How lifespan and body weight vary as a function of diet and genetic differences is not well understood. Here we quantify the impact of differences in diet on lifespan in a genetically diverse family of female mice, split into matched isogenic cohorts fed a low-fat chow diet (CD, n = 663) or a high-fat diet (HFD, n = 685). We further generate key metabolic data in a parallel cohort euthanized at four time points. HFD feeding shortens lifespan by 12%: equivalent to a decade in humans. Initial body weight and early weight gains account for longevity differences of roughly 4–6 days per gram. At 500 days, animals on a HFD typically gain four times as much weight as control, but variation in weight gain does not correlate with lifespan. Classic serum metabolites, often regarded as health biomarkers, are not necessarily strong predictors of longevity. Our data indicate that responses to a HFD are substantially modulated by gene-by-environment interactions, highlighting the importance of genetic variation in making accurate individualized dietary recommendations.
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Fig. 1 | Study overview. a, Balanced sets of females from 73 BXD strains and their parents were assigned to the low-fat chow diet (CD) or the high-fat diet (HFD) and weighed every other month. b, HFD modulates lifespan. When grouped by diet—independent of genetic background—median lifespan of CD (n = 663) cohort exceeded by 77 days that of the HFD (n = 685) cohort (see inset for a box plot that spans from the 25th to 75th percentile, with the centerline at the median and the whiskers extending to maximum and minimum data points that are no more than 1.5 times the length of the box away from the box). Red and blue dots represent individuals on CD and HFD, respectively. For strain averages of lifespan in the two cohorts, see Extended Data Fig. 1a,b.

phenomes across multiple environments—what we refer to as a family polyphenome.

In this study, we have measured lifespan, body weight, weight gain and selected metabolites across balanced cohorts of up to 73 fully sequenced BXD family members and their progenitors (C57BL/6, DBA/2, and their F1 progeny D2B6F1). We studied cases and controls on diets that differed greatly in fat content—controls on a standard low-fat chow diet (CD, 17% of calories from fat) and cases on a very high fat diet (HFD, 60% calories from fat). This study involved a large and very rigorously controlled GXE experiment on the effects of diet on lifespan and weight change.

Here we address a set of questions about the impact of diet on lifespan across highly diverse genotypes of mice. How does a HFD modulate variability in lifespan within and between strains? Put more simply: what is the strength of evidence in favour of GXE effects on lifespan? We ask if youthful adult body weight (roughly 120 days) predicts lifespan. Is the change in body weight in adults in response to a HFD a causal predictor of lifespan? Finally, we ask whether levels of classic serum metabolites or metabolic hormones in response to a HFD a causal predictor of lifespan? Finally, we ask whether levels of classic serum metabolites or metabolic hormones measured in middle-age or old-age predict variation in lifespan? Our focus is both on overall effects and on strain-specific difference in effect of diet on lifespan and weight gain, rather than on specific genetic modifiers or loci of lifespan.

## Results

### HFD shortens lifespan with wide strain variation

Balanced sets of females from 73 BXD strains and their parents were assigned to either CD (n = 663) or HFD (n = 685) at an average of 120 days of age, weighed every other month and followed until natural death or extreme morbidity (Fig. 1a). The HFD has a strong average effect on lifespan across the family. Mean lifespan decreased significantly (P < 6.6 × 10⁻²¹, CD:HFD r = 0.55) from 690 ± 8 s.e.m. (±199 s.d.) days on the control diet to 605 ± 6 s.e.m. (±169 s.d.) days on the HFD. Median longevity decreased 77 days—from 703 to 626 (Fig. 1b, box plot inset). The 75% quantile change in longevity decreased from 796 to 699 days. Assuming linear scaling and that a 77- to 97-day difference would compare roughly to a 8–10-year loss of longevity in humans.

Using a mixed-effects Cox model with diet as a fixed effect and strain as a random effect, we estimated a hazard ratio of 2.0, indicating that animals on HFD have a twofold higher age-adjusted risk of death compared to their matched CD-fed controls (Fig. 2a). The hazard ratio is relatively constant throughout the study and there is no crossing of the cumulative hazard curves (Fig. 2b).

While HFD decreased lifespan at the family level, individual strains differed greatly in how they reacted to diet (Extended Data Fig. 1a,b). The parents exemplified this marked difference—the lifespan of the DBA/2 paternal strain was unaffected by diet, whereas the C57BL/6J maternal strain lost 76 days on the HFD (Fig. 2c). In fact, some progeny lived even longer on the HFD, demonstrating that the longevity–diet relation was modulated by a forceful GXE component (Fig. 2d). Overall, lifespan in 21 out of 67 strains (those with four or more natural deaths in both cohorts) was significantly affected by the HFD at a nominal P threshold of 0.05. To correct for multiple testing (n = 67), we computed q values (Fig. 2d), and with this correction only 15 strains had significantly different lifespans on the two diets. BXD8 had a significantly longer lifespan on the HFD, with a median increase of 208 days (t = 4.0, P = 0.0052, q < 0.05, two-tailed). One other strain also trended in the same direction—BXD172 (146-day increase on HFD, P = 0.073), but with a high q value of 0.85. As expected, the HFD reduced longevity for most strains (Fig. 2d, right side)—most prominently for BXD65, with a decrease in median longevity of nearly a year (345 days, t = 9.3, P = 9.0 × 10⁻², q = 6.0 × 10⁻²). Of interest, this strain ages most rapidly based on its epigenetic clock on a HFD. Adjusting for strain differences, mean lifespan is decreased by 89 ± 14 s.e.m. days on the HFD (P < 0.0001). Genetic variation explained 30% of the total variance in lifespan whereas diet accounted for 5%, and genetics-by-dietary interaction accounted for 6%.

### Modulation of variability in lifespan by HFD

We computed coefficients of variation (CV) (s.d. divided by mean) for all strains in the two cohorts with sample sizes of greater than six cases (GeneNetwork (GN) traits BXD_21533 and BXD_21534). The mean CV on the control diet is 0.27 ± 0.015 s.e.m. (n = 52) compared to 0.25 ± 0.013 s.e.m. on HFD (n = 50)—a small drop in CV on the HFD, but not significant. Using a random-effects model, we estimated the lnCV ratio between CD and HFD to be 0.0757 (s.e.m. 0.0653); the estimate of between-strain heterogeneity (F) was 48.2%. The CVs of inbred strains are slightly higher on average than those of outbred female mice of about 0.21 (Miller et al., 2012, GN2 Trait ITP_10001).

