previous studies of ad libitum fed C57BL/6J mice[60,61] by approximately 25%.

Lack of detrimental or beneficial effects of prolonged BCAA restriction on cardiovascular function. Blood levels of BCAAs are associated with increased risk of cardiovascular diseases[62], cardiac dysfunction and remodeling following myocardial infarction and an increased incidence of subsequent adverse events[63–65]. To determine if a low BCAA diet might prevent age-associated cardiac dysfunction, or alternatively if lifelong low BCAA feeding might lead to impairment in cardiovascular structure or function, we performed echocardiograms on a subset of lifelong control-fed and low BCAA-fed mice of both sexes at 16 months of age (Supplementary Table 12). We observed no significant differences in cardiovascular parameters, including FS and ejection fraction, in low BCAA-fed mice (Supplementary Table 12). We also found a significant sex × diet interaction in parameters related to aortic stiffening with age (Supplementary Table 12). Thus, lifelong low BCAA diet feeding did not clearly improve or impair cardiac function.

Transcriptional profiling of skeletal muscle identifies sex-specific changes in longevity-associated signaling pathways. To obtain insight into the sex-specific effects of a low BCAA diet, we performed transcriptional profiling of the quadriceps muscle of control and low BCAA lifelong fed mice at 16 months of age (Extended Data Fig. 4a and Supplementary Tables 13 and 14). Principal-component analysis showed that while the muscle transcriptome of male and female control-fed mice substantially overlapped, it diverged upon low BCAA diet feeding (Fig. 6a and Extended Data Fig. 4b,c). Approximately 80% more genes were significantly altered by a low BCAA diet in males than females, with relatively little overlap between the sexes, as visualized by a scatter plot of the effect size of low BCAA feeding (Fig. 6b and Supplementary Tables 13a and 14a).

To identify pathways altered by a low BCAA diet in each sex, we performed KEGG overrepresentation analysis (ORA) on the differentially expressed genes (DEGs), regardless of directionality. In males, we found that a low BCAA diet resulted in significantly altered multiple Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways related to metabolism and longevity, including the Forkhead box class O (FoxO), insulin and mTOR signaling pathways (Supplementary Table 13B). A low BCAA diet altered a distinct and nonoverlapping set of pathways in females (Supplementary Table 14b). Visualizing the individual transcriptional changes in pathways of interest, we noted that several KEGG pathways identified as significantly altered only in a single sex appeared to be

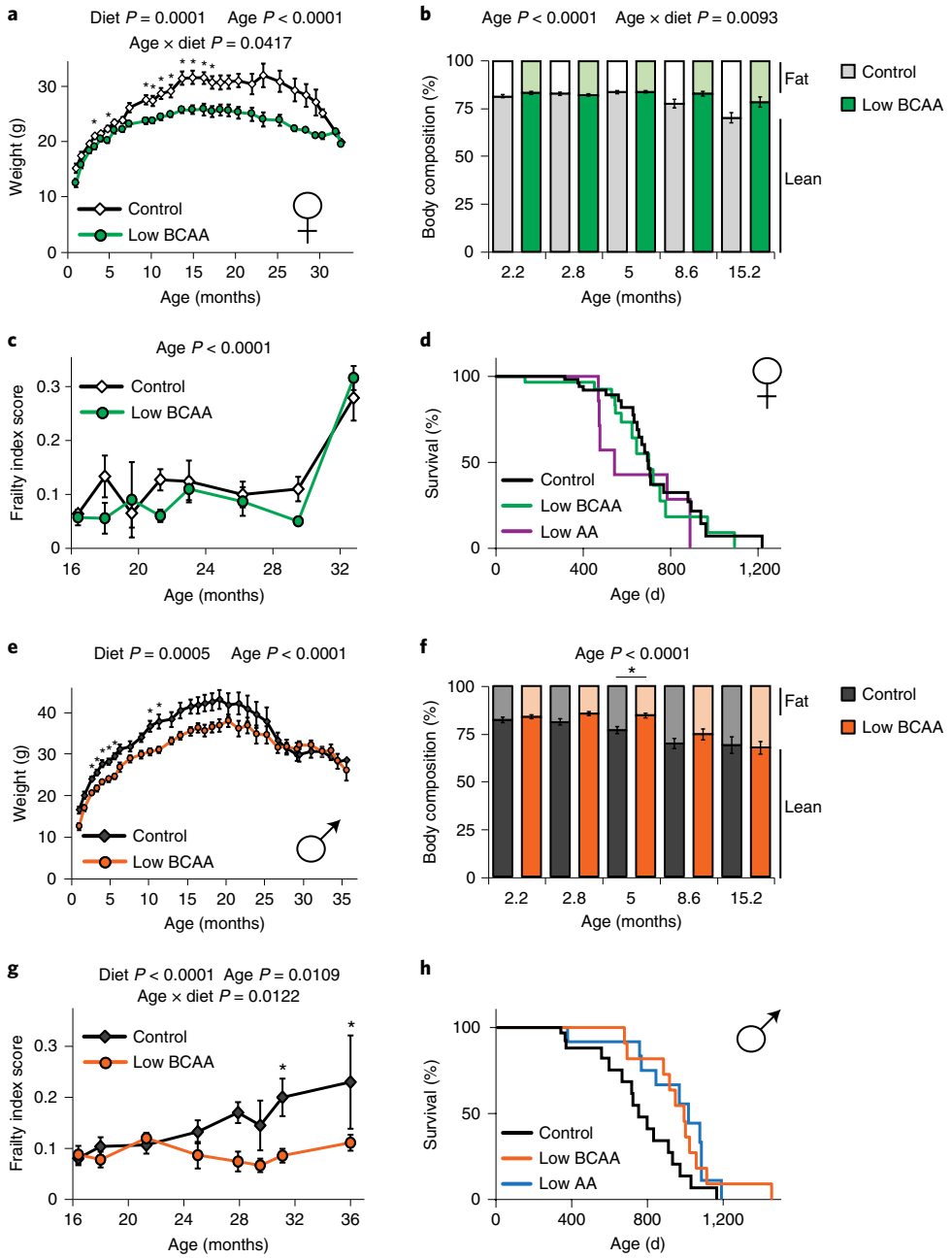


Fig. 5 | Lifelong consumption of a low BCAA diet promotes health span and extends male life span. Wild-type female (**a**–**d**) and male (**e**–**h**) mice were placed on either control or low BCAA diets at weaning. **a**, The weight of the female mice was tracked longitudinally (*n* varies by month, maximum $n = 35$ (control) and 22 (low BCAA) biologically independent animals; $^*P < 0.05$, adjusted *P* values by month of age: 3.25 mo, 0.0015; 4.75 mo, 0.0136; 9.5 mo, 0.0102; 10 mo, 0.0341; 11 mo, 0.0065; 12.25 mo, 0.0393; 13.5 mo, 0.0047; 15 mo, 0.0057; 16.25 mo, 0.0150; 17.25 mo, 0.0323). **b**, Female body composition (*n* varies by month, maximum $n = 15$ (control) and 12 (low BCAA) biologically independent animals). **c**, Female frailty was assessed longitudinally starting at 16 months of age (*n* varies by month, maximum $n = 25$ (control) and 18 (low BCAA) biologically independent animals); average frailty by index measured is available in Supplementary Table 9. **d**, Kaplan–Meier plot showing the survival of females that were fed the indicated diets from weaning ($n = 60$ (control), 29 (low BCAA), 9 (low AA) biologically independent animals; Supplementary Table 10). **e**, The weight of the male mice was tracked longitudinally (*n* varies by month, maximum $n = 22$ (control) and 23 (low BCAA) biologically independent animals; $^*P < 0.05$, adjusted *P* values by month of age: 2.5 mo, 0.0014; 3.25 mo, 0.0190; 4 mo, 0.0014; 4.75 mo, 0.0028; 5.5 mo, 0.0005; 10 mo, 0.025; 11.25 mo, 0.0283). **f**, Male body composition, (*n* varies by month, maximum $n = 19$ (control) and 18 (low BCAA) biologically independent animals; $^*P = 0.0154$). **g**, Male frailty was assessed longitudinally starting at 16 months of age (*n* varies by month, maximum $n = 13$ biologically independent animals for both groups; $^*P < 0.05$, adjusted *P* values by month of age: 31 mo, 0.0409; 36 mo, 0.0199); average frailty by index measured is available in Supplementary Table 11. **h**, Kaplan–Meier plot showing the survival of males that were fed the indicated diets from weaning ($n = 54$ (control), 30 (low BCAA) and 21 (low AA) biologically independent animals; Supplementary Table 10). In **a**–**c** and **e**–**g**, statistics for the overall effects of diet, age and the interaction represent the *P* value from a mixed-effects model (REML) or two-way repeated-measures ANOVA, with multiple comparisons by two-sided Sidak's post hoc test. Data are the mean ± s.e.m.

