Video Article

Measurement of Metabolic Rate in *Drosophila* using Respirometry

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Abstract

Metabolic disorders are a frequent problem affecting human health. Therefore, understanding the mechanisms that regulate metabolism is a crucial scientific task. Many disease causing genes in humans have a fly homologue, making *Drosophila* a good model to study signaling pathways involved in the development of different disorders. Additionally, the tractability of *Drosophila* simplifies genetic screens to aid in identifying novel therapeutic targets that may regulate metabolism. In order to perform such a screen a simple and fast method to identify changes in the metabolic state of flies is necessary. In general, carbon dioxide production is a good indicator of substrate oxidation and energy expenditure providing information about metabolic state. In this protocol we introduce a simple method to measure CO_2 output from flies. This technique can potentially aid in the identification of genetic perturbations affecting metabolic rate.

Video Link

The video component of this article can be found at https://www.jove.com/video/51681/

Introduction

The biochemical Kreb's cycle generates ATP through the oxidation of acetate derived from carbohydrates, fats, and proteins producing CO_2. In *Drosophila*, O_2 input is directly correlated with CO_2 output and reflects the level of metabolism[1]. Thus, measurement of CO_2 output has successfully been used in studies related to aging and metabolism[2-5]. Here our laboratory has modified previously designed experimental setups, allowing measurement of CO_2 production in up to eighteen samples without requiring any specialized equipment. Others and we have previously used this method to show differences in metabolic rates in flies that are deficient in the muscular dystrophy associated protein, Dystroglycan (*Dg*)[6-8].

O_2 used for oxidative metabolism is converted into CO_2, which is expelled as respiratory waste. The construction of hand-made respirometers is described that allows for the determination of the rate of O_2 consumed. Flies are placed in a sealed container with a substance that absorbs expelled CO_2, efficiently eliminating it from the gaseous phase. The change in gas volume (decreased pressure) is measured by the displacement of fluid in a glass capillary attached to the closed respirometer.

The main advantage of this technique over others is the cost. Previous studies have measured CO_2 production by *Drosophila* using gas analyzers and technically advanced respirometry systems[1,9]. Despite the more complex equipment, the sensitivity of the method described here is similar to reported values (**Table 1**). Additionally, several other groups have used variations of this technique to determine relative metabolic rates in *Drosophila*[4-6]. Therefore, this assay can be used to generate reliable, reproducible data relevant to *Drosophila* metabolism without the purchase of specialized equipment which can be setup in any lab and can be used for educational purposes.

In general, the accepted techniques to determine the metabolism of an organism is to measure the CO_2 produced, the O_2 consumed, or both[3,4,9]. Though, it can be assumed that one equivalent of O_2 generates one equivalent of CO_2, the precise ratio of CO_2 generated is dependent on the metabolic substrate utilized[10]. Thus, to accurately determine the metabolic rate in energy units it is necessary to measure both O_2 consumed and CO_2 produced. Due to this, the method described here is specifically relevant to comparing differences in CO_2 production between animals and not the absolute value. Our technique integrates multiple animal CO_2 production over a period of time (1-2 hr) and therefore returns an average of the animals' activity. If there is reason to believe that the experimental animals are less active than the control animals the measurement could reflect different levels of activity and not necessarily metabolism.

Protocol

1. Preparation of Respirometers

1. Cut the 1,000 µl pipette tip with a razor blade to allow insertion of the 50 µl capillary micropipette, try to get the pipette tip as straight as possible.
2. Place a piece of foam into the pipette and push it down in the pipette tip.

3. Add a small amount of CO_2 absorbent and contain it by a second piece of foam.
4. Apply glue at the place where the micropipette is inserted into the pipette tip.
5. Leave the respirometer overnight to allow the glue to dry.
   A schematic of a respirometer is shown in **Figure 1A**.

2. Preparation of the Measurement Chamber

1. Prepare the chamber solution by mixing water with eosin in a ratio that will result in visible colorization.
2. Pour the eosin/water solution into the chamber.
3. Label one of the sides of the chamber with a centimeter scale.

