SUPPLEMENTAL INFORMATION: ENERGY BUDGET OF DROSOPHILA EMBRYOGENESIS

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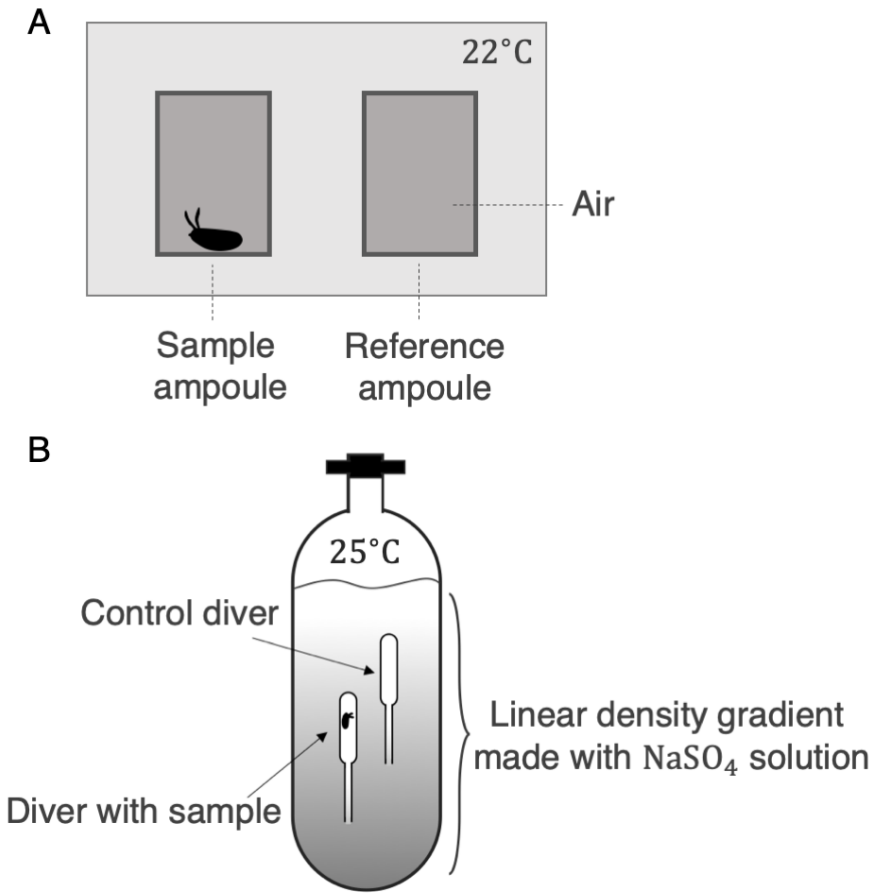

Figure S1. Calorimetry and respirometry setup. Related to Figure 1. A: Schematic of the isothermal calorimetry setup as previously reported in [S1]. The ampoules are kept under isothermal condition at 22 °C. The heat dissipation from the control and sample containing ampoule are read out through thermopiles. B: Schematic of the respirometry setup used in [S2]. A linear density gradient of $NaSO_4$ solution is setup in a pressure controlled column. As the embryo consumes oxygen within the diver, the diver moves down the density gradient. The oxygen consumption rate is calculated from the relative position of the embryo containing diver from that of the control diver.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Fly stocks and husbandry

For the fuel depletion measurements, we used the *Drosophila* stock OregonR provided by the Bloomington Stock Center. Adult flies were matured in room temperature on fresh yeast paste for at least two and at most 14 days to collect embryos. We did not determine the sex of embryos used for this study. All embryos were grown on apple juice plates at 25 °C. All fly stocks were maintained by standard methods at room temperature, and were grown on a standard cornmeal, sucrose, and yeast media. For calorimetry experiments, Canton-S flies were used, and the adults were reared in 25 °C with a standard cornmeal, sucrose, and yeast media.

Isothermal calorimetry

Heat dissipation throughout embryogenesis was measured through isothermal calorimetry at Osaka University using the same setup that was previously used to measure frog embryo heat dissipation. Freshly laid embryos were first washed with deionized water to remove yeast. Then, a small number of embryos (n=4-5) were loaded onto a wet filter paper and enclosed in a humidified closed volume ampoule filled with air gas, which was kept under isothermal condition at 22 °C. Heat dissipation rate was reported every 10 seconds and the recorded values were two minute averages (Figure S1) [S1].

Glycogen and triglyceride quantification

Embryos were collected at one hour intervals on apple juice plates with small amounts of yeast paste at room temperature. Pools of ~20 embryos were dechorionated in bleach, washed thoroughly with water, and transferred to sterile microcentrifuge tubes.

For glycogen quantification, glycogen was extracted as described by Parrou and Francoise, 1997 [S3] with minor modifications. Embryos in microcentrifuge tubes were suspended in 50 µM of 0.25 M Na_2CO_3 and boiled at 95-98 C° for four hours. Then, 30 µL of 1 M acetic acid and 120 µL of 0.2 M sodium acetate was added to this solution. This solution was incubated overnight at 57 C° with 1.0 U/mL of amyloglucosidase (Sigma A7420). The next day, we used Glucose Quantification Assay kit (Sigma GAGO20) to determine the amount of glucose present in the sample. In brief, glucose is oxidized to gluconic acid and hydrogen peroxide by glucose oxidase. Then, hydrogen peroxide reacts with a probe that eventually forms a stable colored product. The intensity of the resulting signal is measured through a colorimeter.

For triglyceride quantification, we used the Triglyceride Assay kit (Abcam ab65336) where we used the protocol specified in the kit for tissue samples. In brief, triglycerides are converted to free fatty acids and glycerol, and the glycerol is oxidized into a product. This product reacts with a probe and generates fluorescence that is measured through a microplate reader.

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AUTHOR CONTRIBUTIONS

Conceptualizaton, Y.S., J.O.P., L.T., Y.N., J.D.R. and S.Y.S.; Investigation, Y.S., J.O.P., L.T., and Y.N.; Writing, Y.S. and S.Y.S. with input from all authors; Funding Acquisition, S.Y.S.

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