

Comment 1:

S2 shows that the median lifespan of mice on *none* of 25 diets exceeded 140 weeks, let alone 150 weeks. Yet **Figure 2** in the main text (chart below) suggests median lifespans beyond 150 weeks; **Figure 2B** shows a Kaplan-Meier curve featuring the *oldest* mice (outliers >150 weeks) while obscuring the range of *median* lifespans (all <140 weeks) over the 30-diet experiment.

Response 1:

This comment indicates confusion around median and maximum lifespans and the nature of survivorship curves. Median lifespans per diet treatment (Table S2) are used as the basis for the response surface in Figure 2A, mapped onto mean nutrient intakes for the mice on each diet. The full survivorship analyses in the remainder of Fig. 2 includes lifespans of all mice for a given dietary category (dietary protein to carbohydrate ratio or energy density), which of course include cases both shorter and longer than the median.

Comment 2:

The authors claim **falsely** that “Median lifespan was greatest” on diets “low in protein and high in carbohydrate”. You can see (Table S2) that median lifespan was greatest on a diet *high* in protein (42%) and *low* in carbohydrate (29%): 139 weeks is 10% better than the next-best median, also from a *high*-protein diet. Alas, in **Figure 2A** the authors carefully suppressed any possible sign of the two best diets (median lifespan 126-139 weeks).

Response 2:

The conclusion that lower protein, higher carbohydrate diets supported longest lifespans and best mid-late-life cardiometabolic health in the mice was derived from the entire dataset - and was statistically robust. The power and novelty of this study was that it systematically measured many combinations and quantities of protein, carbohydrate and fat and tested the responses of mice across all of these – *not* diet by diet. In fact, to pick out one or two diets for special attention is invalid – equivalent to refuting a statistically significant regression based on individual points below or above the fitted line.

To further explain (and at the risk of belaboring the point – please skip to comment 3 if so), we visualized the response surfaces using thin-plate spline regressions and used generalized additive models (GAMs) to estimate the main and interactive effects of macronutrients on lifespan and health outcomes. As is typical for any statistical model of biological data, the model does not fit the data perfectly, thus when assessing a single individual data point against a model prediction one will see a discrepancy (a residual). However, plotting the residuals for all diets (below) against their respective P:C ratios clearly show that there is no systematic bias in over- or under-estimation of lifespan as a function of dietary macronutrient content (in the case shown below, the protein to carbohydrate ratio).

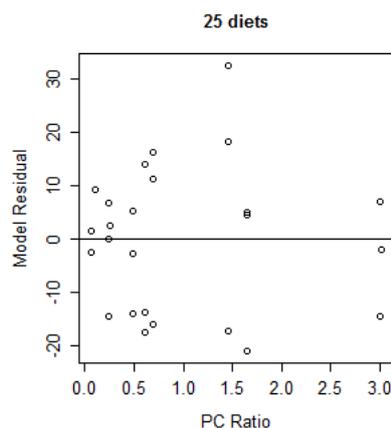
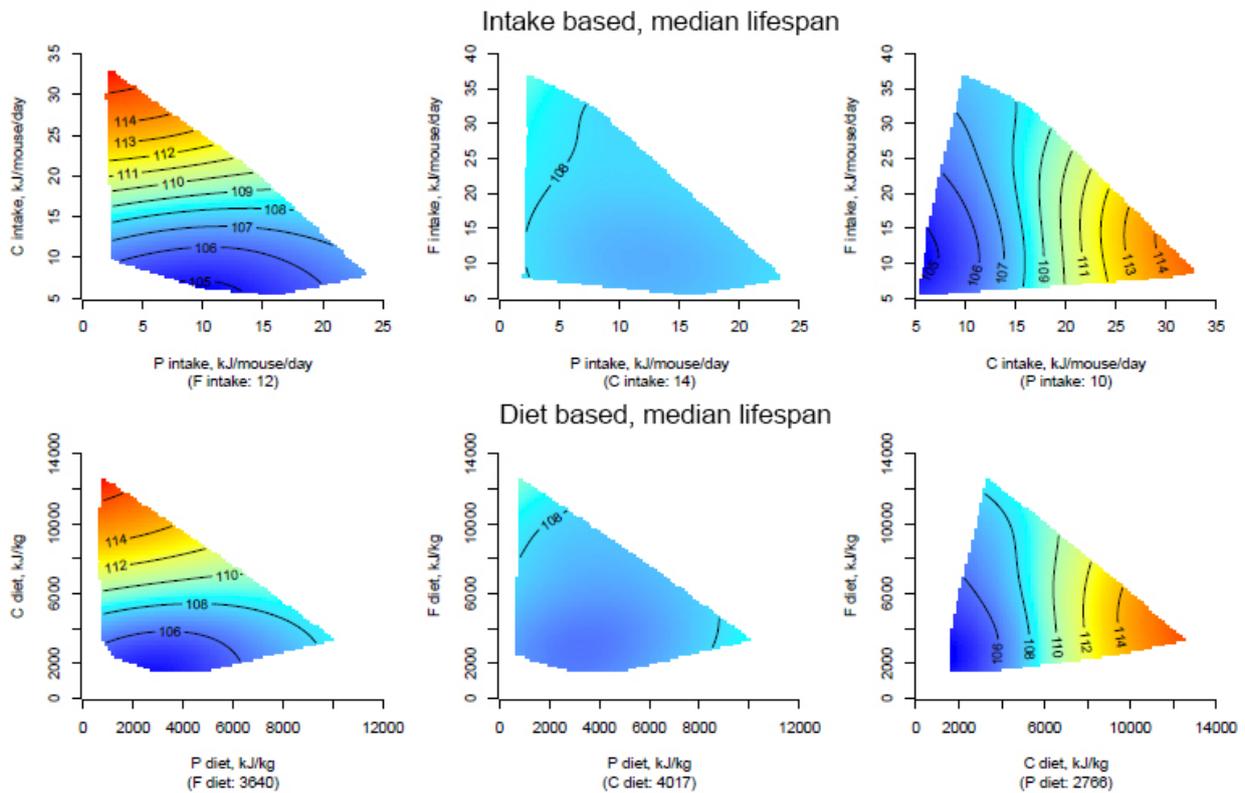