Youthful adult body weight is a strong predictor of lifespan. Individuals were weighed bi-monthly intervals throughout their lifespan. As expected, those on HFD typically gained far more weight than those on CD (Fig. 3a). Initial weights recorded at point of entry into the ageing colony at about 120 days of age had a significant influence on lifespan after adjusting for differences in age at start of the HFD (P = 0.0006, r = −0.5) (Fig. 3b). A 1g increase in
Age at death (days)

Diet significantly alters lifespan, not weight gain per se. We chose to focus on two time points for body weight analyses—100 days on the diet as a point to evaluate early weight gain on HFD, and 400 days on the diet, a stage that is close to the maximal weight on both diets. The mean weight of the population plateaus around 500 days of age and declines thereafter on both diets. By 500 days of age, cases had been on HFD for 400 ± 44 days and gained an average of 29.5 g. Those on the CD gained only 6.2 g (mean weight on CD, 29.7 ± 0.35 s.e.m. g, n = 447; mean weight on HFD, 52.6 ± 0.63 s.e.m. g, n = 447).

However, the substantial increase in body weight on the HFD for 400 days is not significantly associated with decrease in lifespan (Fig. 3e). Only 10% of the effect of diet on lifespan is mediated through body weight gain after adjusting for strain-specific differences in lifespan. Mirroring this interesting observation, sustained weight gain after prolonged feeding of HFD (Fig. 3f) has no predictive value, emphasizing that the diet itself, rather than weight gain per se, modulates lifespan. Adiposity in harvested females on both diets (n CD = 309, n HFD = 292, 47 BXD strains), calculated from adipose tissue weights (subcutaneous, perirenal and perigonadal fat pads) as a percentage of body weight, was significantly increased on high-fat feeding (mean CD 4.4 ± 0.22, HFD 14.4 ± 0.41, P < 2.2 × 10⁻¹⁶ Welch’s t-test). The increased adiposity in HFD-fed animals showed a moderate negative association with lifespan (P=0.03, r=-0.39).

Further, we examined the difference between subcutaneous and visceral adiposity (calculated from perirenal and perigonadal adipose tissue weights), which are known to exhibit different intrinsic properties that make visceral fat a more pathogenic depot with greater impact on metabolic health than subcutaneous fat⁴⁷,⁴⁸. In our HFD cohort, visceral adiposity tended to have a negative impact on lifespan (P=0.09). Overall, 53 out of 67 strains had significant weight gain on the HFD after 100 days (P<0.05, q<0.1, t values ranging from −3.03 to −13.43) (Fig. 3g). At 500 days of age, 45 out of 57 strains gained significant weight on the HFD over 400 days (P<0.05, q<0.1, t values ranging from −3.03 to −13.14) (Extended Data
Fig. 3 | Effect of body weight on lifespan. Effects of body weight on lifespan across all strains were analysed using fixed-effects linear model in R. Correlations are Pearson’s r. a. Body weight (BW) by diets and age (CD, red line and HFD, blue line). Data are presented as mean ± s.e.m. Single and double asterisks denote significance at \( P < 0.05 \) and \( < 0.001 \). Body weight declines on both diets after about 500 days of age. b. Initial body weight—the weight taken at entry into colony before the point of any animal being shifted to the HFD—has a modest but consistent negative slope with lifespan (−6 d per g, \( P = 0.0006, r = 0.1 \)) that is not exacerbated by the HFD (n = 659 on CD, 685 on HFD). c. Body weight after 100 days on both diets (roughly 260 d of age) correlates negatively with lifespan (−4 d per g, \( P < 0.001, r = 0.3 \), see the line labelled c in a) (n = 626 on CD, 665 on HFD). d. Early weight change in response to HFD (blue line)—the difference from baseline after 100 days on the diet—was negatively related with lifespan (−4 d per g, \( P = 0.004, r = 0.1 \)), but this is not true of cases remaining on CD. e. After 400 days on diet (roughly 500 d of age), body weight does not predict variance in lifespan (see the line labelled d in a) (\( P = 0.63, r = 0.01 \) (n = 447 on CD and HFD)). f. Substantial weight change after prolonged HFD feeding—difference from baseline to 400 days on diet (blue line)—does not predict lifespan (\( P = 0.26, r = 0.02 \)). g. Strain-wise changes in median weight after 100 days on diets. Red points represent lifespan of cases on CD and blue points those on HFD. Lines represent median body weight (left y axis). Grey bars represent differences in median body weight on the diets (right y axis). Parental strains and F1 hybrid are denoted by bold font. Strain averages of body weight at 500 days of age in the two cohorts are shown in Extended Data Fig. 1c,d.

Fig. 1c,d). Diet had a substantial impact on body weight accounting for 45% of variance in body weight versus 19% explained by genotypetype, 10% by gene–diet interaction and 25% as unexplained residual.

Association of lifespan with serum metabolites and hormones. In a separate subsample of animals on both diets harvested at approximately 6, 12, 18, and 24 months of age, the HFD elevated circulating levels of glucose, cholesterol, and triglycerides (n = 255 CD, 254 HFD, ~30 strains). Glucose was elevated from 10.34 ± 0.89 on the CD to 11.54 ± 0.22 mmol/L on the HFD (p < 4.77E−5 Welch’s t-test, see GN traits BXD_21607 and BXD_21608). Total cholesterol climbed from 1.7 ± 0.46 to 2.4 ± 0.06 mmol/L (p < 2.2E−16 Welch’s...
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Serum glucose and cholesterol levels increased. Glucose (\(P = 0.04\) Welch's t-test, BXD_24379 and BXD_24380, Fig. 4a–d).

In contrast, levels of free fatty acids did not change significantly.

Glucose (\(P < 0.0001\), \(r = -0.28\)) and total cholesterol (\(P = 0.0077\), \(r = -0.17\)) showed a weak negative association with age at death, on the HFD. Almost all strains trended towards a shorter lifespan as serum glucose and cholesterol levels increased. Glucose (\(P = 0.03\), \(r = 0.29\)) and total cholesterol (\(P = 0.07\), \(r = 0.44\)) showed a moderate positive association with final body weight.

In another subsample of animals on both diets (\(n = 312\), \(n = 302\) on HFD = 302, roughly 50 BXD strains), collected at 6, 12, 18 and 24 months, serum insulin levels were significantly higher on a HFD with mean values of CD 7.03 ± 0.04 pg ml\(^{-1}\), HFD value of 7.56 ± 0.05 pg ml\(^{-1}\), \(P = 1.051\times 10^{-14}\) Welch's t-test (GN trait BDL_10055). Homeostatic model assessment-insulin resistance (HOMA-IR), a measure of insulin sensitivity, was also significantly higher in HFD-fed mice compared to CD (2.195 versus 2.839 log units, \(P < 2.2 \times 10^{-16}\) Welch's t-test).