3. Placing Flies into Respirometers

1. Label the individual respirometers with a marker.
2. Anesthetize flies using an alternative method to CO_2 and place 3-5 flies of the desired genotype inside each respirometer.
3. Seal the respirometers tightly at the top using plasticine putty.
4. Allow flies to recover from anesthetization for approximately 15 min.
5. Prepare one respirometer without flies, which will be used as the atmospheric control.

4. Performing the Experiment

1. Hang the respirometers in the chamber by attaching a 1.5 ml Eppendorf tube holder that is open on the top and the bottom at the top of the chamber.
2. Insert respirometers with the micropipette tip down into the chamber allowing the tip to submerge into the colored solution.
3. Add Vaseline between the lid cover and the chamber to provide stronger isolation from temperature and pressure fluctuations.
4. Close the lid and allow the system to equilibrate for 15 min.
5. Take a photograph of the chamber making sure that the level of liquid within each micropipette is visible and so is the scale (see example shown in **Figure 1B**).
6. After 1-2 hr, take another picture.
7. When experiment is finished, remove the flies from respirometers and weigh if desired or transfer them back to the vial if needed further.

5. Analysis of Results

1. Open acquired images using ImageJ software[11].
2. Using the scale in each picture, set the pixel scaling in the software.
3. Measure the distance (Δd) that the liquid traveled from a determined reference spot in images taken at the beginning (d1) and end of experiment (d2). A schematic example is shown in **Figure 1C**.
4. Calculate the amount of produced CO_2 (µl/hr/fly) with the formula:

$$\frac{\left((\pi \times R^2) \times (\Delta d) - (\Delta c)\right) \times 1000}{n \times h}$$

R= radius of micropipette tube in centimeters
Δd= distance the liquid has moved up in the micropipette of test samples measured in centimeters
Δc= distance the liquid has moved up in the micropipette of the negative control sample (without flies)
n = number of flies used
h= hours

Representative Results

In order to show that the method is sensitive we measured CO_2 production from wild type (*Oregon R*) male flies at 18, 25, and 29 °C and flies mutant for *Dg*. Flies were raised at 25 °C and then shifted to the experimental temperature for 5 days prior to measurement. As expected for this ectothermic species, the amount of CO_2 produced increased with temperature (**Figure 2**). We have in the past shown that a sugar free diet reduces the metabolic rate of both wild type and *Dg* mutant flies[7]. The loss of *Dg* leads to increased metabolic levels (**Figure 2**).