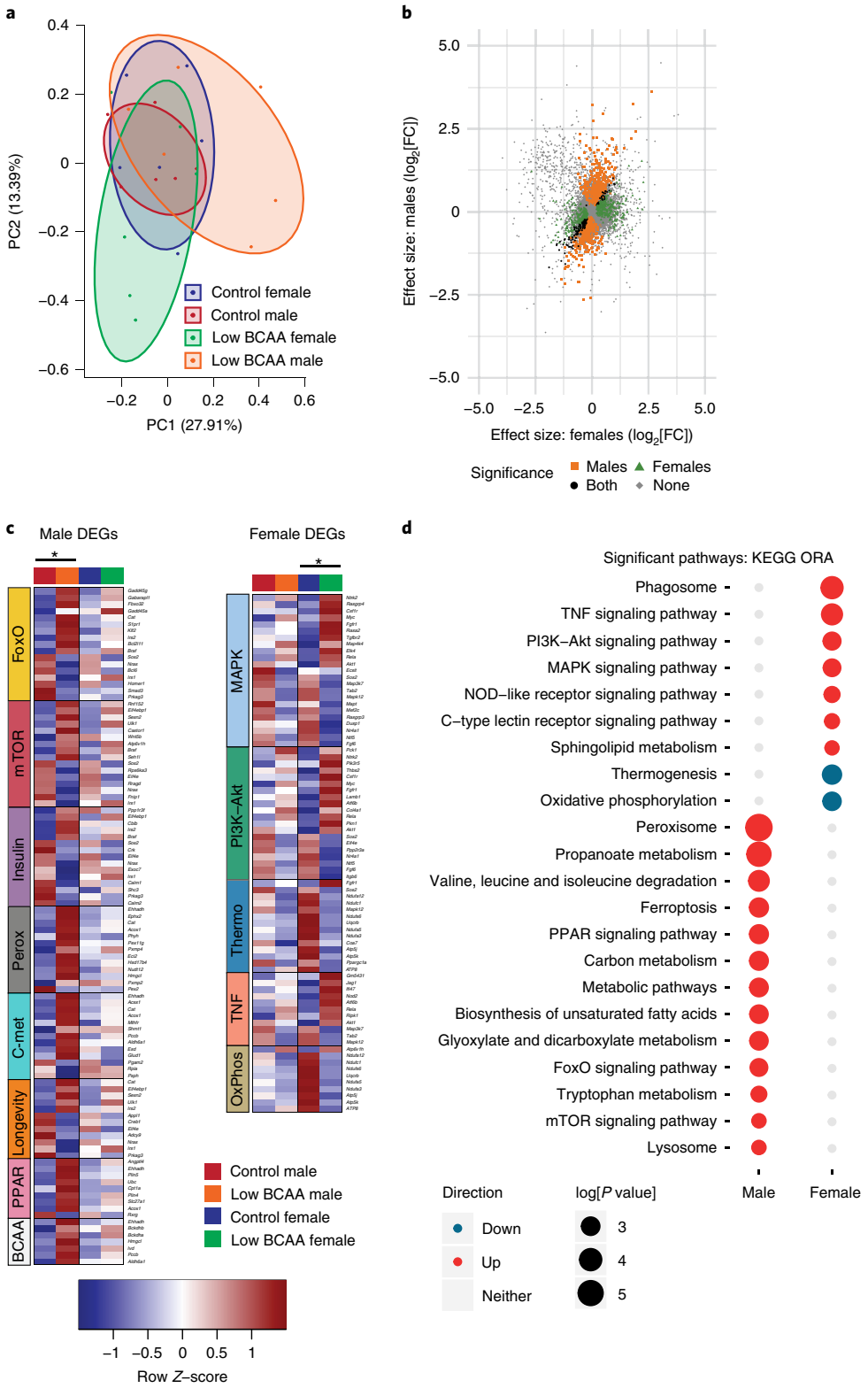


Fig. 6 | Transcriptional profiling of skeletal muscle identifies male-specific changes in longevity-associated signaling pathways. Transcriptional profiling was performed on mRNA from the skeletal muscle of male and female mice that consumed either control or low BCAA diets from weaning until 16 months of age ($n = 6$ biologically independent animals for all groups; Supplementary Tables 13 and 14). **a**, Plot of the first two principal components (PC1 and PC2) obtained from the principal-component analysis. **b**, Scatter plot showing effect size of low BCAA diet feeding in males and females, colored according to significance in one, both or neither group. FC, fold change. **c**, Heat maps of DEGs from significant KEGG ORA pathways of interest identified in Supplementary Tables 13b and 14b. DEGs were identified using an empirical Bayes moderated linear model. *Two-sided *P* values adjusted with the Benjamini–Hochberg procedure. **d**, Dot plot of significant pathways and directionality changes from KEGG ORA in Supplementary Tables 13c and 14c. MAPK, mitogen-activated protein kinase; NOD, nucleotide-binding and oligomerization domain; PPAR, peroxisome proliferator-activated receptor; TNF, tumor necrosis factor. ORA was performed on DEGs (designated by an adjusted *P* value of 0.3 for female and 0.2 for male contrasts) using a one-sided hypergeometric test, and *P* values were adjusted using the Benjamini–Hochberg procedure.

altered to a lesser degree in the opposite sex (Fig. 6c and Extended Data Fig. 4d).

We also performed KEGG ORA with gene directionality (Fig. 6d and Supplementary Tables 13c and 14c). Many pathways centered around metabolism and longevity were significantly upregulated in males subjected to low BCAA diet feeding, and again, a distinct set of nonoverlapping metabolic pathways were altered in females. Notably, although the mTOR signaling pathway was listed as upregulated, several of the upregulated genes, including *Sesn2* and *Castor1*, encode negative regulators of mTORC1 (refs. [66,67]).

As inhibition of mTORC1 robustly extends longevity in mice, and this pathway was significantly altered only in males, we examined mTORC1 signaling in more detail. We fed an additional cohort of young and aged mice control and low BCAA diets from 16 months of age until an age at which we expected 10% mortality, that is, 22 months of age (females) and 25 months of age (males). Young mice were fed in parallel for an equivalent length of time beginning at 6.5 months of age. Mice were then euthanized and tissues collected for analysis of mTOR signaling.

We found that males fed a low BCAA diet had significantly decreased phosphorylation of the mTORC1 substrate S6K1 T389 in skeletal muscle, with a 47% decrease in young males and a 78% decrease in aged males (Extended Data Fig. 5a). Phosphorylation of S6 S240/S244, a downstream readout of mTORC1 activity, was likewise reduced by 55% in young males. There was a statistically significant overall effect of diet on the phosphorylation of both S6 and S6K1 (Extended Data Fig. 5a). We did not observe any changes in the phosphorylation of the mTORC2 substrate AKT S473 (Extended Data Fig. 5a), demonstrating that these effects were specific to mTORC1. No changes in the phosphorylation of S6K1 T389, S6 S240/S244, ULK1 S757 or AKT S473 were observed in low BCAA-fed females (Extended Data Fig. 5b).

We also examined the phosphorylation of these mTOR substrates in the liver. We observed that phosphorylation of S6K1 T389 was decreased by 97% in young, low BCAA-fed males, and phosphorylation of S6 S240/244 was similarly decreased by 64% (Extended Data Fig. 6a). As in skeletal muscle, there was a statistically significant overall effect of diet on the phosphorylation of both S6 and S6K1 in male liver, as well as a statistically significant age × diet interaction on the phosphorylation of ULK1. We observed no effect of a low BCAA diet on mTOR signaling in female liver (Extended Data Fig. 6b).

Discussion

Reduced consumption of dietary protein is associated with increased metabolic health and longevity in organisms including flies, mice, rats and humans[11–13,16,18,15,19–22]. The beneficial effects of PR may be mediated by reduced consumption of specific AAs. Based on emerging evidence that reducing dietary levels of the three BCAAs recapitulates the metabolic benefits of a protein-restricted diet, and that elevated levels of BCAAs promote insulin resistance, obesity and mortality[15,18,32,36,38], we tested the hypothesis that restricting dietary BCAAs would promote health span and longevity.

Here, we determined the effect of restricting dietary BCAAs on the longevity of both progeroid and wild-type C57BL/6J mice, as well as multiple measures of health span. We show that a reduced BCAA diet promotes longevity in two distinct progeroid mouse models. A reduced BCAA diet begun in middle-aged C57BL/6J mice promotes metabolic health, keeping mice lean and glucose tolerant, decreases frailty and also reduces cancer in female mice. Lifelong consumption of a reduced BCAA diet improves the metabolic health of wild-type mice, reduces frailty in males and significantly extends male but not female life span. Finally, we show that a reduced BCAA diet has sex-specific effects on the skeletal muscle transcriptome, reducing mTORC1 signaling specifically in males.

We first utilized short-lived progeroid mice to rapidly analyse the effect of these diets; several other geroprotective interventions also promote the health of progeroid mice[47,68,69]. We used two distinct mouse models: first, *Lmna*$^{-/-}$, a model of Emery–Dreifuss muscular dystrophy[70]; and second, a recently developed model of HGPS, *Lmna*$^{G609G/G096G}$, which has the same mutation as most humans with HGPS[50]. The low BCAA and low AA diets increased survival in both models, increasing the overall survival and maximum life span of *Lmna*$^{G609G/G096G}$ mice.

The effect on the survival of *Lmna*$^{-/-}$ mice was limited to females and was significant only by the Wilcoxon test, which gives greater weight to early deaths than the log-rank test and does not assume proportional hazards; median but not maximum life span was increased in low BCAA-fed *Lmna*$^{-/-}$ female mice. Although we did not conduct a detailed study of the physiological or molecular impacts of a low BCAA diet on *Lmna*$^{-/-}$ mice, the shape of the survival curves suggest that the hazard ratio varies during the life span. The benefit of a low BCAA diet to the survival of *Lmna*$^{-/-}$ female mice may therefore result from improved health during midlife rather than a reduction in the rate of aging. Alternatively, the inability of a low BCAA diet to extend maximum life span may be related to the fact that *Lmna*$^{-/-}$ mice suffer from muscular dystrophy. While further research will be required to determine how low BCAA and low AA diets extend the survival of these progeroid strains, and to assess if these diets may provide insight into the treatment of muscular dystrophy or HGPS, these findings in progeroid mice support our overall conclusion that a low BCAA diet promotes healthy aging.

We next tested the effects of a low BCAA diet using wild-type C57BL/6J mice, beginning the diet at 16 months of age. The diet promotes metabolic health, keeping mice lean and glucose tolerant. A limitation of our study is that control-fed mice gained weight and fat for several months, achieving a peak weight similar to that of other aged chow-fed mice in our animal facility[71–73]. The weight gain we observed in control-fed mice may be due to transition of these animals, previously housed at the NIA Aged Mouse Colony, from a normal chow (NIH-31) diet; further research will be required to clarify the effect of a low BCAA diet on the weight and adiposity of aged mice.

Low-protein intake is associated with increased frailty in adults[9,11], and the role of dietary protein and BCAA intake on sarcopenia, frailty and longevity in the aged has been debated extensively[74]. Despite the importance of dietary protein in building and maintaining muscle, there were no negative effects of a low BCAA diet on grip strength or rotarod performance in either sex, and a low BCAA diet led to a reduction in age-associated frailty. Unexpectedly, the chemokine MCP-1, which has been suggested as a circulating biomarker of age and frailty[53], was increased in low BCAA-fed males. Understanding the relationship between dietary BCAAs, MCP-1 and frailty during aging will require additional research; examining MCP-1 and frailty in low AA-fed animals would also be informative.