Among other metabolites analysed, the HFD significantly elevated the circulating levels of leptin with a mean CD value of 7.52 ± 0.08 pg ml\(^{-1}\), HFD value of 8.82 ± 0.07 pg ml\(^{-1}\), \(P < 2.2 \times 10^{-16}\) Welch's t-test (GN trait BDL_10059) and C peptide with mean CD value of 5.94 ± 0.05 pg ml\(^{-1}\), HFD value of 6.56 ± 0.06 pg ml\(^{-1}\), \(P = 9.1 \times 10^{-14}\) Welch's t-test (GN trait BDL_10056) (Fig. 5a–d).

Insulin (\(P = 0.0004\), \(r = 0.49\)), leptin (\(P = 0.01\), \(r = 0.36\)) and HOMA-IR (\(P = 0.03\), \(r = 0.40\)) showed a moderate positive association with final body weight on the HFD. In contrast, only HOMA-IR (\(P = 0.07\), \(r = -0.19\)) displayed a weak negative association with age at death on the HFD.

We learned a consensus Bayesian network model that links serum metabolites to both body weight at 500 days and to lifespan. We tested several versions of these causal models, grouping strains by diet or by the age at which serum metabolites were measured. Here we define two age groups—between 6 and 12 months of age (middle-aged) or between 18 and 24 months of age (old-aged). Our models included up to six metabolites (Methods) as predictors of lifespan—(1) serum glucose, (2) total cholesterol, (3) triglycerides, (4) high-density lipoprotein cholesterol, (5) low-density lipoprotein cholesterol and (6) free fatty acids. The consensus model highlighted a potential causal effect of diet on peak body weight measured relatively late in life (500 days), acting through circulating levels of total and high-density lipoprotein cholesterol measured in the old-age group (Extended Data Fig. 2). The Bayesian network analysis, as we structured it, failed to show any causality between serum metabolites and variability in lifespan.

Association between lifespan and metabolic organ weights. We measured weight of certain metabolic organs and tissues of a subsample of cases on both diets at roughly 500 days of age. HFD mice (\(n = 63\)) had 84% greater fat mass, 25% greater heart mass, 19% greater liver mass and 18% greater kidney mass at roughly 500 days compared to controls (\(n = 71\)). However, HFD did not influence brain mass (Supplementary Table 1). The correlation between lifespan and organ weights at 18 months is not significant, except for liver weight on HFD, which shows a weak negative association with lifespan (\(P = 0.03\), \(r = -0.1\)).

Major morbidities contributing to death in the ageing colony. We carried out gross necropsies, with or without histopathology, for a total of 155 individuals. Seventy-six CD cases from 45 strains had a single cause of morbidity; the remaining seven had multiple likely causes of death. Similarly, 71 HFD cases from 43 strains had a single cause of morbidity; the remaining eight had multiple causes. The likely cause of death was clear at necropsy or following histopathology in 87% of cases. Haematopoietic neoplasias—lymphomas and histiocytic sarcomas—were leading causes of death, accounting for roughly 35% on both diets. We also detected miscellaneous non-neoplastic conditions in 24% of CD- and 32% of HFD-fed animals.

Diet appeared to modulate causes of morbidity and mortality (Table 1). In the HFD cohort there was a higher prevalence and severity of cardiovascular disease and lesions, including aortic stenosis, myocardiumyopathy and cardiac dilation sometimes with hepatic steatosis and cerebrovascular atrophy than in the CD cohort. Nineteen percent of these cases (15 out of 79) had heart pathologies that we rated moderate to severe. In contrast, only a single CD cases had a heart lesion rated at least moderately severe (two-tail Fisher's exact test \(P = 0.0003\)). Four HFD cases, but no CD cases, had systemic polyarteritis. Conversely, some pathologies were evidently more pervasive in the CD cohort. Thirty-six percent displayed non-haematopoietic malignant neoplasia (27 of 76 CD—11 sarcomas, 15 carcinomas and one teratoma) compared to 13% HFD cases with non-haematopoietic malignancy (ten out of 79 HFD, six sarcomas and four carcinomas). While this contrast is nominally significant (Fisher \(P = 0.0012\)), we have not corrected for multiple comparisons.

Discussion We have studied effects of a HFD on lifespan in a large and genetically diverse family of mice. In the introduction, we pose three sets
of questions for which we now have good answers. A diet that is high in fat reduces longevity by an average of 12% in female members of the BXD family, and as in humans, the risk of cardiovascular disease is elevated on the HFD. However, this is not a universal response. Differences among genomes and GXE effects are strong for both lifespan and weight gain. Even after we correct for multiple comparisons, one strain lives significantly longer and another strain gains no weight on the HFD. We confirm that lower weight at an early age is linked to longer lifespan, and that this effect is also true on a HFD. There is at best only a modest association between weight gain after maturity and lifespan. Weight gain measured later in life—between ages of 12–18 months—accounts for minimal variation in lifespan. Diet modulates lifespan and has a stronger effect than weight gain per se. Key serum metabolites, including glucose and total cholesterol exhibit a modest negative association with lifespan. Similar effects are seen with key serum hormones such as insulin and leptin. While these specific biochemical assays can be linked causally to body weight, they cannot be linked causally to lifespan. By far the strongest effect on lifespan and weight gain is genometype.

In the interest of avoiding overly broad generalization from this study of GXE effects, we highlight three limitations. First, we only studied females—the main reason being that unlike males, they can be housed without overt and detrimental aggression. Males generally do not live as long as females, and in the large NIA Interventional Testing Program the overall difference is 10%: 808 ± 2.88 days for males (n = 7147) and 894 ± 2.43 days for females (n = 6139) (GeneNetwork Trait ITP_10001). Of even greater practical importance to us, variability of lifespan is much higher in males than females—a CV of 0.30 versus 0.21. As a result, studies of lifespan in females are statistically twice as efficient as those in males—the ratio of the squares of CVs22,30. At this point we can only presume that the same GXE effects of diet are likely to apply to males22,46. Second, we have studied only one dietary contrast. Each diet has the potential to reveal new GXE effects as a function of macro- and micronutrients12. The dietary difference in this study unequivocally causes differences in weight gain and lifespan as a function of genotypetotype (more correctly, genometype), but we are cautious in terms of attributing effects strictly to fat content. Differences in fibre content—150 versus 66 g kg−1 on CD and HFD, respectively—and other differences in cholesterol and micronutrients are also likely to contribute31. Third, 75 of 76 genotypes of mice we have studied here—all except D2B6F1 hybrids—are fully inbred individuals of the type now used pervasively in biomedical research. One might expect this unusual genetic architecture to have both generally deleterious effects on lifespan, and possibly in higher variability within and between strains than in outbred stock such as used in the NIA ITP program. This last caveat is discussed in more detail below.