Fig. A. Residuals of median lifespan plotted against P:C ratios. Horizontal line at zero added for reference and does not indicate a line of best fit.

It is also worth noting that the response surfaces and associated statistical analyses shown in Figure 2A and elsewhere in the paper are mapped onto mean nutrient intakes for each diet (rather than diet composition). This is because we were interested in the impacts of nutrient intakes on health and longevity outcomes in later life. It is clear in Figure 1A that mice altered their food intake based on nutrient density, such that those fed diets with higher nutrient concentrations (especially protein and carbs, less so for fat) reduced food intake. In geometric terms, this means that the dietary

composition (i.e. g/kg of protein, carbs and fat in the food) constrains the vector in protein-carb-fat intake space along which an animal confined to that diet can travel as it consumes food, whereas the distance it travels from the origin into nutrient space is a function of how much food it chooses to eat.

Nonetheless, if median lifespans were mapped onto *diet compositions*, the resulting surfaces were much the same, with the statistical model again indicating greatest median lifespans under low protein, high carbohydrate diets (heat maps below).



Comment 3:

Table 3 (on p.6, below) confirms that the authors have skilfully misrepresented their 30-diet longevity results, including by obscuring 100+ dead mice on five low-protein diets.

Response 3:

As we pointed out at the time of publication in an online response to Mr Robertson, these diets were discontinued within the first 10-23 weeks of the study because the young mice assigned to them from weaning were not growing, and according to the independent veterinary office overseeing the study, would soon have died from malnutrition. Under the terms of the ethics protocol this mandated their immediate removal from the experiment.

Consideration of the composition of the excluded diets reveals the reason. As can be seen in Table S1 (and visualized in Figure S1), the 5 diets excluded from the 30 all combined a low or very low protein macronutrient ratio with high cellulose content (hence low energy content):

- Diet 2 Low energy density 5:75:20 (P:C:F, i.e. very low protein, high carb, low fat)
- Diet 3 Low energy 5:20:75 (very low protein, low carb, high fat)
- Diet 6 Low energy: 5:48:48 (very low protein, medium carb, medium fat)
- Diet 3 Medium energy: 5:20:75 (very low protein, low carb, high fat)
- Diet 6 Medium energy: 5:48:48 (very low protein, medium carb, medium fat).

To have attained sufficient nutrient intakes for growth would have required the mice on these low-energy, low-protein diets consuming more food than they were able to achieve. In short, these diets were not viable for a young, growing mouse.

Furthermore, as we pointed out five years ago to Mr Robertson, excluding the data from those discontinued mice was conservative in relation to our conclusions. This is because the nutrient intakes of mice on the excluded diets were low (because mice were unable to compensate sufficiently for dilution of nutrients by increasing food intake). As a result of a) their low nutrient intakes, and b) what would have been very short lifespans had they not been removed from the experiment, including them in the analysis would simply have deepened the foothills of the response landscape near the origin, with no change in the response surface at higher nutrient intakes. Hence, our conclusions in relation to macronutrient balance and calorie intake would have been strengthened by inclusion of the five diets.

Finally, there seems to be an implication in Mr Robertson's comments that we are somehow advocates for a high carb diet. We are not – we are scientists. As he could see by reading Solon-Biet et al. 2015 (PNAS), reproductive function in the same male and female mice was maximised on a higher protein, higher fat diet. The message from these and other experiments is that titrating macronutrient ratios (and varying their quality) can achieve many and various health and life-history outcomes – but not all outcomes are optimised on a single diet composition.