Our results support the idea that reducing dietary BCAAs promotes multiple aspects of health span in mice. Beginning a reduced BCAA diet at midlife had no effect on male life span. While there was also no effect on overall life span in females, we observed significant excess mortality in the low BCAA-fed females following the diet switch. A similar effect was observed in previous studies that examined the life span of AA-restricted mice[27,31], as well as some studies of calorie-restricted animals[58,73]. Future studies, perhaps using a gradual 'step-down' of BCAAs rather than an abrupt 67% restriction to avoid any stress related to the diet switch, will be needed to unambiguously establish the effects of midlife BCAA restriction on female life span.

BCAAs are linked to the development and progression of cancer[75,76], and we observed a 50% reduction in tumors in low

BCAA-fed female mice. As cancer is the predominant cause of death in C57BL/6J mice, a reduction in the rate or progression of neoplasia would be expected to correlate with increased longevity. We did not identify cancer types or perform in-depth analysis of pathology, and more in-depth necropsies should be performed in future studies to better understand the effect of a low BCAA diet on cause of death, cancer incidence and progression. Future studies would also benefit from the inclusion of a chow-fed control group, which would enable any effects of AA-defined diets on life span or health span to be detected.

Dietary interventions often have a stronger effect on longevity when begun early in life[59]. We observed that lifelong consumption of a low BCAA diet improves metabolic health, and does not impair rotarod performance, grip strength or cardiac function. A low BCAA diet blunts the age-associated increase in frailty in males. Finally, we found that lifelong consumption of a low BCAA diet robustly extends the median life span of male mice by 32% and the survival of the longest-lived quartile was increased by 12%, recapitulating the effect of restricting all dietary AAs on life span.

Lifelong consumption of a low BCAA or low AA diet did not extend female life span. Although we did not anticipate that PR or BCAA restriction would have sex-specific effects, female and male mice benefit maximally from different levels of CR[58], and in *Drosophila*, males and females have optimal reproduction on low- and high-protein diets, respectively[19,77]. As most existing research on PR has focused on males, a sexually dimorphic response to a PR diet may have been overlooked; a recent study suggests that female mice have a blunted metabolic response to PR[78]. One early study found that PR extends the life span of female rats when PR was started at 120 d of age[79]. It is possible that the precise level of PR and BCAA restriction used here, or the age of initiation, was not optimal for female longevity.

Another limitation of the research presented here is the use of mice on a single genetic background, C57BL/6J. It has become clear that the effects of a calorie-restricted diet vary not only by sex, but also by strain[58,80]. Strain-dependent effects have been observed with respect to the metabolic effects of a series of different healthy diets[81], as well as the phenotypes relating to genetic modifications[82]. Genetic background will likely play a role in determining the effect of BCAA restriction on health span and longevity, and examining BCAA restriction in different strains or in a heterogeneous genetic background such as HET3 mice will be crucial in understanding whether these effects are strain independent and relevant to the genetically heterogeneous human population.

Our results broadly agree with a recent study showing that excess dietary BCAAs shorten life span[38]. However, Solon-Biet and colleagues did not observe increased longevity or a statistically significant improvement in glucose tolerance in mice fed a reduced BCAA diet[38]. Key differences from our study include the age at which restriction was initiated, diet design and the degree of restriction. Solon-Biet and colleagues examined 50% and 80% restriction starting at 12 weeks of age, and the approach used for diet construction increased levels of other essential AAs, including methionine and tryptophan, that impact longevity[27,28,30]. Our study specifically restricted BCAAs by 67%, with dietary levels of all other essential AAs kept constant; the level of restriction was selected based on our previous work demonstrating that restricting dietary AAs to a greater degree led to a progressive loss of lean mass[15]. Additional research will be required to define the optimal level of dietary BCAAs for health span and longevity in each sex. Finally, while we see an overall benefit in survival of the longest-lived quartile, we do not have enough information to draw conclusions about survival of the longest-lived decile, which could be addressed in future, larger studies powered to detect these differences.

Since a low BCAA diet reduces frailty, we performed transcriptional profiling of the skeletal muscle of both male and female mice fed either a low BCAA or a control diet. In agreement with the male-specific effects of a low BCAA diet on frailty and life span, we found that substantially greater changes were induced by a low BCAA diet in males, and there was no overlap in the KEGG pathways significantly enriched in either sex.

One KEGG pathway enriched in low BCAA-fed males is the mTOR signaling pathway, and we found that low BCAA-fed males, but not females, had reduced phosphorylation of mTORC1 substrates. In particular, there were significant reductions in the phosphorylation of S6K1 and its substrate S6; reduced S6K1 signaling extends the life span of mice, as well as other model organisms[42,45]. A trend towards increased mTORC1 signaling in aged male muscle, which we and others have previously observed[83,84], was not observed in low BCAA-fed males. Low BCAA-fed males, but not females, upregulate genes associated with BCAA degradation; as BCAA degradation is negatively associated with mTORC1 activity[85,86], the male-specific decrease in mTORC1 activity that we observed could result in part from male-specific alterations in BCAA catabolism. Additional work will be required to examine how BCAA requirements, utilization and catabolism differ between the sexes.

Reduced mTORC1 signaling in skeletal muscle delays sarcopenia and improves markers of muscle health[84], and skeletal muscle mTORC1 signaling regulates life span[87]. There are some similarities between the effects of a low BCAA diet and the effects of rapamycin, including a decrease in cancer and a trend towards improvements in cardiovascular parameters, including ejection fraction and FS[88,89]. While the effects of a low BCAA diet and rapamycin are not equivalent, it is likely that the beneficial effects of a low BCAA diet are mediated in part by inhibition of mTORC1.

FGF21 is elevated in low BCAA-fed male mice. Transgenic expression of FGF21 extends the life span of mice[90], and as this hormone is a key mediator of many metabolic effects of PR[16], it may also contribute to both the life span and metabolic phenotypes of low BCAA-fed males. Additional research will be required to elucidate the role of reduced mTORC1 signaling and FGF21 in the beneficial effects of a low BCAA diet. Further, it will be important to determine if, like rapamycin, the beneficial effects of reduced dietary BCAAs persist after resumption of a normal diet.

In summary, we have shown that dietary BCAAs regulate both health span and life span in progeroid and wild-type mice. Restricting dietary BCAAs broadly improves metabolic health and decreases frailty without overt negative consequences on muscle performance or cardiac function. Additional research will be required to determine if there are potentially negative effects of BCAA restriction, and to examine how optimal levels of BCAAs for life span and health span vary with age. We find that lifelong BCAA restriction reduces mTORC1 signaling, reduces frailty and extends the life span of wild-type males, while BCAA restriction begun in midlife promotes health span in both sexes and reduces the incidence of cancer in females. Our results demonstrate that dietary levels of BCAAs are critically important in healthy aging, and provide new evidence that protein quality—the specific AA composition of dietary protein—is as important as the amount of dietary protein consumed. Finally, while additional research and clinical trials will be required to determine how our findings apply to humans, our results support an emerging model that suggests that limiting dietary levels of BCAAs may be a key to a long and healthy life.

Methods

Animal use and care. All procedures were performed in conformance with institutional guidelines and were approved by the Institutional Animal Care and Use Committee of the William S. Middleton Memorial Veterans Hospital. C57BL/6J.Nia mice were obtained from the NIA Aged Rodent Colony. *Lmna*$^{-/-}$ mice were cryorecovered by The Jackson Laboratory (stock 009125)[70,91], and heterozygous parents were bred to produce *Lmna*$^{-/-}$ progeny. Homozygous male *Lmna*$^{G609G/G609G}$ mice[50] were obtained from B. Kennedy at The Buck Institute for Research on Aging by the kind permission of C. López-Otín and their sperm

used for in vitro fertilization of female C57BL/6J mice to obtain *Lmna*$^{G609G/+}$ heterozygous mice suitable for breeding. *Lmna*$^{G609G/+}$ heterozygous mice were then crossed to obtain experimental *Lmna*$^{G609G/G609G}$ mice and wild-type (*Lmna*$^{+/+}$) mice. Mice were housed in a specific pathogen-free mouse facility with a 12:12-h light/dark cycle maintained at 22 °C, with free access to food and water. Specific pathogen-free status was monitored by the use of sentinel animals and serology testing every 4 months, as well as a parasitology and *Helicobacter pylori* screening twice yearly, and these tests remained negative for the duration of these experiments. Animals were group housed in static microisolator cages, except when temporarily housed in a Columbus Instruments Oxymax/CLAMS metabolic chamber system.

Diets. All AA-defined diets were obtained from Envigo: control (TD.140711), low AA (TD.140712) and low BCAA (TD.140714 in Fig. 1a,b and TD.150662 in all other figures) diet compositions are provided in Supplementary Table 1. Mice not consuming experimental diets were fed standard chow diet (Purina LabDiet 5001), and breeding mice were fed breeder diet (Purina LabDiet 5015).

Life spans and necropsy. *Lmna*$^{-/-}$ progeny from parent heterozygous crosses were identified at weaning (confirmed with genotyping) and co-housed with a wild-type or heterozygous littermate of the same sex. *Lmna*$^{G609G/G609G}$ mice were obtained from parent heterozygous crosses and identified by genotyping before removing excess littermates and co-housing with a wild-type littermate of the same sex. C57BL/6J.Nia mice were obtained from the NIA Aged Mouse Colony at 15 months of age and allowed to acclimate to the animal facility for 1 month. Mice were fed control, low BCAA or low AA diets. As *Lmna*$^{-/-}$ mice have a particularly short life span, rigorous daily inspection of welfare, hydration and body temperature were assessed. Mice were euthanized for humane reasons if moribund, if the mice developed other specified problems (for example, excessive tumor burden) or upon the recommendation of the facility veterinarian. No mice in these studies contracted dermatitis requiring treatment or removal. Mice found dead were noted at each daily inspection and saved in a refrigerator for gross necropsy, during which the abdominal and thoracic cavities were examined for the presence of solid tumors, metastases, splenomegaly and infection; on the basis of this inspection, the presence or absence of observable cancer was recorded. A subset of mice was removed from the study for cross-sectional analysis (for example, echocardiography) or to control colony size; these mice were selected on the basis of age and cage grouping without visual inspection of the animals or reference to their health status or frailty. Mice were censored as of the date of death if removed for cross-sectional analysis, to control colony size, or if death was due to experimenter error. The life spans of *Lmna*$^{-/-}$, *Lmna*$^{G609G/G609G}$ and wild-type mice are provided in Supplementary Tables 2, 5, 8 and 10.

