

Introducing cumulative energy balance for CalR 1.3.

Simplifying experimental visualization.

Small differences in food intake or energy expenditure can accumulate to cause changes in body weight over time. However, the sensitivity of the indirect calorimeter to detect these small changes is limited by the biological variability between animals, the noise in measurements, and sample size. However, the cumulative differences in food intake and energy expenditure can alter energy balance, which when visualized, is helpful to reveal trends over time. CalR now includes cumulative energy balance and cumulative energy expenditure, to join the existing cumulative food intake visualization under the time plots tab.

We define energy balance as:

$$\text{Energy balance (kcal/hr)} = \text{Food consumed (kcal/hr)} - \text{Energy Expenditure (kcal/hr)}$$

Example 1. In the first example of cumulative energy balance, we examine freely fed groups of mice A and B. We examine data collected over 48 hours for mice on a standard chow diet (Formulab diet 5008). The mice in group B have significantly greater energy expenditure than the mice in group A (Figure 1A, $p < 0.01$ by ANCOVA for total body mass). However, the mice in group B have a trend towards lower food intake (Figure 1B, $p = 0.52$ by ANCOVA). While overall differences in hourly energy balance are not overtly apparent (Figure 1C, left) but become evident when plotted over time (Figure 1C, right). In the cumulative energy balance plot, we can observe that the mice in group B are in neutral energy balance, eating nearly the amount of food to counter the increased energy balance. The mice in group A have a greater food intake than their energy expenditure leading to approximately a positive 11 kcal gain in energy balance which would correspond to just over 1 gram of fat mass gained for each animal.

The downside of the energy balance calculation and visualization is that the noise and variability is combined from both food consumed and energy expenditure. The larger error bars in the cumulative energy balance plot are apparent.

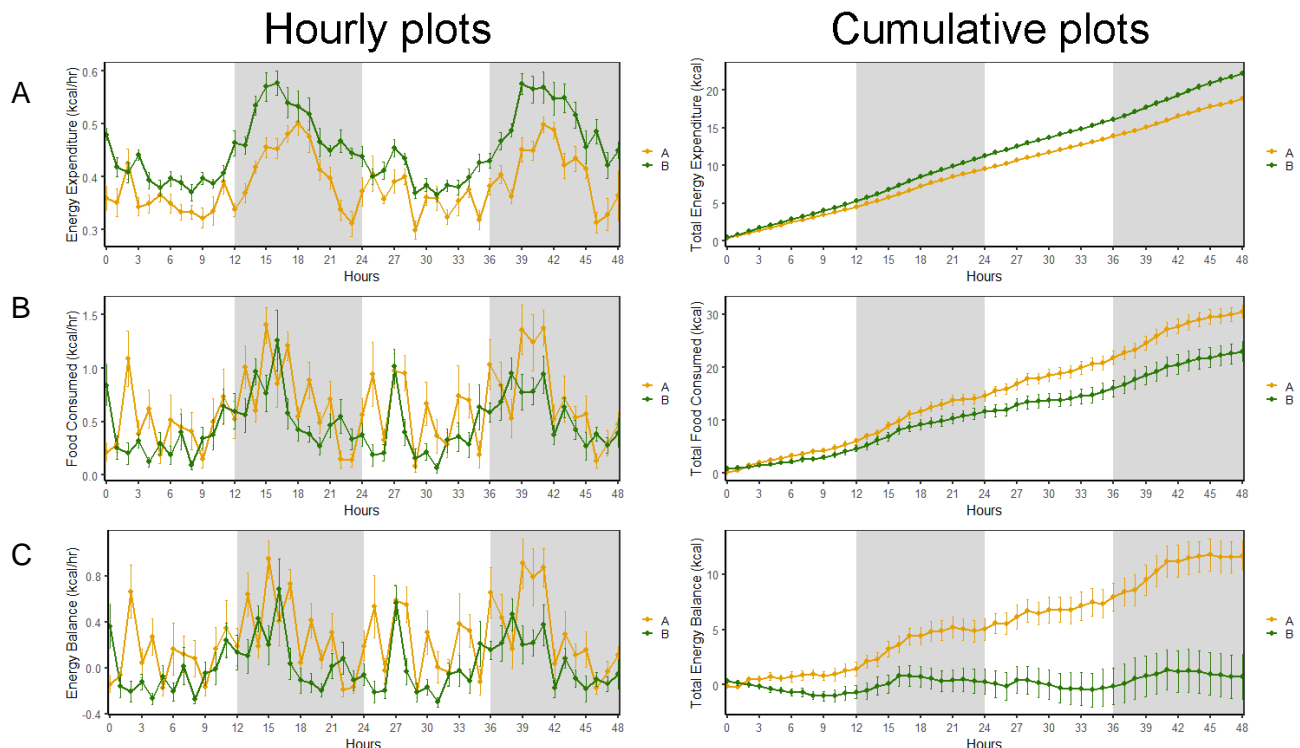


Figure 1. New cumulative visualizations for CalR 1.3. Left: hourly plots, right: cumulative plots. A) Energy expenditure vs time. B) Food intake vs time. C) Energy balance vs time. Groups A & B $n = 7$ & 9 respectively.