A HFD decreases lifespan by an average of almost three months across BXD females, roughly scaling to a decade decrease in humans. The HFD is associated with an average twofold higher age-adjusted risk of death compared to the control CD. Lifespan under the two diets correlates moderately well (r = 0.55, GN traits BXD_18435 and 18441). However, the strains of mice display wide variation in responses to diet, and despite the strong effect, diet only accounts for 5% of the total variance in lifespan. In comparison, strain as a factor accounts for 30% of variance. Combined across the two diets, lifespan varies from a low of 307 ± 37 days in BXD13 (n = 21) to a high of 852 ± 33 days in BXD168 (n = 23). Some strains are fully resistant to the negative effects of HFD on body weight and lifespan while others are strongly affected. While mean lifespan is shortened by an average of 10% on a HFD, genetic factors account for roughly a twofold range. At least one strain—BXD8—actually lives significantly longer on the HFD (+37%). Lifespans of other strains, including BXD16 and BXD73, are apparently unaffected by the HFD challenge. Of course, lifespan of most strains is adversely affected (n = 67), and in the case of BXD65 is cut in half. Weight gain is also characterized by a forceful GXE effect—at least four BXD family members are resistant to weight gain, including BXD16, BXD77, BXD87 and BXD91, gaining at most 5% over 100 days of the HFD. There is a mild positive correlation (r = 0.20, n = 63 strains) between weight change after 100 days on the HFD and median lifespan differences (control minus HFD). This again indicates that that weight gain accounts for only 4–5% of the change in lifespan.

Our findings can be compared to strain variation and GXE effects in response to dietary restriction. Dietary restriction without malnutrition is regarded as having an almost universal benefit on longevity22–34. One exception is a pair of studies on the impact of moderately intense restriction—a 40% reduction in caloric intake—across a large family of LXS strains of mice (n of up 44 strains with 10–20 replicates per strain)17,18. Their most notable finding was high variation in strain-specific changes in lifespan—shortened in some strains by up to 671 days, but lengthened in others by up to 300 days (GN trait LXS_10164). Both the Liao and Rikke papers were controversial18, but this puzzle is mirrored to some extent in similar studies of non-human primates35. Given these contrasts in outcome, it would be worthwhile extending the analysis of HFD to dietary restriction and other modifications in the expanded BXD family (now 150 strains19). Given what we now know of the precise quantitative effects of one dietary intervention on lifespan, with what level of accuracy can we predict the impact of other interventions?

Variations in lipid metabolism mediates differences in lifespan acting via different dietary, genetic, pharmacological and surgical interventions in model organisms37. Age-related changes in glucose

**Fig. 5** | Diet effect on serum metabolic hormones. a–d, Violin plots of insulin (a), HOMA-IR (b), leptin (c) and C peptide (d) on CD (n = 312) and HFD (n = 302), across roughly 50 BXD strains. HOMA-IR was calculated as fasting serum insulin (uIU ml−1) multiplied by fasting glucose (mg dl−1). See inset for a box plot that spans from 25th to 75th percentile, with centerline at median and whiskers extending to maximum and minimum data points that are no more than 1.5 times the length of the box away from the box. HFD significantly elevated circulating levels of serum insulin (P = 1.051 × 10−14), HOMA-IR (P < 2.2 × 10−16), leptin (P < 2.2 × 10−16) and C peptide (P = 9.1 × 10−14). Statistical significance was determined by a two-sided Welch’s t-test.
and fat metabolism have been quantified extensively under tightly controlled experimental conditions using young (3 months) and old (22 months) C57BL/6J males on the CD [74-76]. We have extended this work in the BXD family using high-fat-fed females and by evaluating causal links between serum metabolites and metabolic hormones and lifespan and body weight. The HFD cohort had increased adiposity and higher concentrations of serum metabolites including serum glucose, total cholesterol and triglycerides, with weak negative associations to age at death. All three metabolites are linked to cardiovascular disease [46] and diabetic cardiomyopathy in mice [77]. The HFD cohort had significantly higher serum insulin levels coupled with increased insulin resistance as compared to the CD cohort, with moderate positive associations to final body weight but a much weaker negative association with age at death. Previous work has shown that mice with reduced circulating insulin levels have improved age-dependent insulin sensitivity and metabolic homeostasis, extending lifespan [9,48]. While the role of insulin levels and resistance on health is experimentally well supported and evolutionarily conserved, our results suggest that the linkage to lifespan is complex and surprisingly modest in this family of female mice. There is also evidence that insulin sensitivity and longevity may belong to different causal pathways that are not interconnected [79].

We used Bayesian causal modelling to formulate and test networks that link the diet intervention to key serum metabolites and to two outcome measures—body weight at 500 days and lifespan. The best model indicates that circulating levels of high-density lipoprotein and total cholesterol measured at the older ages (18-24 months) mediate variation in peak body weight measured at 500 days of age. In contrast, the Bayesian causal analysis does not define any similar mediating effects on lifespan for any combination of the six metabolites. We therefore cannot find any support that these common metabolites predict lifespan alone or in combination. A possible reason could be that the Bayesian networks that we explored did not incorporate strain genotypes.

Recent observational studies using Mendelian randomization emphasize the difficulty in evaluating causal links between lipid metabolites and human cardiometabolic diseases [41]. In the cases of low-density lipoprotein cholesterol and triglycerides, the link to coronary heart disease risk was reconfirmed, whereas in the case of high-density lipoprotein cholesterol, a causal link could not be established [46]. Even in elderly populations there is no compelling causality linking triglyceride levels to longevity [90]. Similarly, there is no causal link between C-reactive protein levels and risk of coronary artery disease despite evidence that inflammation plays a key role in the pathogenesis of coronary heart disease [79].