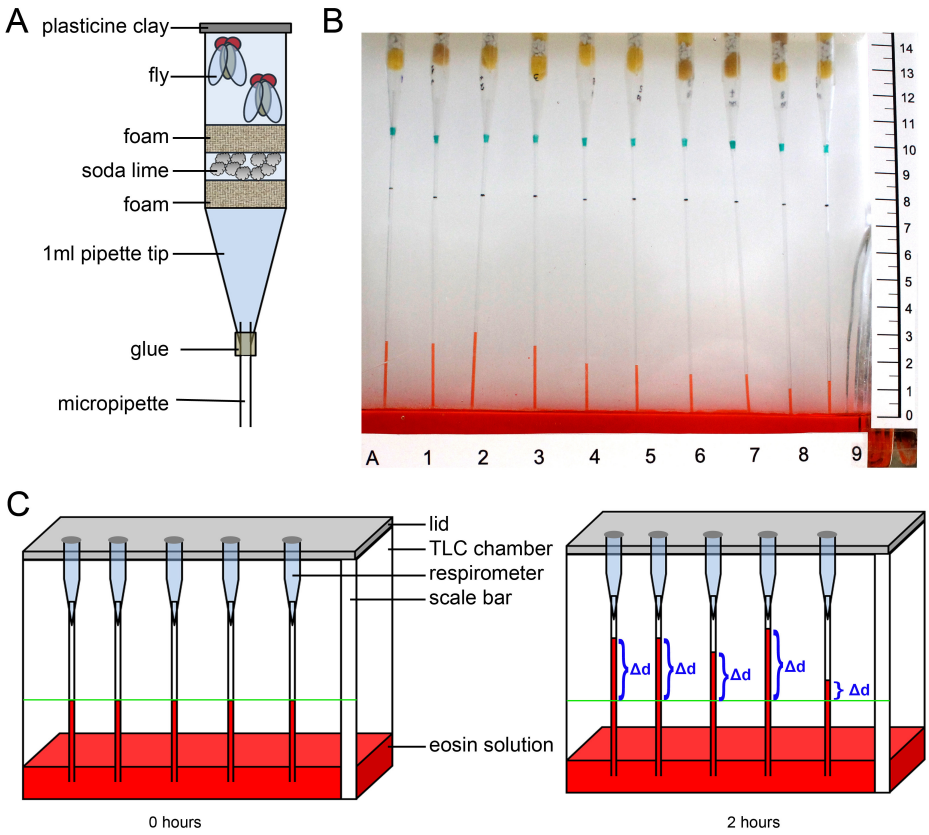

Figure 1. Setup for measuring of CO_2 production in flies. A. Schematic showing the construction of the individual respirometer. **B.** Photograph of the chamber during the CO_2 measurement. Letters mark the position of respirometers. Vertical numbers indicate scale in centimeters. **C.** Schematic showing the changes during the experiment. Green line indicates reference spot (d1), blue indicates the final position of the liquid after 2 hours (d2). Δd is the distance the liquid has traveled in the micropipette.