Genotyping. Genotyping primers were obtained from Integrated DNA Technologies. Genotyping of *Lmna*$^{-/-}$ mice utilized primers 8965 (5′-CAAGT CCCCATCACTTGGTT-3′), 8966: (5′-CTGTGACACTGGAGGCAGAA-3′) and oIMR7415 (5′-GCCAGAGGCCACTTGTGTAG-3′), as recommended by The Jackson Laboratory, with the following PCR program: 1 × 4 min at 94 °C, 35 × (30 s at 94 °C, 30 s at 52 °C and 1 min at 72 °C), 1 × 10 min at 72 °C and hold at 4 °C, using DreamTaq Green PCR Master Mix (Thermo Fisher Scientific). Genotyping of *Lmna*$^{G609G/G609G}$ mice utilized primers as described by Osorio et al.[50]: 5′-AAG GGGCTGGGAGGACAGAG-3′, 5′-AGTAGAAGGTGGCGCGAAGG-3′ and 5′-AGCATGCAATAGGGTGGAAGGA-3′, with the following PCR program as advised by the Kennedy laboratory: 1 × 3 min at 98 °C, 30 × (30 s at 98 °C, 1 min at 64 °C and 30 s at 62 °C), 2 min × 72 °C and hold at 4 °C, using Phusion Taq DNA Polymerase (Thermo Fisher Scientific).

Body composition. Body composition was measured by MRI using an EchoMRI 700 body composition analyser.

Glucose and insulin tolerance tests. GTTs were performed by fasting the mice overnight for 16 h and then injecting glucose (1 g kg^{-1} intraperitoneally[15,92]. ITTs were performed by fasting mice for 4 h starting at lights on, and then injecting insulin (0.75 U kg^{-1}) intraperitoneally. Glucose measurements were taken using a Bayer Contour blood glucose meter and test strips.

Metabolic chambers. To assess metabolic physiology (O_2, CO_2, RER and food consumption) and spontaneous activity, mice were acclimated to housing in a Columbus Instruments Oxymax/CLAMS metabolic chamber system for approximately 24 h, and data from a continuous 24-h period were then recorded and analysed.

Frailty assessment. Frailty was assessed longitudinally in a subset of mice using a 26-item frailty index[51]. This frailty index reflects an accumulation of deficits associated with aging, akin to Rockwood's frailty index in humans[93] and is conceptually distinct from Fried's frailty syndrome[94]. The items scored included alopecia, loss of fur color, dermatitis, loss of whiskers, coat condition, tumors, distended abdomen, kyphosis, tail stiffening, gait disorders, tremor, body condition score, vestibular disturbance, cataracts, corneal opacity, eye discharge/swelling, microphthalmia, vision loss, menace reflex, nasal discharge, malocclusions, rectal prolapse, vaginal/uterine/penile prolapse, diarrhea, breathing rate/depth, mouse grimace score and piloerection. Average scores per group over time of these specific items can be found in Supplementary Tables 6 and 7 for the midlife intervention and Supplementary Tables 9 and 11 for the lifelong intervention.

Rotarod and grip strength. Forelimb grip strength was measured using a grip strength meter (Columbus Instruments, 1027SM) and calculated as the average of three tests. Performance on a rotating rod was calculated as the average of two runs after two acclimating runs the day before, using a Rota Rod Rotamex 5 (Columbus Instruments, 0890M). The rotarod was initially set to 3 r.p.m., with speed increasing by 1 r.p.m. every 3 s.

Echocardiography. Mice used for echocardiography were selected on the basis of age without reference to frailty status and were euthanized following the procedure, with animals censored from the life span study on the date of euthanasia. Transthoracic echocardiography was performed using a Visual Sonics Vevo 770 ultrasonograph with a 30-MHz transducer as detailed previously[95]. For acquisition of two-dimensional guided M-mode images at the tips of papillary muscles and Doppler studies, mice were sedated with 1% isoflurane administered through a facemask, hair removed, and maintained on a heated platform. Blood velocities across the mitral, aortic and pulmonary valves were measured using Doppler pulsed-wave imaging, angling the probe to obtain a nearly parallel orientation to the blood flow.

End diastolic and systolic left ventricular (LV) diameter, as well as anterior and posterior wall (AW and PW, respectively) thickness were measured on line from M-mode images obtained in a parasternal long-axis view using the leading-edge-to-leading-edge convention. All parameters were measured over at least three consecutive cardiac cycles and averaged. Left ventricular FS was calculated as: $((\text{LV diameter}_{dias} - \text{LV diameter}_{sys})/\text{LV diameter}_{dias}) \times 100$; ejection fraction: $((7.0/(2.4 + \text{LV diameter}_{dias})(\text{LV diameter}_{dias})^3 - (7.0/(2.4 + \text{LV diameter}_{sys})(\text{LV diameter}_{sys})^3/(7.0/(2.4 + \text{LV diameter}_{dias})(\text{LV diameter}_{dias})^3 \times 100$; and LV mass: $(1.05 \times ((\text{PW}_{dias} + \text{AW}_{dias} + \text{LV diameter}_{dias})^3 - (\text{LV diameter}_{dias})^3))$. Heart rate was determined from at least three consecutive intervals from the pulsed-wave Doppler tracings of the LV outflow tract. Ejection time was measured from the same outflow track tracings from the onset of flow to the end of flow. Isovolumic relaxation time was measured as the time from the closing of the aortic valve to the opening of the mitral valve from pulsed-wave Doppler tracings of the LV outflow tract and mitral inflow region. The same investigator obtained all images and measures.

Transcriptional profiling. Animals used for transcriptional profiling were euthanized after an overnight fast of 16 h. RNA was extracted from the quadriceps muscle as previously described[18]. The concentration and purity of RNA were determined using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific), and RNA was diluted to 100–400 ng µl^{-1} for sequencing. The RNA was then submitted to the University of Wisconsin-Madison Biotechnology Center Gene Expression Center and DNA Sequencing Facility, and RNA quality was assayed using an Agilent RNA NanoChip. RNA libraries were prepared using the TruSeq Stranded Total RNA Sample Preparation protocol (Illumina) with 250 ng of mRNA, and cleanup was performed using RNA Clean beads (no. 17225200). Reads were aligned to the mouse (*Mus musculus*) with genome-build GRCm38.p5 of the NCBI database under accession GCA_000001635.7, and expected counts were generated with Ensembl gene IDs[96].

Analysis of significant DEGs was completed in R (version 3.6.1)[97] using edgeR[98] and limma[99]. Gene names were converted to gene symbol and Entrez ID formats using the mygene package. Initially, 51,912 transcripts were identified across all genes, and those with zero counts per million in four or more individuals were removed, leaving 16,602 transcripts. To reduce the impact of external factors not of biological interest that may affect expression, data were normalized to ensure the expression distributions of each sample were within a similar range. We normalized using the trimmed mean of M values method, which scales to library size. Heteroscedasticity was accounted for using the voom function, DEGs were identified using an empirical Bayes moderated linear model, and log coefficients and Benjamini–Hochberg adjusted *P* values were generated for each comparison of interest[100]. DEGs (designated based on an adjusted *P* value of 0.3 for female and 0.2 for male contrasts) were used to identify enriched pathways, and KEGG-enriched pathways were determined for each contrast.

Plasma hormone measurements. Plasma MCP-1 and FGF21 were quantified using a mouse MCP-1 ELISA kit (MJE00B) and mouse/rat FGF21 ELISA kit (MF2100), respectively, from R&D Systems. Plasma insulin (90080) and IGF-1 (80574) were quantified using ELISA kits from Crystal Chem.

Immunoblotting. Animals used for western blotting were euthanized following a 4-h refeeding period after an overnight fast. Tissue samples from muscle were lysed in cold RIPA buffer supplemented with phosphatase inhibitor and protease inhibitor cocktail tablets (Thermo Fisher Scientific) as previously described[83] using a FastPrep 24 (M.P. Biomedicals) with bead-beating tubes (16466–042; VWR) and

zirconium ceramic oxide bulk beads (15340159; Thermo Fisher Scientific). Protein lysates were then centrifuged at 13,300 r.p.m. for 10 min and the supernatant was collected. Protein concentration was determined by Bradford assay (Pierce Biotechnology). Approximately 20 µg of protein was separated by SDS–PAGE on 8%, 10% or 16% resolving gels (Thermo Fisher Scientific) and transferred to poly(vinylidene fluoride) membrane (EMD Millipore). pT389-S6K1 (9234), S6K1 (9202), pS240/244-S6 (2215), S6 (2217), pS757-ULK1 (6888), ULK1 (8054), pS473-AKT (9271), AKT (9272) and HSP90 (4877) were purchased from Cell Signaling Technologies and used at a dilution of 1:1,000. Imaging was performed using an ImageQuant LAS 4000 imaging station (GE Healthcare). Quantification was performed by densitometry using the National Institutes of Health (NIH) ImageJ software.

Blinding. Investigators were blinded to diet groups during data collection whenever feasible, but this was not usually possible or feasible as cages were clearly marked to indicate the diet provided, diets were color coded to prevent feeding mistakes, and the size and body composition of the mice was altered by genotype and diet. However, blinding is not relevant to the majority of the studies conducted here, as the data are collected in numeric form, which is not readily subject to bias due to the need for subjective interpretation. Investigators were not blinded during necropsies.