Example 2: In the next experiment we observe mice that underwent a 17 hour fasting period. In the cumulative food intake plot we clearly see the fasting event taking place from hours 17-34 (Figure 2).

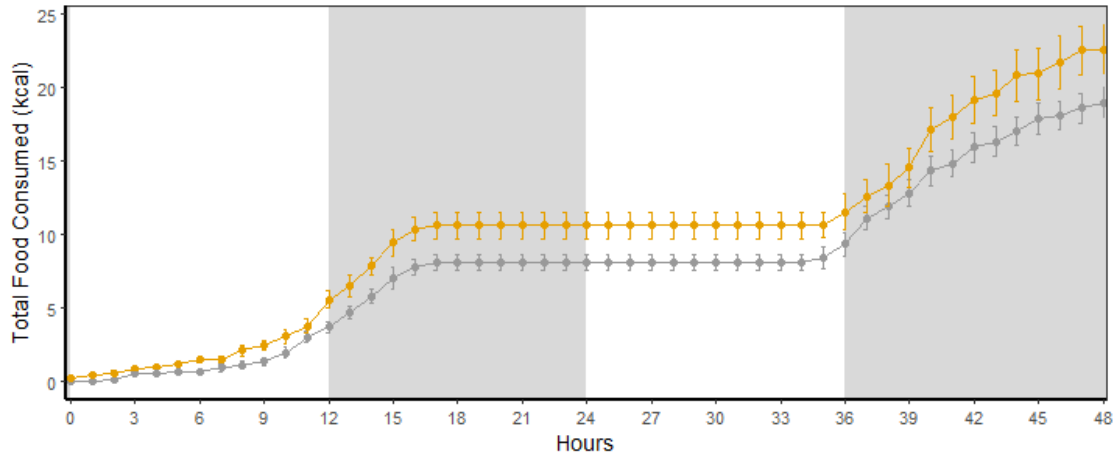


Figure 2. Cumulative food intake.

When examining hourly energy expenditure in Figure 3 it is possible to observe a relatively normal—if somewhat smaller—energy expenditure phenotype while the mice are going through a fasting event. In addition, we can verify that there is no visual difference in energy expenditure between groups by inspecting the cumulative energy expenditure plot in Figure 4.