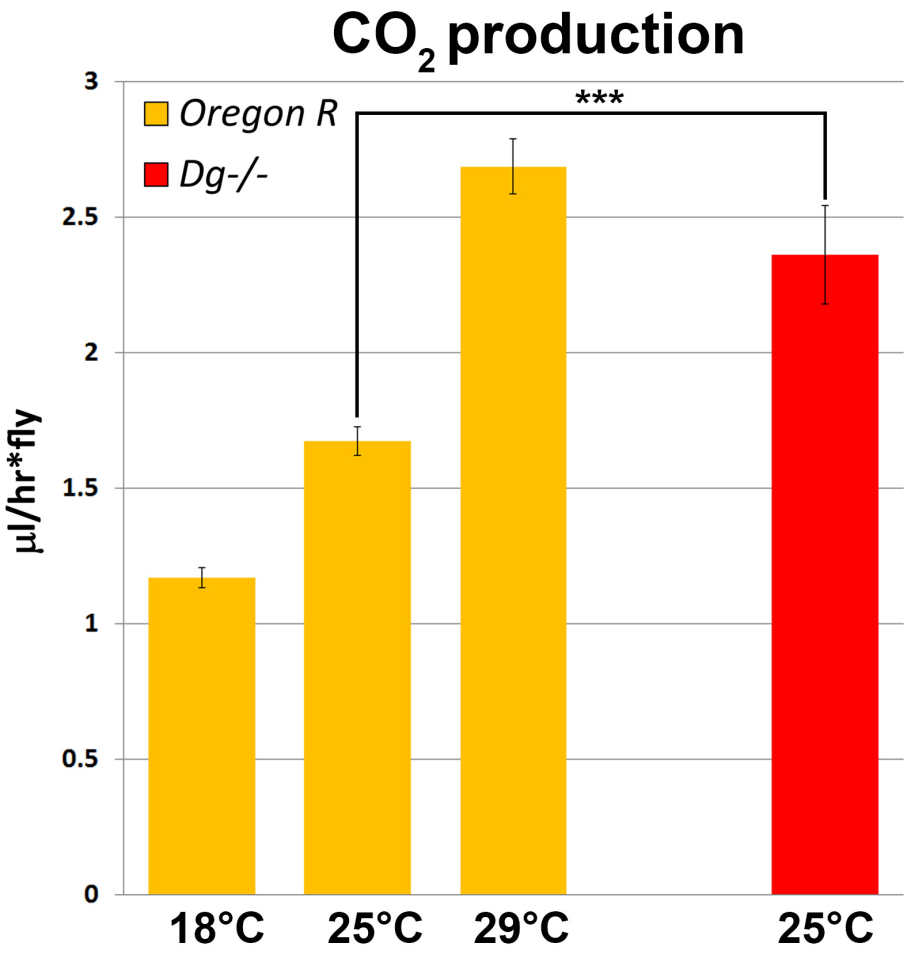

Figure 2. CO_2 production in flies at different temperatures and in *Dg* mutants. Housing temperature positively correlates with CO_2 production in *Drosophila* and is significantly increased in *Dg* mutants. Error bars indicate SEM from four individual experiments, ***p≤0.01.

(µl /fly x hour)	Equipment used	Reference
2-3 CO_2	CO_2 gas analyzer	(Van Voorhies, Khazaeli *et al.* 2004)
4.68 ± 1.04 CO_2	CO_2 respirometry system	(Khazaeli, Van Voorhies *et al.* 2005)
2.9-6.2 O_2	Similarly designed respirometers	(Hulbert, Clancy *et al.* 2004)
2.20 ± 0.15 O_2	Described here handmade respirometers	(Kucherenko, Marrone *et al.* 2011)
		* table values are from studies that report numerical values of flies measured at 25 °C

Table 1. Comparison of different techniques used to measure CO_2 production by *Drosophila*.

Discussion

In this protocol, we describe an inexpensive and reliable method for measuring CO_2 production in flies. We found that this experiment is easy, quick to conduct and generates reproducible data that is in agreement with other studies[1,6,9]. The protocol outlined here can be easily modified to fit any laboratory's budget and available materials. The construction of each individual respirometer can be adapted as long as the chamber remains airtight. However, the longer, thinner micropipettes offer more precision over shorter ones. The use of the outer chamber can be optional as long as there are no significant ambient temperature and pressure changes to compromise the experiment. This can be determined by analysis of the negative control and a large variability within biological sample measurements. Additionally, the CO_2 absorbent can be of any variety as long as it is not toxic to the flies (*e.g.*, potassium hydroxide). It is of utmost importance to have a negative control to ensure that the technique is working properly. The most common problem is for the respirometer to not be sealed completely. If this is the case, then the measurement will be comparable to that of the negative control. Additional problems could arise due to flies that have not had time to recover from anesthetization or have perished.

The most critical step in this protocol is the construction of the respirometer. As noted above, the respirometer must be airtight. The attachment of the micropipette to the larger pipette chamber must be done with the correct glue. A more rubber-like adhesive has worked best. It is

recommended to inspect the respirometers under a microscope to observe any compromises in the glued structure after completion. Additionally, the sealing of the respirometer after flies have been placed inside is very important. The use of putty has been found to work best and fails infrequently. Paraffin has been found to work very poorly and should be avoided. The liquid that is used to measure gas volume is also very important and should be manometric. Water is therefore the best choice. The use of ink is not recommended because it can harden in the capillary making the respirometer useless. It is also critical to not use reproducing females, as we noticed that their CO_2 production can be highly variable. In addition the age of flies is important, therefore the flies that are compared should be of the same age. In our test we used 5 days old males. The genetic background of the flies is also important. The control flies should be of the same genetic background when compared to mutants. The data can also be presented as (µl/hr/mg) by measuring the mass of the flies after performing the assay if there are significant differences in size of flies. In our hands, one wild type fly weighs 0.80 ± 0.11 mg (n = 180). It should also be pointed out that during timing of the experiment, the values of the average metabolic rate are obtained. We have also found that it is possible to measure an individual fly, but we achieved the highest precision using 3-5 flies. The space available in respirometer for flies is enough that they do not feel overcrowded but at the same time they do not have space to walk intensively: therefore defective geotaxis should not have any effect on the level of CO_2 production.