Statistics. Statistical analyses were conducted using Prism 8 (GraphPad Software). Statistical analyses were performed by one-way ANOVA or, in the case of factor analysis, two-way ANOVA. Dunnett's post hoc test comparisons were used in one-way ANOVA analyses, and Sidak's post hoc test comparisons were used in two-way ANOVA analyses. Where measurements were taken longitudinally, a mixed-effects model (REML) or two-way repeated-measures ANOVA was used. Energy expenditure data were analysed by linear regression of energy expenditure by body weight (ANCOVA). Statistical significance for cancer incidence at death was calculated using a two-sided Fisher's exact test. Life span comparisons were calculated by log-rank test or Gehan–Breslow–Wilcoxon test, as specified in figure legends. A log-rank test was performed unless the assumption of proportional hazards was not satisfied; in these cases, a Gehan–Breslow–Wilcoxon test, which does not rely on this assumption, was used as a fallback. Transcriptomics data were analysed using R (version 3.6.1). Additional comparisons, if any, were corrected for multiple comparisons using the Benjamini–Hochberg method. Maximum life span calculations were made by generating a cutoff of the top 25% longest-lived animals in each cohort, coupled with Boschloo's test for significance between groups[48]. All statistical analyses were performed as specified in the figure legends. Sample sizes for longevity studies were determined in consultation with previously published power tables[101]. Sample sizes for metabolic studies was determined based on our previously published experimental results regarding the effects of low BCAA diet feeding[18,15], with the goal of having >90% power to detect a change in the area under the curve during a GTT ($P < 0.05$). Data distribution was assumed to be normal, but this was not formally tested.

Randomization. Middle-aged animals obtained from the NIA were randomized into groups of equivalent weight before the beginning of the in vivo studies; all other animals, which were bred in-house, were enrolled by birth order. Mice removed from the life span studies for cross-sectional analyses were selected from a database without information about the health status of the mice, as they reached the age previously chosen for phenotyping and there was availability at the cardiac phenotyping core. Young animals removed from the life span study were removed at the cage level without assessing the health status of individual mice.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

RNA-sequencing data have been deposited with the Gene Expression Omnibus and are accessible through accession number GSE155064. The data that support the plots and other findings of this study are available from the corresponding author upon reasonable request. Full scans of western blot images are provided as source data.

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Author contributions

Experiments were performed in the Lamming and Hacker laboratories. N.E.R., T.H. and D.W.L. conceived and designed the experiments. All authors participated in performing the experiments. N.E.R., T.H. and D.W.L. analysed the data and prepared the manuscript.

Competing interests

D.W.L. has received funding from, and is a scientific advisory board member of, Aeovian Pharmaceuticals, which seeks to develop new, selective mTOR inhibitors for the treatment of various diseases. The University of Wisconsin-Madison has applied for a patent for the use of BCAA-restricted diets to promote metabolic health, for which N.E.R. and D.W.L. are inventors.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s43587-020-00006-2.

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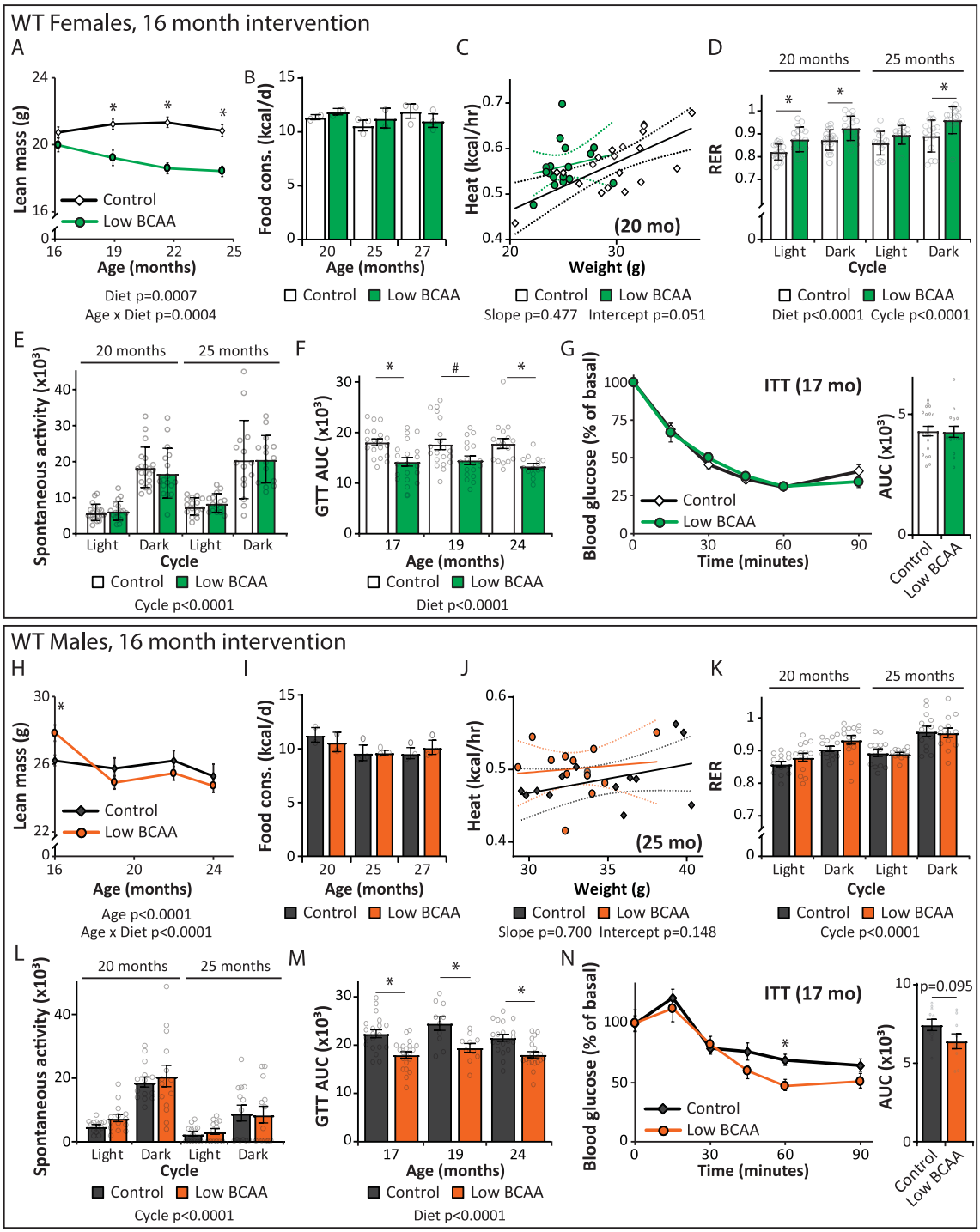
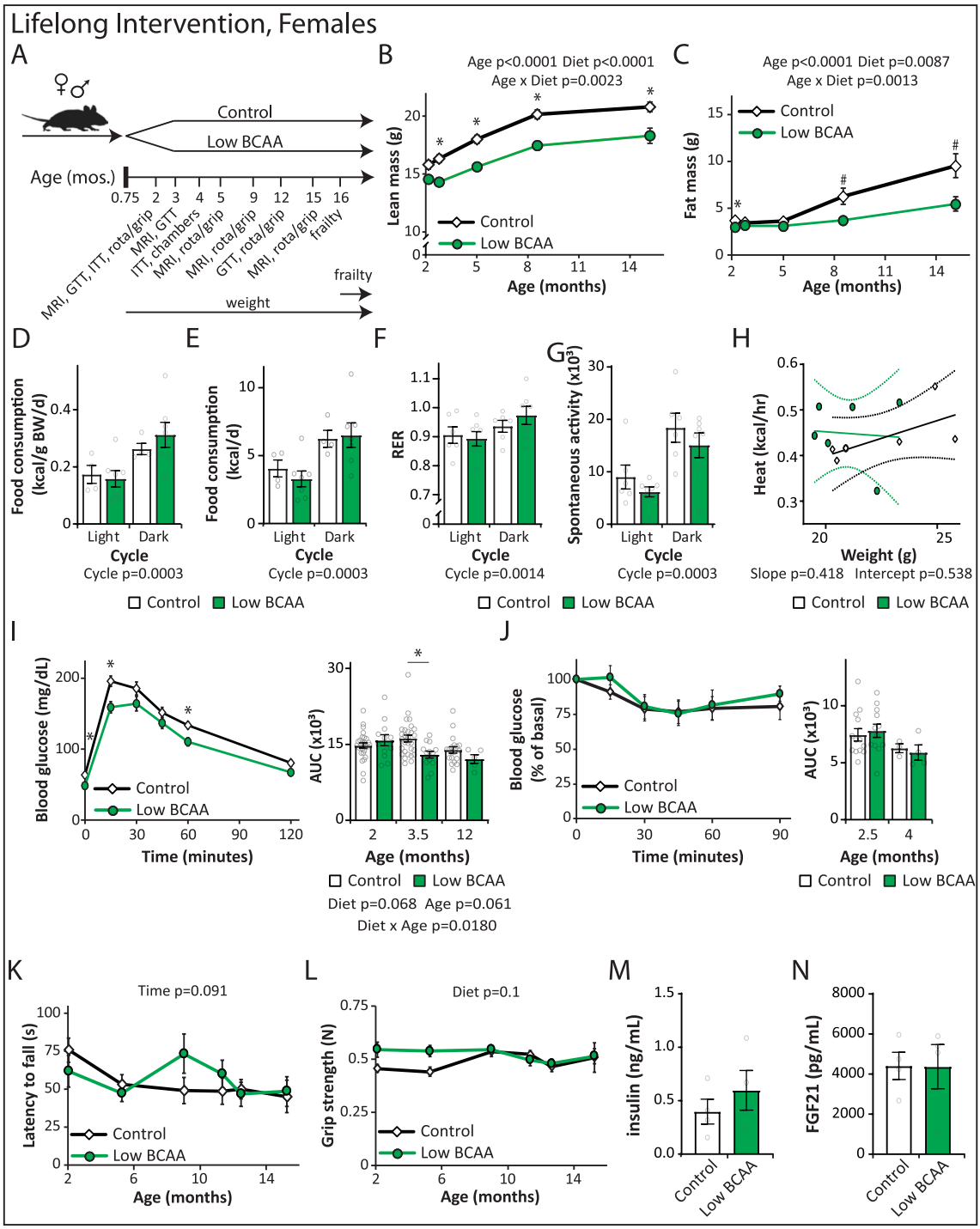


