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Modeling the *Manduca sexta* