fed flies ($P < 0.0001$, Wilcoxon rank sum test; **Supplementary Methods** online). When we exposed flies briefly to dye-labeled food, we found that the dye took less than 50 min to start appearing in feces. Thus, by 30 min, the amount of dye accumulated in the fly reflected feeding rate alone, while after 50 it reflected the rate of label ingestion, the rate of egestion and the gut capacity. Our measurements of crop size showed that the latter was increased by dietary restriction[4]. The use of radioactive labels[2] involves further potential confounding processes than those for a non-absorbed dye because the amount of isotope present will also depend upon the capacity of the body for the labeled element[2,5].

Using data from Geer *et al.*[5] for ^{14}C-choline labeled food accumulation by *Drosophila* (**Supplementary Fig. 1** and **Supplementary Methods** online), we generated a model:

$$m(t) = -\left(\frac{c}{s}\right) \times [1 - \exp^{(s \times t)}]$$

where $m(t)$ is the amount of label in the fly at time t; c is the feeding rate; and s is the fraction of labeled material removed from the fly (rate of label removal divided by the internal label capacity of the fly). We assigned arbitrary values to these parameters and observed their effect on label accumulation (**Fig. 1b–d**). The accumulation profile (**Fig. 1b**) consists of an 'initial' phase when label is taken in and not egested, an 'intermediate' phase where label ingestion rate exceeds egestion rate, and an 'equilibrium' phase when label egestion and ingestion rates are equal. The amount of label in the fly gives a reliable estimate of feeding rate only during the 'initial' phase. During the 'intermediate' phase, the amount of label in the fly will underestimate the extent of a genuine difference in feeding rate (**Fig. 1b–d**) and will fail to detect the difference once 'equilibrium' is reached. For a fly with a greater internal capacity, the amount of dye present will overestimate feeding rate relative to controls once egestion has started ('intermediate' phase), to an extent that reaches a maximum at the 'equilibrium' phase (**Fig. 1c**).

Fitting this model to the data presented in **Figure 1a**, diet-restricted and fully fed flies consumed food at equal rates, but fully fed flies turned over 32% of their gut capacity per hour, whereas diet-restricted flies turned over only 14%. At equilibrium, the absolute amount of material egested must equal the amount eaten, and therefore diet-restricted flies have an approximately twofold larger gut capacity for labeled food than do fully fed flies. Thus the conclusion that fruit flies compensate for dietary restriction by increasing their feeding rate[2] was inaccurate because of inappropriate use of the method. As an alternative for longer-term measurements, we have developed an assay that, when appropriately calibrated, offers a more accurate measurement of food intake[6].

Note: Supplementary information is available on the Nature Methods website.

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Carvalho *et al.* reply: In their Correspondence, Wong *et al.*[1] argue that dye labeling is not a satisfactory technique to address the issue of compensatory feeding and try to generalize this inadequacy to all food labeling methods. While some valid points are raised, others are off the mark.

As the authors point out, long-term measurements made with food labels reflect not only ingestion, but also internal capacity for the label and elimination rates. Capacity is a major limitation of non-absorbable dyes but not of ^{32}P or ^{14}C radiolabeling. Whereas dye measurements quickly plateau[1], ^{32}P and ^{14}C levels accumulate near-linearly for several days[2,3], indicating that internal capacity is not rate-limiting over 24 h, the time point used in our study[2]. In contrast, as emphasized in our report, the absorbable nature of isotopes precludes us from discerning whether compensation takes place at the level of intake or absorption. Using the capillary feeder (CAFE), a direct, real-time assay of ingestion in undisturbed animals involving no food labels, it has been recently demonstrated that flies can sense nutrient variation and adapt their intake accordingly[4], supporting the behavioral over the metabolic mechanism for compensatory feeding. As for elimination, the fact that diluted media stimulate excretion[5] suggests that compensatory feeding may be even more dramatic than our results indicated. However, this does not directly address isotope turnover. It will be interesting to dissect this issue using methodology developed since the publication of our report[6].

Radiolabeling and the CAFE are currently the most reliable and sensitive assays of feeding behavior in adult *Drosophila melanogaster*. With the advent of these methods, it is, in our opinion, no longer justifiable to infer feeding rate from surrogate assays such as egg laying or indirect behavioral observations[7].

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