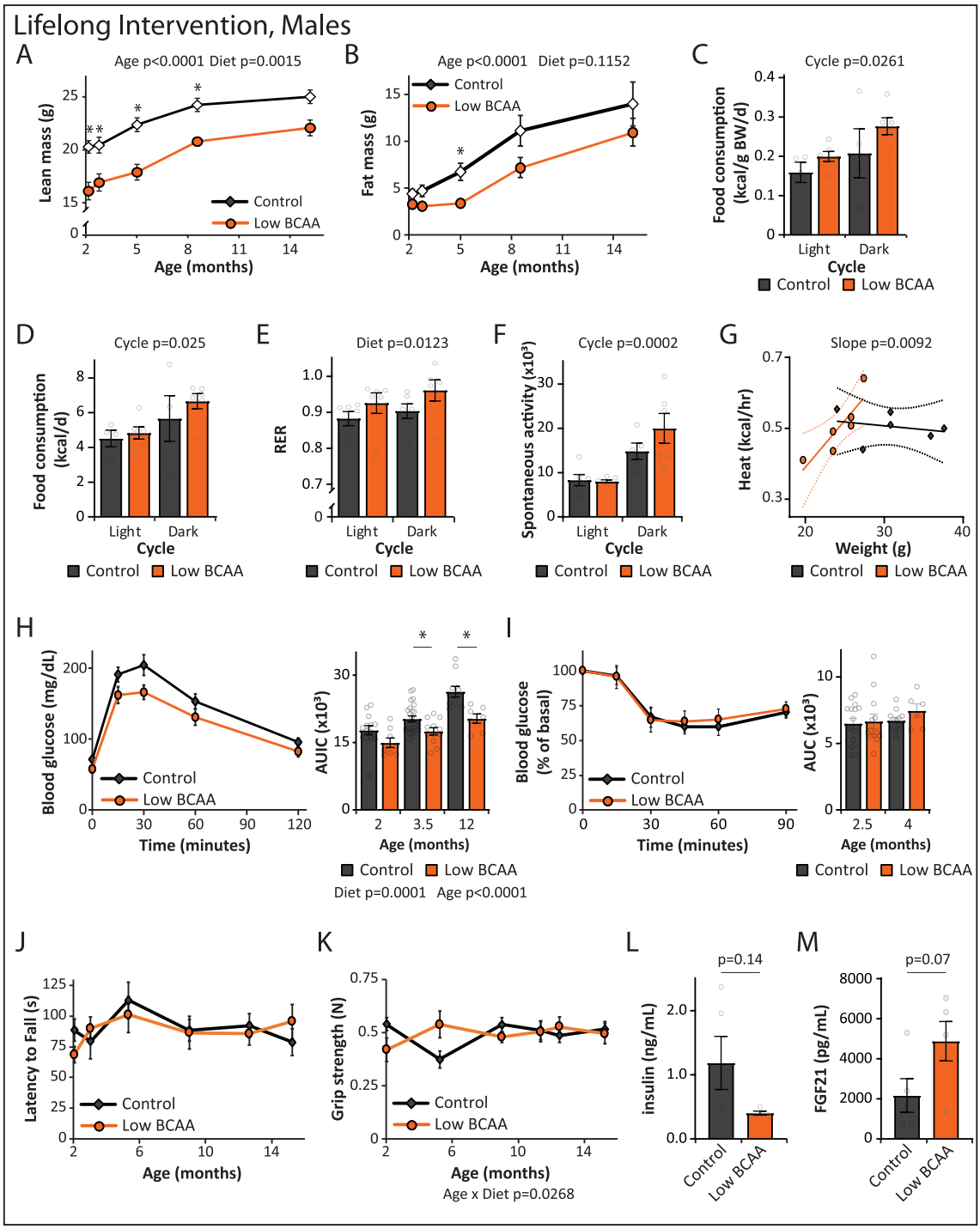
Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | A Low BCAA diet promotes the metabolic health of aged wild-type mice. a-g, Female and **h-n**, Male C57BL/6 J.Nia mice were fed the indicated diets beginning at 16 months of age. **a**, The lean mass of a subset of female mice was tracked (n varies by month; maximum N = 10 biologically independent animals for both groups; * $p < 0.05$ (p value by month of age: 19 mo. = 0.0091, 21.5 mo. <0.0001, 24.5 mo. = 0.0004). **b**, Food consumption over time calculated as total kcal/d (maximum N = 3 independent cages for both groups). **c**, Energy expenditure (Heat) was assessed using metabolic chambers at 20 months of age (N; Control = 20, Low BCAA = 17 biologically independent animals). **d**, Respiratory exchange ratio and **e**, ambulatory movement was assessed using metabolic chambers at 20 and 25 months of age (maximum N; Control = 20, Low BCAA = 17 biologically independent animals; * $p < 0.05$ (p value for **d** by month of age: 20 mo. light=0.0056, dark=0.0105, 25 mo. dark=0.0012). **f**, Area under the curve (AUC) corresponding to the glucose tolerance test in Fig. 2g as well as repeat tests performed at 19 and 24 months of age (maximum N = 20 biologically independent animals for both groups; * $p < 0.05$, # $p < 0.1$ (p value by month of age: 17 mo. = 0.0034, 19 mo. = 0.0714, 24 mo. = 0.0016). **g**, Insulin tolerance test and corresponding area under the curve after four weeks of diet feeding (N; Control = 20, Low BCAA = 15 biologically independent animals). **h**, The lean mass of a subset of male mice was tracked (n varies by month; maximum N = 20 biologically independent animals for both groups, * $p = 0.0066$). **i**, Food consumption over time calculated as total kcal/d (maximum N = 3 independent cages for both groups). **j**, Energy expenditure (Heat) was assessed using metabolic chambers at 25 months of age (N = 13 biologically independent animals for both groups). **k**, Respiratory exchange ratio and (**l**) ambulatory movement was assessed using metabolic chambers at 20 and 25 months of age (maximum N = 14 biologically independent animals for both groups). **m**, AUC corresponding to glucose tolerance test in Fig. 2m as well as repeat tests performed at 19 and 24 months of age (maximum N = 20 biologically independent animals for both groups; * $p < 0.05$ (p-value by month of age: 17 mo. = 0.0006, 19 mo. =0.0240, 24 mo. = 0.0026). **n**, Insulin tolerance test and corresponding area under the curve after four weeks of diet feeding (N; Control = 10, Low BCAA = 9 biologically independent animals; * $p = 0.0192$). **a**-**b**,**d**-**f**,**h**-**i**,**k**-**m**, Statistics for the overall effects of diet, age, and the interaction represent the p value from a mixed-effects model (restricted maximum likelihood [REML]) or two-way repeated measures ANOVA, multiple comparisons by two-sided Sidak's post-test. **c,j**, Energy expenditure data was analysed by linear regression of energy expenditure by body weight (ANCOVA). **g,n**, AUC comparisons were made by two-sided t-test, * $p < 0.05$. Data are represented as mean ± SEM.



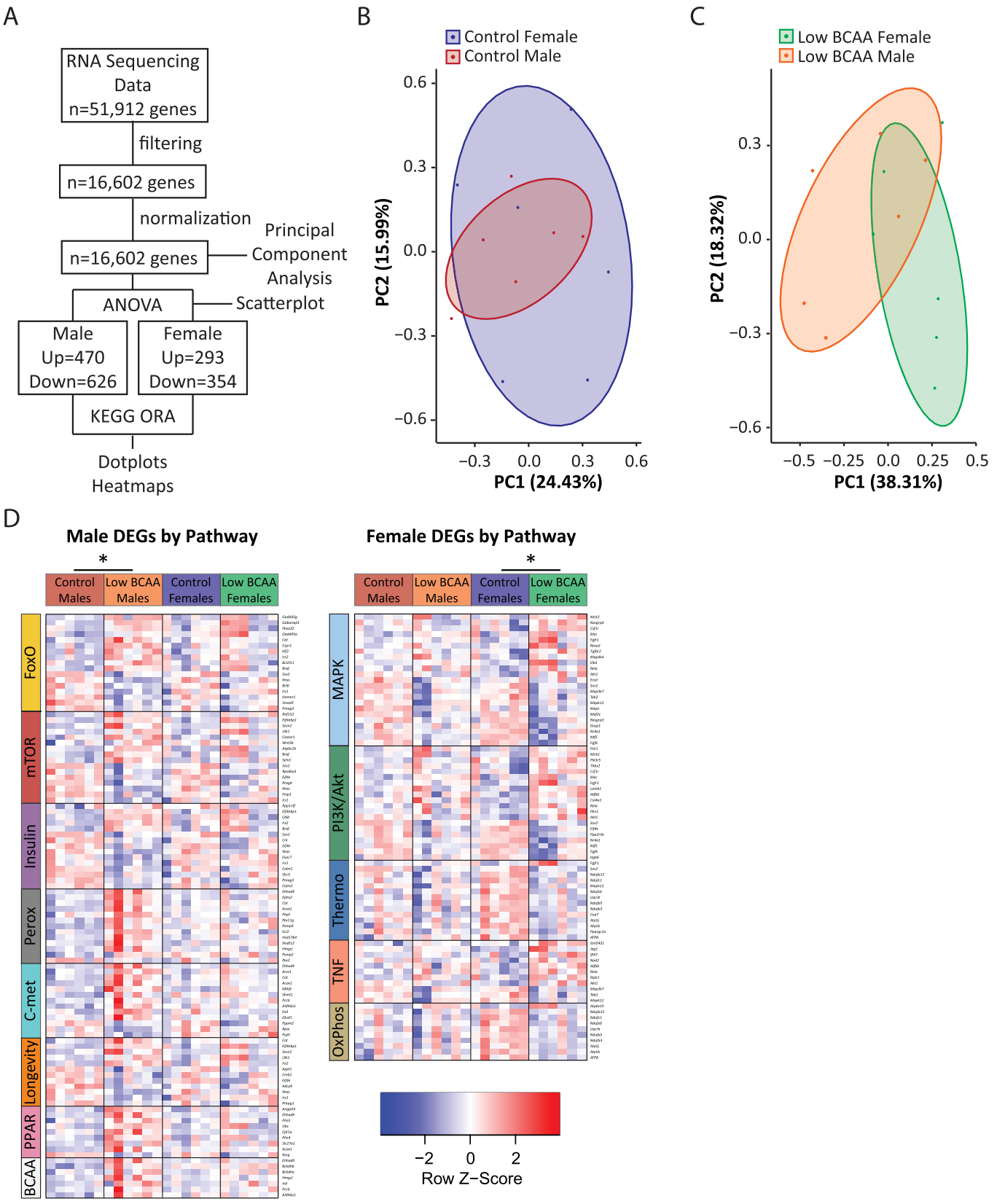
Extended Data Fig. 2 | See next page for caption.