Major morbidities and likely causes of death among different members of the BXD family are influenced by diet. Females on the HFD have an increased prevalence and severity of cardiovascular disease and lesions. However, the effects of a HFD on cardiovascular disease incidence is modest in mice, unlike that observed in human populations [46,90]. The incidence of sarcomas and carcinomas in the BXD females are higher on the CD than HFD. Large prospective studies of humans have failed to detect strong associations between dietary fat and cancer risk [91]. The impact of dietary fat on increasing cancer risk depends on tumour type, location and context, making it difficult to glean any conclusive data in the setting of large trials. Combining data from prospective cohort trials with mechanistic studies in mice reveals that certain tumours such as prostate cancer respond directly to dietary fat, while breast cancer is affected indirectly by the resulting obesity and related complications such as inflammation and insulin resistance [79]. Evaluating a causal role of diet-induced obesity in the aetiology of several chronic diseases and cancers has been difficult due to correlations with numerous lifestyle factors and resulting confounding biases. Obesity dramatically modifies the adipose tissue microenvironment, which may present a hospitable environment to adjacent developing tumours and facilitate metastatic progression [92]. Mendelian randomization provides a practical way to assess causal links between risk factors and diseases [93]. Well-controlled animal experiments can similarly provide additional understanding of causes and mediators underlying these complex relations, while also enabling well-controlled interventions of specific replicable genotypes.

Chronically high levels of fat consumption lead to substantial weight gain, associated metabolic disorders and shortened lifespan in humans and mice, but causal interactions among these critical variables remain controversial. In general, a diet high in fat leads to obesity and reduced lifespan in diverse species including Drosophila, Caenorhabditis elegans and mice [46,79]. In our study, higher youthful body weight—between 100 to 200 days of age—is associated with a reduction in lifespan of roughly 4 days per gram. Higher body weight in young adulthood has been associated with accelerated epigenetic ageing in a separate subsample of BXDs on both diets [46]. This corroborates much previous work that demonstrates that larger body size within a species is generally associated with a shorter lifespan. For example, in outcrossed mice, for each gram increase in weight at 180 days, lifespan is reduced by 10 to 15 days (their table 1). Among breeds of dog, for each kilogram increase in weight, lifespan is reduced by roughly 15 days [49-52]. In young adult and middle-aged humans, for each kilogram increase in body weight, lifespan is reduced by 80 to 146 days (their fig. 6). In these three species, a 5% gain in young adult body weight is associated with a 1-3% loss in lifespan. In both mice and dogs, the relation between early weight gain and longevity is in part a well-known function of growth hormone (GH)–insulin-like growth factor 1 (IGF1) activity, although other mechanisms and gene variants are likely to

Table 1 | Major morbidities contributing to death in BXD family

| Morbidity type       | Diet   | n with single morbidity | n with multiple morbidities | P value |
|----------------------|--------|------------------------|-----------------------------|---------|
| Unnatural causes     | CD     | 7                      | 1                           | 0.1257  |
|                      | HFD    | 3                      | 0                           |         |
| Non-neoplastic       | CD     | 16                     | 2                           | 0.3715  |
|                      | HFD    | 22                     | 2                           |         |
| Heart/cardiovascular | CD     | 0                      | 1                           | 0.0003* |
|                      | HFD    | 8                      | 7                           |         |
| Polyaetoritis (autoimmune) | CD    | 0                      | 0                           | 0.1204  |
|                      | HFD    | 3                      | 3                           |         |
| Lymphoma             | CD     | 25                     | 3                           | 0.7402  |
|                      | HFD    | 26                     | 1                           |         |
| Sarcoma              | CD     | 9                      | 2                           | 0.2042  |
|                      | HFD    | 4                      | 2                           |         |
| Carcinoma            | CD     | 12                     | 3                           | 0.0065* |
|                      | HFD    | 4                      | 0                           |         |
| Renal                | CD     | 0                      | 1                           | 0.3673  |
|                      | HFD    | 1                      | 3                           |         |
| Teratoma             | CD     | 0                      | 1                           | 0.4903  |
|                      | HFD    | 0                      | 0                           |         |

*Significant with Bonferroni correction for multiple tests. **Significant at nominal alpha of 0.05.

Unnatural causes refer to a flooded cage, foot injury in wire lid, eye abrasion leading to euthanasia and others. Miscellaneous refers to non-neoplastic conditions such as inflammation, infection, amyloidosi and ulcerative dermatitis. Heart refers to a major contribution of cardiovascular failure other than polyarteritis. Polyarteritis refers to systemic multiforme arteritis suggestive of autoimmune disease. Lymphoma refers to haematopoetic neoplasia including lymphoma and histiocytic sarcoma. Sarcoma refers to non-histioptic sarcoma, spindle cell, hemangiosarcoma, leiomyosarcoma, sarcoma NOS. Carcinoma refers to carcinomas (hepatocellular, squamous cell). Renal refers to renal failure due to severe nephropathy.
contribute. In humans the causes of this relation are probably a result of an interplay of many factors, especially nutritional composition, smoking, mean activity levels and health care systems. For example, tall stature protects against cardiovascular disease due to lower levels of adiposity, lipid fractions, blood pressure and better lung function\(^a\). The linkage of longevity to IGF1 levels is also more tenuous in humans than in either mice or dogs\(^b\), although short stature and lower GH/IGF1 signalling is associated with resistance to most types of cancer\(^c\).

In humans, an increase in body mass index from 27 to 42 kg m\(^{-2}\) increases all-cause mortality hazard ratio 1.6-fold\(^d\). We see an even greater effect in the BXD family—the HFD diet increases weight 1.8-fold and the mortality hazard ratio twofold. But these averages mask impressive modulation by genetic differences. While 45 of 57 family members gain significant weight, another 12 gain only a modest and statistically insignificant weight. Even after 400 days on the HFD, BXD16 is only 1.05-fold heavier than control, whereas BXD24 is 2.1-fold heavier. Only 10% of the effect of diet on lifespan is mediated through weight gain. HFD itself exerts a stronger direct effect on lifespan in BXDs than weight gain per se.