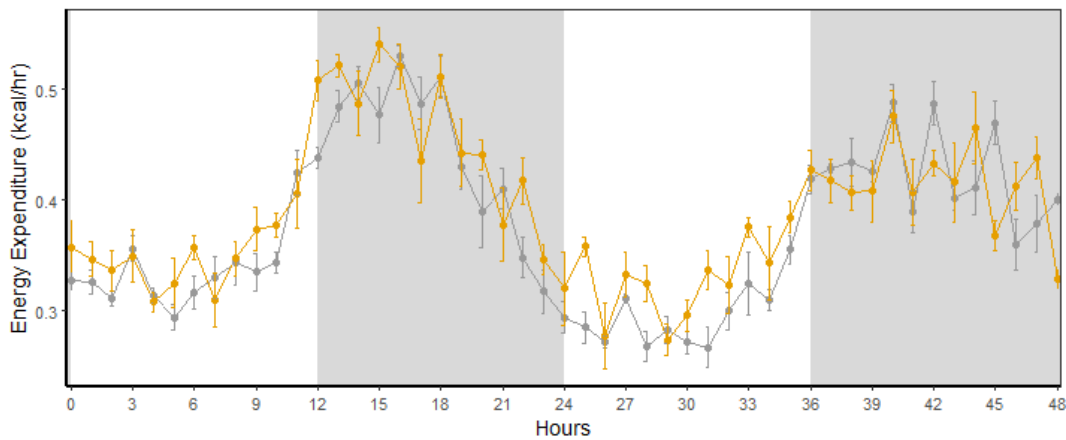


Figure 3. Hourly energy expenditure

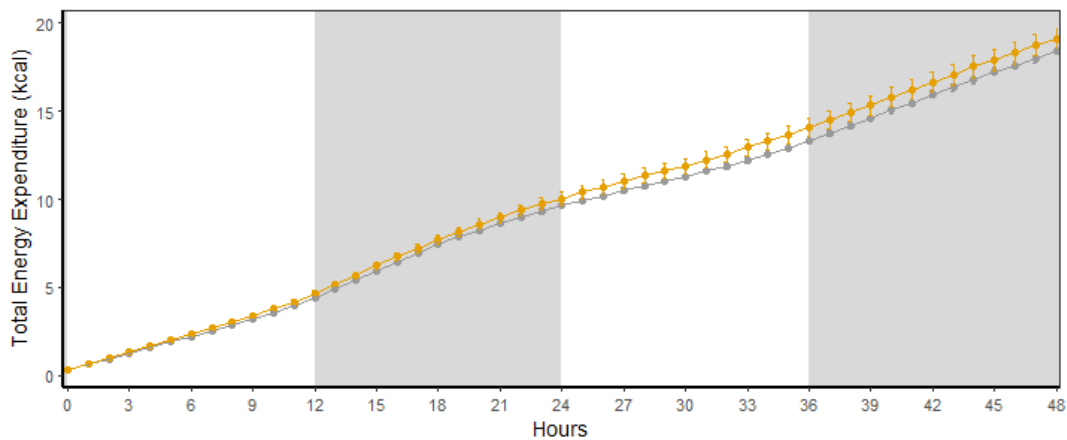


Figure 4. Cumulative energy expenditure.

Based on the results in Figures 2-4, we anticipate a difference between groups as the food intake was different but the energy expenditure was similar. However, when we examine the hourly energy balance plot in Figure 5 it is difficult to distinguish that there is any identifiable divergence between groups. When we examine Figure 6 which depicts cumulative energy balance, we can clearly see the difference between groups driven by food intake and how it affects overall energy balance. Once again, the larger error bars in the cumulative energy balance plot are apparent.

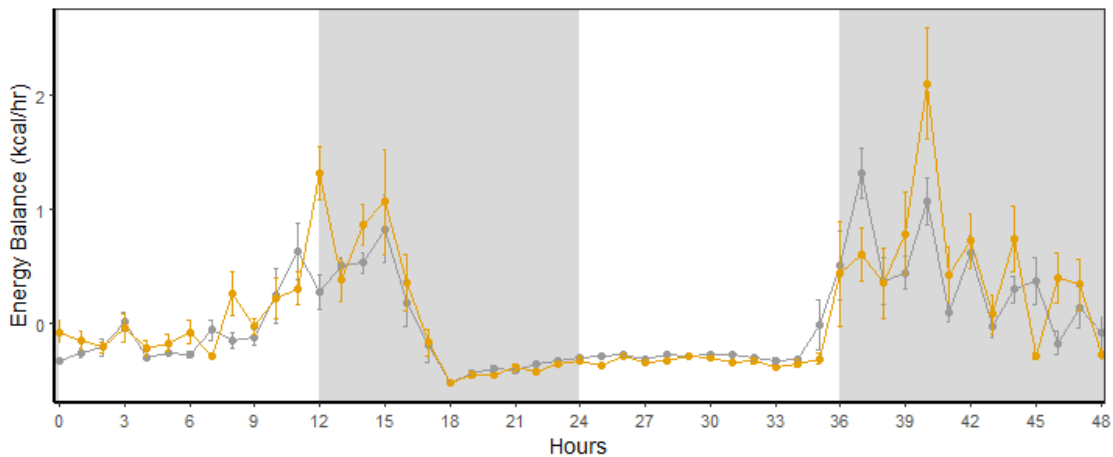


Figure 5. Hourly energy balance.

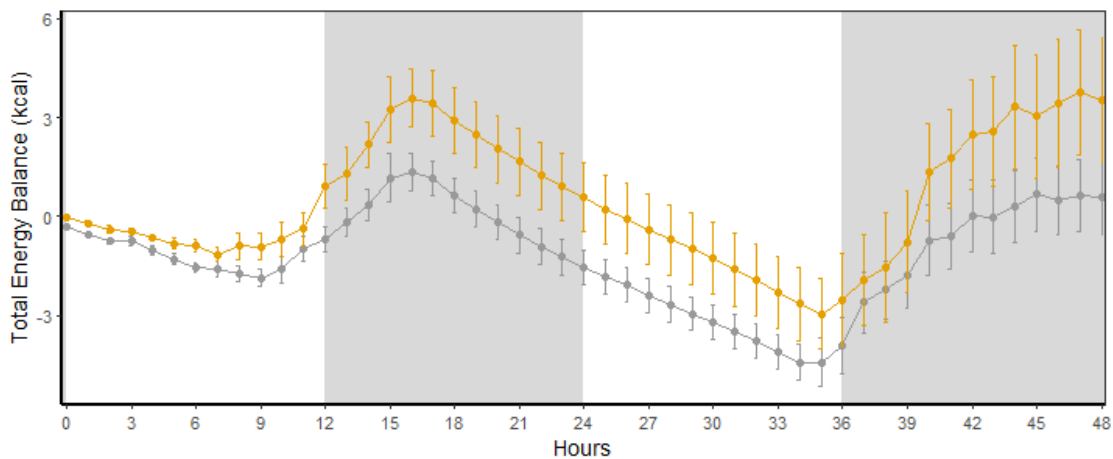


Figure 6. Cumulative energy balance