Extended Data Fig. 2 | Effects of a lifelong Low BCAA diet on the health span of female mice. a, Schematic showing timeline of measurements taken from male and female mice fed Control or Low BCAA diets, relevant to Fig. 5 and Extended Data Figs. 2 and 3. **b-n**, Wild-type female mice were placed on either Control or Low BCAA diets at weaning. **b**-**c**, The **b**, lean mass and **c**, fat mass of a subset of mice was tracked (n varies by month, maximum N; Control = 15, Low BCAA = 12 biologically independent animals). **b**, * $p < 0.05$ (p-values for by month of age: 2.75 mo. = 0.0004. 5 mo. < 0.0001, 8.5 mo. = 0.0003, 15 mo. = 0.0341). **c**, * $p < 0.05$, # $p < 0.1$ (p-values by month of age: 2 mo. = 0.0102, 8.5 mo. = 0.0695, 15 mo. = 0.0652). **d-e**, Food consumption was calculated per gram of body weight **d**, and by animal **e**, (N; Control = 4, Low BCAA = 7 biologically independent animals). **f-h**, Respiratory exchange ratio (**f**), ambulatory movement (**g**), and energy expenditure (heat) (**h**) were assessed using metabolic chambers at 5 months of age (N; Control = 6, Low BCAA = 7 biologically independent animals). **i**, Glucose tolerance test at 2 months of age (N; Control = 18, Low BCAA = 16 biologically independent animals; * $p < 0.05$ (p-value by time: 0 m = 0.0005, 15 m = 0.0120, 60 m = 0.0267), and corresponding area under the curve (AUC), also from tests performed at 3.5 and 12 months of age (maximum N; Control = 24, Low BCAA = 18 biologically independent animals; * $p = 0.0063$). **j**, Insulin tolerance test at 2.5 months of age (N; Control = 13, Low BCAA = 12 biologically independent animals), and corresponding area under the curve, also from a test at 4 months of age (N; Control = 3, Low BCAA = 4 biologically independent animals). **k**, Rotarod performance (n varies by month, maximum N; Control = 12, Low BCAA = 8 biologically independent animals) and **l,** grip strength (n varies by month, maximum N; Control = 15, Low BCAA = 12 biologically independent animals) was assessed longitudinally. **m-n**, Levels of **m**, insulin and **n**, fibroblast growth factor 21 (FGF21) were measured in serum by ELISA (16 months of age; N = 4 biologically independent animals per group). **b**-**n**, Statistics for the overall effects of diet, age, and the interaction represent the p value from a mixed-effects model (restricted maximum likelihood [REML]), two-way repeated measures ANOVA, or a two-tailed, unpaired t-test in **m**-**n**; multiple comparisons by two sided Sidak's post-test. Data are represented as mean ± SEM.



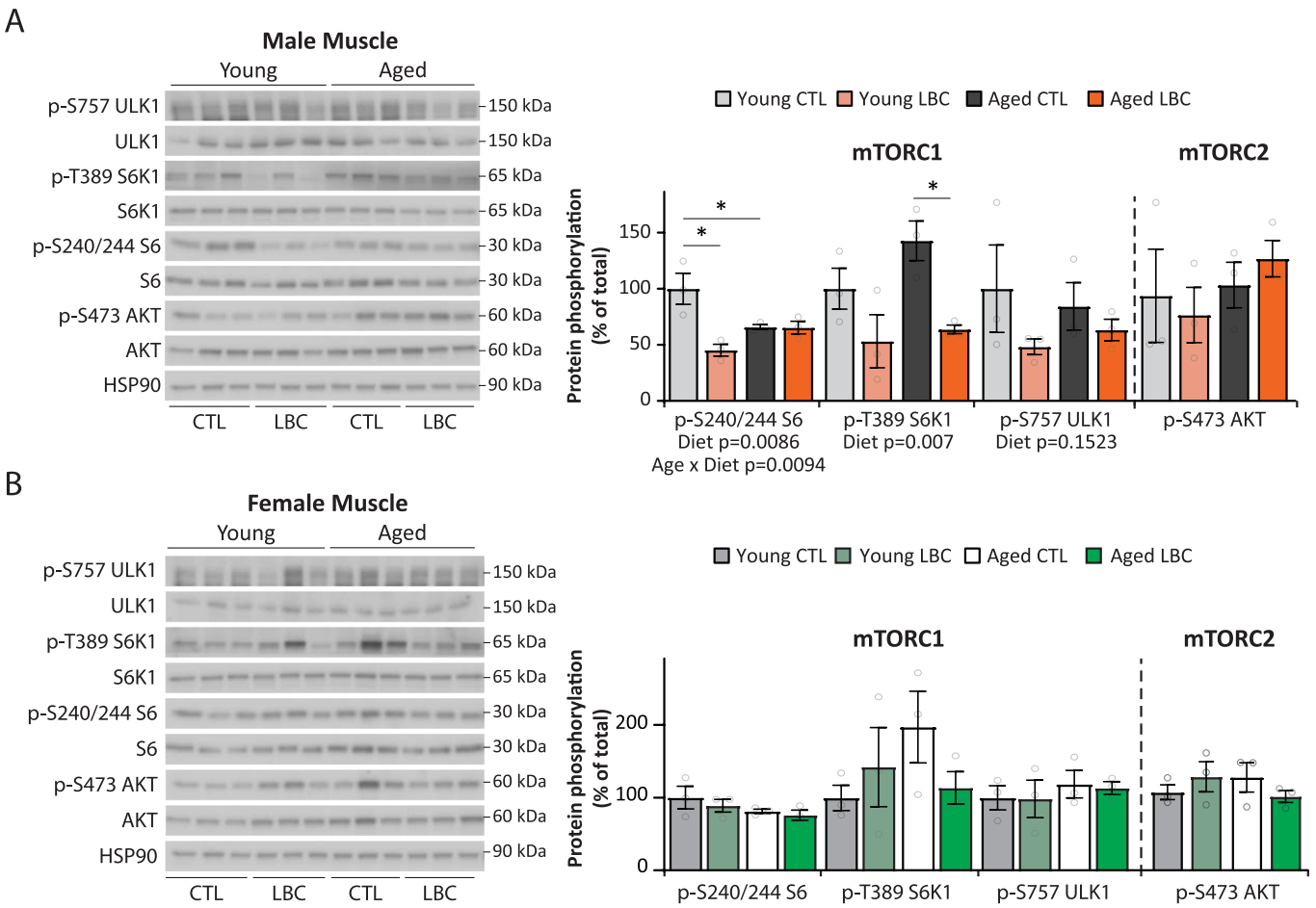
Extended Data Fig. 3 | See next page for caption.

Extended Data Fig. 3 | Effects of a lifelong Low BCAA diet on the healthspan of male mice. a-m, Wild-type male mice were placed on either Control or Low BCAA diets at weaning. **a**-**b**, The **a,** lean mass and **b**, fat mass of a subset of mice was tracked (n varies by month, maximum N; Control = 11, Low BCAA = 8 biologically independent animals; * $p < 0.05$ (p-values for **a** by month of age: 2 mo. = 0.0028, 2.75 mo. = 0.0194, 5 mo. = 0.0011, 8.5 mo. = 0.0246; for **b** * $p = 0.0095$). **c**-**d**, Food consumption (N; Control = 4, Low BCAA = 6 biologically independent animals), was calculated **c,** per gram of body weight and **d**, by animal. **e**, Respiratory exchange ratio, **f**, ambulatory movement, and **g**, energy expenditure (heat) were assessed using metabolic chambers at 5 months of age (**e**-**g**; N = 6 biologically independent animals for both groups). **h**, Glucose tolerance test at 2 months of age (N; Control = 14, Low BCAA = 8 biologically independent animals), and corresponding area under the curve (AUC), also from tests performed at 3.5 and 12 months of age (maximum N; Control = 23, Low BCAA = 12 biologically independent animals; * $p < 0.05$ (p-values by month of age: 3.5 mo. = 0.0379, 12 mo. = 0.0054). **i**, Insulin tolerance test at 2.5 months of age (N; Control = 15, Low BCAA = 13 biologically independent animals), and corresponding area under the curve, also from a test at 4 months of age (maximum N; Control = 15, Low BCAA = 13 biologically independent animals). **j**, Rotarod performance (n varies by month, maximum N; Control = 15, Low BCAA = 12 biologically independent animals) and **k**, grip strength (n varies by month, maximum N; Control = 12, Low BCAA = 8 biologically independent animals) were assessed longitudinally. Levels of **l**, insulin and **m**, fibroblast growth factor 21 (FGF21) were measured in serum by ELISA (16 months of age; N; Control = 5, Low BCAA = 4 biologically independent animals per group). **a-m**, Statistics for the overall effects of diet, age, and the interaction represent the p value from a mixed-effects model (restricted maximum likelihood [REML]), two-way repeated measures ANOVA, or a two-tailed, unpaired t-test in **l**-**m**; multiple comparisons by two-sided Sidak's post-test. Data are represented as mean ± SEM.

