Pitfalls of measuring feeding rate in the fruit fly *Drosophila melanogaster*

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To the editor: Dietary restriction (DR), a reduction of food intake without malnutrition, extends lifespan in many organisms, including the fruit fly *Drosophila*, where DR can be implemented by food dilution [1]. Flies could compensate for reduced nutrient content of food by increasing their feeding rate. Food intake is difficult to measure in *Drosophila* due to their small size. A recent study used radioactively labelled food to estimate *Drosophila* feeding rate, and reported compensation for food dilution [2]. The authors assumed that the amount of radioactive label in flies depended only upon rate of ingestion. However, retention time of the food can also vary. Without measurement of this variable, a genuine difference in feeding rate could be undetected, or a spurious difference in feeding rate detected. We illustrate this here, with an experiment using food labelled with a non-absorbed food dye [3], and a mathematical model.

We measured dynamics of dye accumulation in flies after transfer to labelled food (**Figure 1a**). Initially, DR and fully-fed flies accumulated label at similar rates. However, rate of accumulation declined faster and label reached lower equilibrium levels in fully-fed than in DR flies. Had dye accumulation reflected only feeding rate, these results would imply that up to 30 minutes there was no feeding rate difference between the groups but that by 3 hours of feeding there was an approximately 1.5-fold higher feeding rate in the DR flies. We therefore considered the possibility of a difference in retention time for the food. We found that DR flies had 45% larger crop size than fully-fed flies ($P < 0.0001$, Wilcoxon rank sum test) **(Supplementary Methods** online). When we exposed flies briefly to dye-labelled food, we found that the dye took less than 50 minutes to start appearing in faeces. Thus, by 30-minutes 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, which our crop measurements show was increased by DR [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 labelled element [2,5].

Using data from Geer et al. (1970) for ^{14}C-choline labelled food accumulation by *Drosophila* (**Supplementary Figure 1 and Supplementary Methods**), we generated a model:

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

where $m(t)$ = amount of label in the fly at time t; c = feeding rate, and ; s = fraction of labeled material removed from the fly (rate of label removal (e) divided by the internal label capacity of the fly (p). We assigned arbitrary values to these parameters and observed their effect on label accumulation (**Figure 1b, c, d)**. The accumulation profile (**Figure 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 (**Figure 1b, c and d**, green versus blue), and will fail to detect the difference once 'equilibrium' is reached. For a fly with a greater internal capacity (red), the amount of dye present will over-estimate feeding rate relative to controls once egestion has started ('intermediate' phase), to an extent that reaches a maximum at the 'equilibrium' phase (**Figure 1c**).