**Future directions**

Impressive GXE differences among BXD family members emphasize the complexity of interactions among diet, weight gain and lifespan. These effects need to be teased apart and then also reassembled and explained at genetic, epigenetic, phenome and even polyphenome levels—work that is now in progress using the same genometypes and cohorts\(^e\).\(^f\)\(^g\)\(^h\)\(^i\).\(^j\)\(^k\)\(^l\).\(^m\)\(^n\)\(^o\)\(^p\)\(^q\)\(^r\)\(^s\)\(^t\)\(^u\)\(^v\)\(^w\)\(^x\)\(^y\)\(^z\). Substantial diversity in outcomes among members the BXD family highlight the fact that population averages can obscure major GXE effects. Not much can be said with certainty from a single genometype or strain of mouse or even from a small number of diverse strains. In our case, detecting strong GXE interactions required data from ten isogenic individuals from each of 76 genometypes under both conditions. Data of this type can be a foundation for specific predictions and for the design a second wave of experiments using diabetess cross progeny and the NIA Interventions Testing Program intended to extend lifespan and vigour\(^1\).\(^2\).\(^3\).\(^4\).\(^5\). Furthermore, these data can be used to map genetic modifiers of lifespan: work that is now in progress.

When extrapolated to humans, our results indicate that conventional dietary recommendations will often be too general to provide genuinely individualized guidance for improved health or lifespan. While general dietary recommendations are well intentioned, and even effective at the population level, there is much to be gained by achieving more refined and accurate recommendations that account for the unique genomes and environmental exposures of each individual.

**Methods**

All experimental procedures were in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health and were approved by the UTHSC institutional Animal Care and Use Committee.

**Animals.** Animals were raised and housed in a specific pathogen-free facility at UTHSC, at 20–24 °C on a 12-hour light cycle. During the course of this study, serum samples from sentinel mice were tested quarterly for the following 12 pathogens: ectromelia virus, epizootic diarrhoea of infant mice, lymphocytic choriomeningitis, Mycoplasma pneumonia, mouse hepatitis virus, murine norovirus, mouse parvovirus, mouse papovavirus, mouse polyomavirus, mouse coronavirus, mouse respiratory enteric virus III (RE03) and Sendai and Theiler’s murine encephalomyelitis (TMEV GDVII). We tested twice a year for endoparasites in intestinal contents, and ectoparasites by direct pelt microscopy. All sentinel tests were negative.

From October 2011 to December 2018, both parental strains (C57BL/6J and DBA/2J), their F1 progeny D2B6F1 and 73 BXD strains were followed from their birth into the ageing colony from a large breeding colony—typically around 120–166 of age but with a wide range, from 26 to 3584—until their death (details below on entry age effects). Animals were inspected daily, and deaths were recorded for each animal with a precision of 1 d. Moribund animals (roughly 10%) were euthanized, and those above the age of 200 d were included in lifespan calculations. Criteria for euthanasia were based on an assessment by our veterinary staff following Association for Assessment and Accreditation of Laboratory Animal Care International guidelines. All animals were initially raised by dams on the standard CD in the breeding colony. On entry into the ageing colony, females were aged in groups of up to ten individuals in polypropylene cages (935 cm\(^2\)) provisioned with Envigo Teklad 7087 soft cob bedding. We provided all cages with enrichment and nesting materials: Bed-r’Nest (www.andersonslabbedding.com), and torn autoclaved paper towels. While the ageing colony at UTHSC is still in operation, for this analysis we only consider individuals with deaths between April 2012 and November 2018. The colony was moved to a new vivarium in the Translational Science Research Building in April 2016 from the Nash Annex Building, both at UTHSC. Approximately 60% of the individuals lived and died in the original vivarium, roughly 35% were born in the new vivaria but lived in the original colony, and both vivaria and around 5% were born and spent their entire lives in the new facility. We evaluated birth and death data over all seasons for both vivaria and have ruled out any site-specific or seasonal effect on lifespan.

**Diet.** Controls were provisioned with a single standard low-fat CD (Envigo Teklad LM-485 7912, 17% calories from fat (fatty acids comprise 0.8% saturated, 1.3% monounsaturated and 2.9% polyunsaturated fats), 25% from protein, 58% from carbohydrates, caloric content of 3.1 kcal g\(^{-1}\)). Cases were provisioned with a HFD (Envigo Teklad TD06414, 60.3% calories from fat (fatty acid profile comprises 37% saturated, 46% monounsaturated, 16% polyunsaturated fats), 21.3% from carbohydrates caloric content of 5.1 kcal g\(^{-1}\)). All individuals had ad libitum access to food and aquifer-sourced autoclaved municipal tap water. We used a conventional CD to align our new data with over 30 years of previous data on conventional diets, and to conform to recent metabolic genetic studies by Almeida-Suhett and colleagues using the same two diets\(^e\).\(^f\).\(^g\).\(^h\). The conventional diet does raise an important question regarding specific macro- and micronutrients likely to be responsible for effects on lifespan or weight. Almeida-Suhett and colleagues demonstrated that conventional non-formulated CD (18% calories from fat) and LF (10% calories from fat) have similar effects on weight gain, glucose tolerance and behavioural outcomes. Only cholesterol levels differ significantly\(^i\).

**Lifespan cohort.** We studied a total of 1,348 female individuals (n = 663 on CD, n = 685 on HFD) from 76 strains (Supplementary Table 1, lifespan cohort). Animals were labelled using ear tags and were randomly assigned to a diet. Body weight was measured at age 16 days after entry into the study. Seventy-seven percent of individuals (n = 527) started on HFD at ages between 50–185 d, but some started on the diet as early as 26 d or as late as 358 d. Only 12 cases were placed on HFD at an age greater than 365 d, and these have been excluded from the results. There was no appreciable correlation between age at which cases were started on the HFD and lifespan (P = 0.22, r = 0.008, n = 685), and this is also true when the analysis was restricted only to those cases that started on the HFD between 26 and 150 d of age (P = 0.60, r = 0.0006, n = 487). Fewer than 20% of animals were retired breeders that entered the study at more than 180 d of age and as shown in the Results, this variable also does not covary with lifespan or weight gain. Individuals were weighed to the nearest 0.1 g every other month until their death (Supplementary Table 1, body weight data).

**Biochemical and body composition cohort.** A separate subpopulation of 662 animals (n = 334 on CD, n = 328 on HFD) from roughly 50 matching strains were euthanized for tissue collection and biochemical evaluation at time points centred on 6, 12, 18 and 24 months (Supplementary Table 1, biochemical cohort). We generated data on selected 17 serum metabolites (Supplementary Table 1, serum metabolites, for example, glucose, total cholesterol, triglycerides, free fatty acids) and major metabolic organ weights (liver, heart, kidneys). Cases and controls were removed from the ageing colony and fasted overnight before euthanasia. The euthanasia started at approximately 9:00 am using the anaesthetic Avertin (0.2 ml per 10 g of body weight), followed by complete blood draw from the vena cava (around 1 ml), and then by perfusion with ice-cold phosphate buffer saline. Blood was collected in lithium-heparin tubes, shaken and stored on ice. Samples were centrifuged at 4,500 p.m. for 10 min at 4 °C before being flash-frozen in liquid nitrogen. Blood serum was stored at −80 °C. Serum metabolite measurements and analysis were performed as described in Williams and colleagues\(^i\).