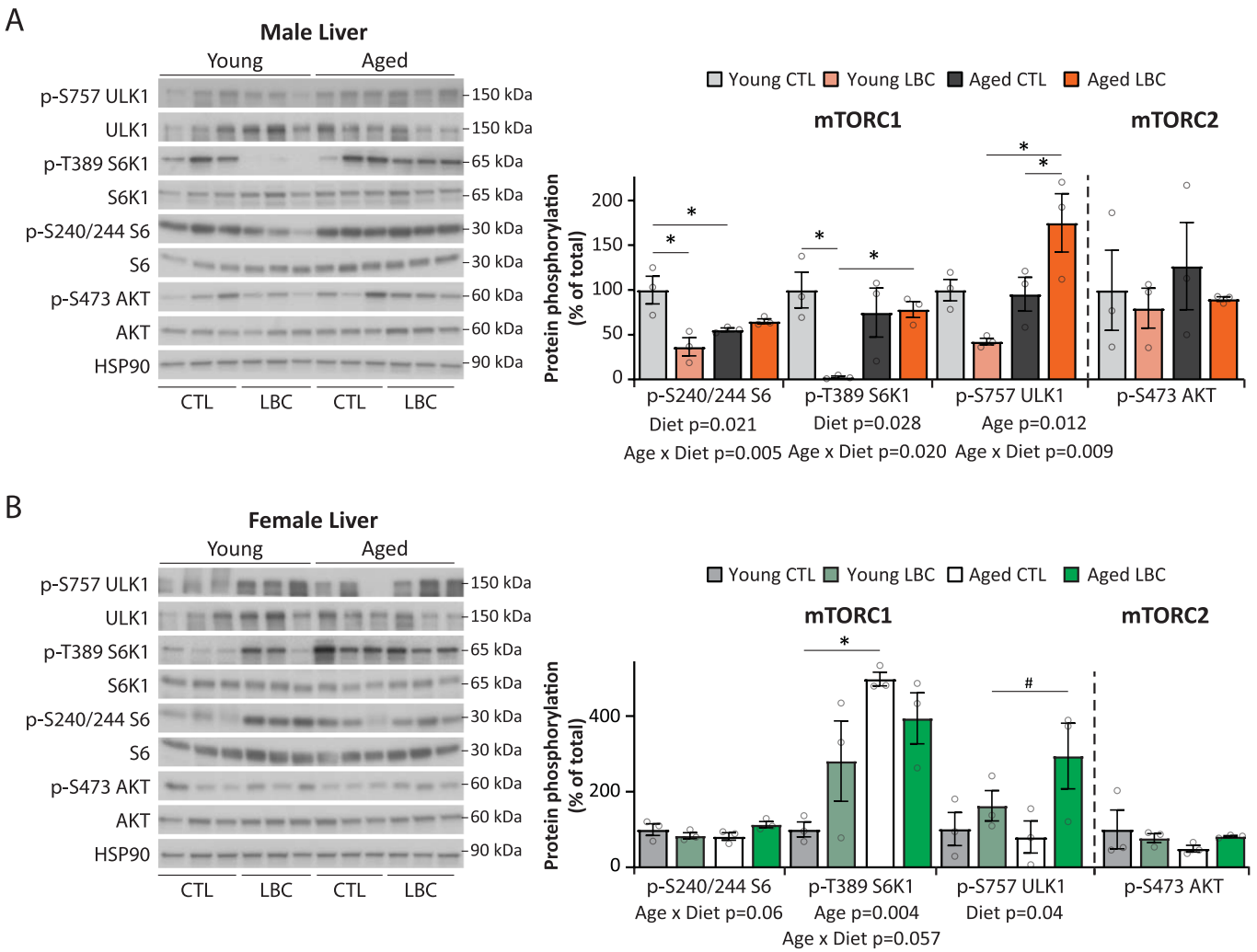


Extended Data Fig. 4 | See next page for caption.

Extended Data Fig. 4 | Transcriptional profiling of skeletal muscle. Transcriptional profiling was performed on mRNA from the skeletal muscle of male and female mice that consumed either Control or Low BCAA diets from weaning until 16 months of age (N = 6 biologically independent animals for all groups; Supplementary Tables 13 and 14). **a**, Workflow from raw RNA sequencing reads through data analysis and data representation. **b-c**, Principal component analysis for **b**, Control and **c**, Low BCAA fed groups. **d**, Heatmaps of differentially expressed genes by mouse from significant KEGG over-representation analysis (ORA) pathways of interest identified in Supplementary Tables 13b and 14b. DEGs were identified using an empirical Bayes moderated linear model. *Two-sided *P* values adjusted with the Benjamini–Hochberg procedure ORA was performed on DEGs (designated by an adjusted *P* value of 0.3 for female and 0.2 for male contrasts) using a one-sided hypergeometric test, and P values were adjusted using the Benjamini–Hochberg procedure.



Extended Data Fig. 5 | A Low BCAA diet reduces mTORC1 activity in male, but not female, muscle. a-b, mTORC1 activity determined by Western blotting and quantification of muscle tissue lysates from male and female mice. Young (12 months females; 15 months males) and aged (22 months females; 25 months males) mice were fed either a Control or Low BCAA diets from 6.5 months of age for young and 16 months of age for aged mice, then sacrificed following an overnight fast followed by 4 hours of refeeding. **a**, Male and **b**, Female muscle. Quantification was by ImageJ (N = 3 biologically independent animals for all groups). **a-b**, * $p < 0.05$ (p-values for (**a**); pS6/S6, Young CTL vs. Young LBC = 0.0025; Young CTL vs. Aged CTL = 0.0327; pS6K1/S6K1, Aged CTL vs. Aged LBC = 0.0254). Statistics for the overall effects of diet, age and the interaction represent the p value from a two-way repeated measures ANOVA, multiple comparisons by two-sided Sidak's post-test. Full scans of the cropped western blots shown here are provided as Source Data files. CTL = Control, LBC = Low BCAA. Data are represented as mean ± SEM.



Extended Data Fig. 6 | A Low BCAA diet reduces mTORC1 signaling in the liver of male mice. a-b, mTORC1 activity determined by Western blotting and quantification of liver tissue lysates from male and female mice. Young (12 months females; 15 months males) and aged (22 months females; 25 months males) mice were fed either a Control or Low BCAA diets from 6.5 months of age for young and 16 months of age for aged mice, then sacrificed following an overnight fast followed by 4 hours of refeeding. **a**, Male and **b**, female liver. Quantification was by ImageJ (N = 3 biologically independent animals for all groups). **a**, * $p < 0.05$ (pS6/S6, Young CTL vs. Young LBC = 0.0028, Young CTL vs. Aged CTL = 0.021; pS6K1/S6K1, Young CTL vs. Young LBC = 0.0086, Young LBC vs. Aged LBC = 0.0309; pULK1/ULK1, Aged CTL vs. Aged LBC = 0.0432, Young LBC vs. Aged LBC = 0.003). **b**, * p = 0.0047, # = 0.056. Statistics for the overall effects of diet, age and the interaction represent the p value from a two-way repeated measures ANOVA, multiple comparisons by two-sided Sidak's post-test. Full scans of the cropped western blots shown here are provided as Source Data files. CTL = Control, LBC = Low BCAA. Data are represented as mean ± SEM.

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☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
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☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
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☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used except for metabolic chambers (Oxymax v. 5.47 software from Columbus Instruments).
Data analysis	Data in this paper was analysed using R (R version 3.6.1), Graphpad Prism 8, NIH Image J 1.50i

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
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RNA-seq data have been deposited with the Gene Expression Omnibus and are accessible through accession number GSE155064. The data that support the plots within this article and other findings of this study are available from the corresponding author upon reasonable request. Full scans of western blot images are provided as Source Data.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was determined based on our previous experimental results with the effects of Low BCAA diet feeding, as published in Fontana and Cummings et al, 2016, Cell Reports. For in vivo experiments, we utilized power calculations (> 90% power to detect a change in area under the curve during a glucose tolerance test, $p < 0.05$) as based on the results of our previously published research.
Data exclusions	Data was not excluded.
Replication	The assays were chosen based on the evaluation of a number of criteria including good reproducibility, and are widely utilized by multiple groups. The Control AA and Low AA diets successfully reproduce results found in our previous studies as well as those from other laboratories. All data derived from animal experiments represent the results obtained from at least three biological independent animals. As these were long-term longitudinal studies, there were no attempts at replication, but animals were assessed for many phenotypes at multiple time points and these assessments are included in the manuscript.
Randomization	Middle-aged animals obtained from the NIA were randomized into groups of equivalent weight prior to the beginning of the in vivo studies; all other animals, which were bred in-house, were randomized by birth order. Mice removed from the lifespan studies for cross-sectional studies were selected from a database without information about the health status of the mice as they reached the age previously chosen for phenotyping and there was availability at the cardiac phenotyping core. Young animals removed from the lifespan study were removed at the cage level without assessing health status of individual mice.
Blinding	Investigators were blinded to diet groups during data collection whenever feasible, but this was not usually possible or feasible as as cages were clearly marked to indicate the diet provided, diets were color-coded to prevent feeding mistakes, and the size and body composition of the mice was altered by genotype and diet. However, blinding is not relevant to the majority of the studies conducted here, as the data is collected in numeric form which is not readily subject to bias due to the need for subjective interpretation. Investigators were not blinded during necropsies.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Antibodies to pT389-S6K (9234), 56K (9202), p5240/244-S6 (2215), S6 (2217), p-AKT S473 (4060), AKT (4691), p5757-ULK1 (6888), ULKl (8054), and HSP90 (4877) were purchased from Cell Signaling Technologies (CST, Danvers, MA, USA).
Validation	All antibodies were validated for use in mice by the manufacturer and validation descriptions are available on the CST website https://www.cellsignal.com/. Specificity of antibodies has also been validated by the authors in previous publications, including Arriola Apelo et al, 2016, Aging Cell; Lamming et al, 2014, Aging Cell; Baar et al, 2016, Aging Cell.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	C57BL/6J female and male mice at ages ranging from birth to approximately 4 years of age were used, as well as Lmna-/- and Lmna G609G/G609g mice of both sexes between birth to approximately 1.5 years of age were used.
Wild animals	No wild animals were used in the study.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	Animal studies were approved by the Institutional Animal Care and Use Committee of the William S. Middleton Memorial Veterans Hospital, Madison, WI.

Note that full information on the approval of the study protocol must also be provided in the manuscript.