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

Figure 1

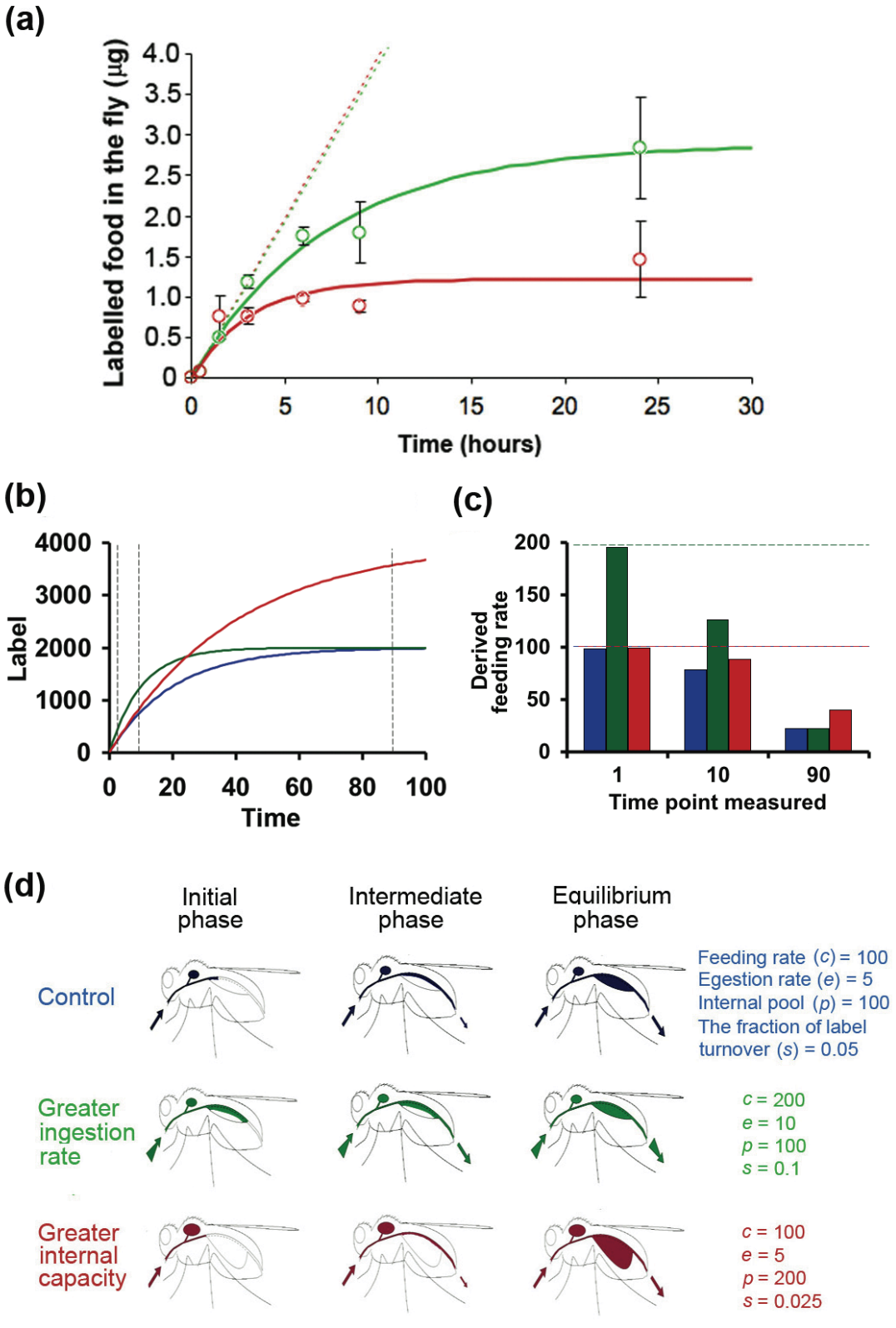
(a)
Labelled food in the fly (µg)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
0
5
10
15
20
25
30
Time (hours)
(b)
Label
4000
3000
2000
1000
0
0
20
40
60
80
100
Time
(c)
Derived feeding rate
200
150
100
50
0
1
10
90
Time point measured
(d)
Initial phase
Intermediate phase
Equilibrium phase
Control
Feeding rate (c) = 100
Egestion rate (e) = 5
Internal pool (p) = 100
The fraction of label turnover (s) = 0.05
Greater ingestion rate
c = 200
e = 10
p = 100
s = 0.1
Greater internal capacity
c = 100
e = 5
p = 200
s = 0.025

Figure 1 legend

(**a**) The amount of labelled food present in DR-fed (green) and fully-fed (red) flies at different times after transfer from unlabelled food to food labelled with blue food-dye. Solid lines represent measured dye accumulation and dashed lines represent the label accumulation profile that would occur if feeding rates were the only factor governing label accumulation. Error bars = s.e.m.

(**b**) Modelled dynamics of accumulation of dye with time for flies assigned arbitrary values of feeding rate (c), internal pool size (p) and fraction of label turned over (s; the rate of egestion (e) divided by the internal pool size (p)).

(**c**) The feeding rates of the three conditions derived by measuring the label accumulated at different times from time 0. If sampled during the 'initial phase' (t = 1) the observed feeding rate reflects the real feeding rate (dashed lines). During the 'intermediate phase' (t = 10), the apparent feeding rate is lower than the real rate and more so in the fly with the higher feeding rate. In the 'equilibrium phase' (t = 90) the apparent feeding rate falsely gives the impression that red-labelled flies have a higher feeding rate than blue or green-lebelled flies. At this point, the measurement only reflects the internal capacity of the fly for the label.

(**d**) A pictorial representation of what may be occurring within the fly for each condition.

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LIST OF SUPPLEMENTARY ITEMS

Supplementary Figure 1 ^{14}C-choline labelled food accumulation in *Drosophila*

Supplementary Methods