An additional 11 metabolites (Supplementary Table 1, serum hormones, such as insulin, C peptide, leptin) were analysed using the Milliplex MAP Mouse Metabolic Hormone Extended Panel (catalogue no. MMHHE-44K) according to the manufacturer’s instructions. Serum samples were analysed on a Magnetic Luminex (Luminex Corp.). We carried out data analysis using the xPONENT software. A curve fit was applied to the standards and the sample concentrations extrapolated from the standard curve using five-parameter logistic method. HOMA-IR was calculated as fasting serum insulin (μU ml\(^{-1}\)) multiplied by fasting glucose (mg dl\(^{-1}\)). Adiposity was calculated using summed weights of subcutaneous, perirenal and pericardial fat pads as a percentage of body weight.

**Bayesian network analysis.** We evaluated causal models linking diet through the subset of metabolites that we measured to the key outcome measures—lifespan
and body weight at 500 d of age. We used Bayesian network modelling software (compbio.uthsc.edu/BNWF5,6). The 1,000 highest scoring networks were averaged, and those directed edges—unidirectional connections between phenotype nodes—that had posterior probabilities greater than 0.5 were retained in final consensus models. For computational efficiency we constrained nodes to have at most four parents. We assigned nodes into one of three tiers: (1) diet in the primary causal intervention tier; (2) serum metabolites in the mediator tier and (3) the outcome measures, lifespan and body weight at 500 d of age (close to maximum body weight for cases and controls). During modeling, we constrained edges to be between diet and the mediator tier, and between the mediator tier and outcomes.

Pathology. Most individuals were fixed by immersion in 10% neutral-buffered formalin within 24 h of death. The body cavity was opened to improve preservation. Evenly balanced but randomly selected cases and controls (45 strains and 76% and 77% HFD and control diets) were selected on the basis of fixation quality for necropsy with histopathology of tissues. A board-certified veterinary pathologist (R.W.R.) performed necropsies and judged probable cause of death and other morbidities.

Statistics. While this study has most of the hallmarks of a classic prospective and intervention randomized controlled trial, in some respects our design is also similar to an observational prospective study. The main complicating factor is that to jump-start this lifespan study in 2013 we accepted females, including retired breeders, into the HFD limb over a broad age range (see Results for details). We used standard methods to observational analyses to adjust for variance in age in entry and to test and eliminate this variable as a confounder. On a positive note, heterogenization of this type can improve robustness of key findings9. Lifespan and body weight data were stratified by diet and by strain. Effects of diet and body weight on lifespan across all strains were analysed using fixed-effects linear model in R (v.4.0.0). Strain-level (genetic) effects were analysed by a random-effects model in R using the metagor package v.2.4-0 (ref. 10). Hazard ratios were calculated using a mixed-effects Cox proportional hazard model using Therneau’s coxme R package v.2.2-10 cran.r-project.org/web/packages/coxme/index.html). Survival analyses were performed using the survival package for R and the data were right-censored (for example, Fig. 2a–c, censored cases CD, n = 32; HFD, n = 80). Survival curves were computed by analysis of variance (ANOVA) and regression analyses were performed using R. The variance explained by strain, diet, GxE and unexplained variance (non-diet, non-genetic) was calculated by fixed-effects two-way ANOVA. Two-sided Welch’s t-test was used to determine statistical significance in the effects of diet on serum metabolites and metabolic hormones. Correlations are Pearson’s r. All graphs were generated in R, and final figures were all prepared with Adobe Illustrator.

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

Data availability
All data conform fully to findable, accessible, interoperable and re usable standards11 and Supplementary Table 1 provides research resource identifiers (www.rirds.org) for all strains. Mean, median and 75% quantile lifespan data from cases and controls are available at GN (www.genenetwork.org) under the headings Species: Mouse; Group: BXD Family; Type: Traits and Cofactors and Dataset: BXD HarvestedPublish. Note that lifespan datasets in GeneNetwork are part of a longitudinal and retrospective study of the BXD family. Some datasets will therefore include additional strains as well as outliers excluded from the fixed Supplementary table. Source data are provided with this paper.

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Acknowledgements
We thank J.F. Nelson for helpful discussion on the LXS dietary restriction datasets.
We thank P. Prins, Z. Sloan and other members of the GeneNetwork team for superb
informatics support. Finally, we thank Dr. Elizabeth A Fitzpatrick and team at the
Regional Biocontainment Laboratory at UTHSC for generating serum hormone
data. This work was supported by grants from the NIH nos. R01AG043930 (R.W.W.),
NIH R01AG070913 (R.W.W.), the University of Tennessee Center for Integrative
and Translational Genomics (L.L.), the Ecole Polytechnique Fédérale de Lausanne,
the European Research Council (AdG-787702) (J.A.), the Swiss National Science
Foundation (310030B-160318) (J.A.) and the AgingX programme of the Swiss Initiative
for Systems Biology (RTD 2013/153) (J.A.). S.S. was supported by NIH grant no. P30
DA044223-04. E.G.W. was supported by NIH F32 Ruth Kirchstein Fellowship (grant
no. F32GM119190). K.M. was supported by NIH grant no. R21 AG055841. R.W.R. was
supported by TriMetis Life Sciences, Memphis, TN, USA. L.M. was supported by the
American Heart Association and Methodist Mission Support Fund. C.K. was supported
by grant no. NIH R01AG054180.

Competing interests
The authors declare no competing interests.

Author contributions
E.G.W., S.R., J.A., L.L. and R.W.W. were responsible for the conceptualization. Aging
colony management and informatics was done by S.R., J.F.I., C.J.C., M.S.M., A.G.C.
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M.K.M., J.D.Z., W.Z., J.H., S.M.N., L.A.W., T.M.S., C.C.K., Y.C., L.L. and R.W.W.
Formal analysis and data curation were done by S.R., D.G.A., M.B.S., P.J., E.G.W.,
A.S., M.H., R.W.R., S.S. and R.W.W. The original draft was written by S.R. and R.W.W.
Review and editing of the paper was done by S.R., M.B.S., E.G.W., K.M., L.M., D.G.A.,
S.S., R.A.M., J.A. and R.W.W. Companion web resources were provided by A.G.C., S.R.,
S.S. and R.W.W.