Flies have long been established as one of the major model organisms to study human diseases that are related to developmental biology, cell biology, and neurobiology. A number of recent studies have shown that flies can easily be used to study energy homeostasis as well (reviewed in [12,13]). The method presented here can easily be used in genetic screens to identify new molecular components possibly involved in controlling the metabolic state prior to purchasing more accurate and expensive equipment. Thus far the majority of such screens were done only in cell culture indicating that more advanced studies are warranted.

Disclosures

We have nothing to disclose.

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References

1. Van Voorhies, W. A., Khazaeli, A. A., & Curtsinger, J. W. Testing the "rate of living" model: further evidence that longevity and metabolic rate are not inversely correlated in *Drosophila melanogaster*. *J Appl Physiol.* **97**, 1915-1922, doi:10.1152/japplphysiol.00505.2004 (2004).
2. Ross, R. E. Age-specific decrease in aerobic efficiency associated with increase in oxygen free radical production in *Drosophila melanogaster*. *Journal of Insect Physiology.* **46**, 1477-1480 (2000).
3. van Voorhies, W. A., Khazaeli, A. A., & Curtsinger, J. W. Selected contribution: long-lived *Drosophila melanogaster.* lines exhibit normal metabolic rates. *J Appl Physiol.* **95**, 2605-2613; discussion 2604, doi:10.1152/japplphysiol.00448.2003 (2003).
4. Hulbert, A. J. *et al.* Metabolic rate is not reduced by dietary-restriction or by lowered insulin/IGF-1 signalling and is not correlated with individual lifespan in *Drosophila melanogaster*. *Experimental Gerontology.* **39**, 1137-1143, doi:10.1016/j.exger.2004.04.006 (2004).
5. Ueno, T., Tomita, J., Kume, S., & Kume, K. Dopamine modulates metabolic rate and temperature sensitivity in *Drosophila melanogaster*. *PLoS ONE*. **7**, e31513, doi:10.1371/journal.pone.0031513 (2012).
6. Takeuchi, K. *et al.* Changes in temperature preferences and energy homeostasis in dystroglycan mutants. *Science.* **323**, 1740-1743, doi:10.1126/science.1165712 (2009).
7. Kucherenko, M. M., Marrone, A. K., Rishko, V. M., Magliarelli Hde, F., & Shcherbata, H. R. Stress and muscular dystrophy: a genetic screen for dystroglycan and dystrophin interactors in *Drosophila.* identifies cellular stress response components. *Developmental Biology.* **352**, 228-242, doi:10.1016/j.ydbio.2011.01.013 (2011).
8. Marrone, A. K., Kucherenko, M. M., Wiek, R., Gopfert, M. C., & Shcherbata, H. R. Hyperthermic seizures and aberrant cellular homeostasis in *Drosophila dystrophic.* muscles. *Scientific Reports.* **1**, 47, doi:10.1038/srep00047 (2011).
9. Khazaeli, A. A., Van Voorhies, W., & Curtsinger, J. W. Longevity and metabolism in *Drosophila melanogaster*: genetic correlations between life span and age-specific metabolic rate in populations artificially selected for long life. *Genetics.* **169**, 231-242, doi:10.1534/genetics.104.030403 (2005).
10. Elia, M. Energy equivalents of CO_2 and their importance in assessing energy expenditure when using tracer techniques. *The American Journal of Physiology.* **260**, E75-88 (1991).
11. Schindelin, J. *et al.* Fiji: an open-source platform for biological-image analysis. *Nature Methods.* **9**, 676-682, doi:10.1038/nmeth.2019 (2012).
12. Bharucha, K. N. The epicurean fly: using *Drosophila melanogaster.* to study metabolism. *Pediatric Research.* **65**, 132-137, doi:10.1203/PDR.0b013e318191fc68 (2009).
13. Rajan, A., & Perrimon, N. Of flies and men: insights on organismal metabolism from fruit flies. *BMC Biology.* **11**, 38, doi:10.1186/1741-7007-11-38 (2013).