Additional information
Extended data is available for this paper at https://doi.org/10.1038/s42255-021-00449-w.
Supplementary information The online version contains supplementary material
available at https://doi.org/10.1038/s42255-021-00449-w.
Correspondence and requests for materials should be addressed to Robert W. Williams.
Peer review information Nature Metabolism thanks the anonymous reviewers for
their contribution to the peer review of this work. Primary handling editor: Christoph Schmitt.
Reprints and permissions information is available at www.nature.com/reprints.
Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in
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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Diet effect on lifespan and body weight at 500 days of age. Related to Fig. 1 and Fig. 3. Diet effect on lifespan and body weight at 500 days of age (A) Data points represent lifespan of animals on low-fat chow diet (CD) in BXDs with n ≥ 4 per strain. Red + denotes the strain median. (B) Data points represent lifespan on the high-fat diet (HFD) in BXDs with n ≥ 4 per strain. Blue + denotes the strain median. (C) Data points represent body weight on CD at 500 days of age in BXDs with n ≥ 4 per strain. Red + denotes the strain median. (D) Data points represent body weight on HFD at 500 days of age in BXDs with n ≥ 4 per strain. Blue + denotes the strain median.
Extended Data Fig. 2 | A Bayesian Network model of the impact of diet on serum metabolites and lifespan and peak body weight at 500 days age. Edge weights in this network are the weighted fraction of best 1000 Bayesian models that have the same edge and polarity. A value of 1.0 means every one of 1000 “top-ranked models” has this edge. The upper 6 nodes are serum metabolites, and lifespan and body weight at 500 days age are the final outcomes.
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- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Software and code

Policy information about availability of computer code

Data collection: Microsoft Excel, FileMaker Pro

Data analysis: R (version 4.0.0) including the metafor package 2.4-0, survival package, coxme R package 2.2-10 ([cran.r-project.org/web/packages/coxme/index.html]), xPONENT software for Luminox. Bayesian Network Webserver http://compbio.uthsc.edu/BNW_1.22. GeneNetwork www.genenetnetwork.org

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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
- A description of any restrictions on data availability

All data conform fully to FAIR standards (findable, accessible, interoperable and reusable, [2]) and Supplementary table S1 provides Research Resource identifiers (RRIDs, www.rrids.org) for all strains. All data are accessible at www.genenetnetwork.org

Mean, median, and 75% quantile lifespan data from cases and controls are available at GeneNetwork (www.genenetnetwork.org, GN) under the headings Species; Mouse; Group: BXD Family; Type: Traits and Cofactors, and Dataset: BXD Published Phenotypes (e.g., GN traits BXD_18435, 18441, 19475, 19452, 21302, 21450). Body weight data at 6, 12, 18 and 24 months is also available in GN (e.g., traits BXD_19126, 19130, 19131, 19167, 19168, 19169, 19170, and 19171). For example,
the following uniform resource locator (URL) with query string parameters will retrieve mean lifespan data for HFD cases: www.genenetwork.org/show_trait?trait_id=18435&dataset=BXD Publish
where the number in bold can be replaced with other ID numbers to obtain and download any data from this work.

Organ weight data for a large subset of cases and controls that were sacrificed between 6 and 24 months-of-age (liver, heart, kidneys, and brain) are available but these data are only covered briefly here (e.g. GN traits BXD_20156, 20157, 20158, 20159, 20353, 20354, 20148, 20149, 20150, 20151, 20146, 20147).

Individual data are also available for all cases, both in the Supplementary table (the precise data used in all analyses here) and in GN under the headings Species: Mouse; Group: BXD NIA Longevity Study; Type: Traits and Cofactors; and Dataset: BXD-NIA-Longevity Phenotypes. For example, individual data for lifespan, irrespective of diet, is accessible here: www.genenetwork.org/show_trait?trait_id=10002&dataset=BXD-HarvestedPublish.

Note that lifespan data sets in GeneNetwork are part of a long-term, and still active genetic study of lifespan in the BXD family, and some datasets will therefore include additional strains as well as outliers excluded from the fixed Supplementary table.

Code availability
Source code and raw data used for the fixed-effects linear model, random effects meta analysis model, and survival analysis in R are available at https://github.com/genenetwork/bxd_gxe longevity_2020. We have generated a Jupyter notebook as well, detailing our source code with computational and statistical output.

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.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

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
We studied a total of 1348 female individuals (n = 663 on CD, n = 685 on HFD) from 76 strains - [Supplemental Table; Lifespan cohort]. While the aging colony at UTHSC is still in operation, for this analysis we only consider individuals with deaths between April 2012 and November 2018.
A separate subpopulation of 662 animals (n = 334 on CD, n = 328 on HFD) from ~50 matching strains were sacrificed for tissue collection and biochemical evaluation at time points centered on 6, 12, 18 and 24 months (Supplemental Table; biochemical cohort).

No sample size calculations were performed before the study. We aimed to have 8-10 animals for each isogenic strain per diet to achieve statistical significance. To the best of our knowledge this is the largest rigorously controlled GxE experiment on the effects of diet on lifespan and weight change.

Data exclusions
Animals above the age of 200 days were included in lifespan calculations. 12 cases were placed on HFD at an age greater than 365 days, and these have been excluded from the Results. Exclusion criteria was established before the analysis was done.

Replication
We use readily available isogenic lines of mice from the Jackson Laboratory [see RRIDs] for which replication is practical. In this work, most isogenic lines were replicated on average about 8 to 10 times per diet with success to ensure robust results with respect to lifespan and body weight differences.

Randomization
Animals were randomly assigned to a diet typically around 120 ± 66 days of age but with a wide range, from 26 days to 358 days.

Blinding
This work mainly focuses on the phenotypic effects of diet on lifespan and body weight. All phenotyping was carried out without blinding. The high fat diet usually has a significant impact on body weight and fat content that prevents phenotyping or necropsy investigator or analysis from being blinded. Researchers performing the biochemical assays were blinded to group allocation.

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 |

Animals and other organisms

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

Laboratory animals: Female mice: parental strains, C57Bl/6j and DBA/2j, D2B6F1, and 73 BXD strains, were used in this study. Animals were followed from their entry into the aging colony from a large breeding colony —typically around 120 ± 66 days of age but with a wide range, from 26 days to 358 days—until their death.

Wild animals: No wild animals were used in this study.

Field-collected samples: No field-collected samples were used in this study.

Ethics oversight: All experimental procedures were in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health and were approved by the UTHSC Institutional Animal Care and Use Committee.

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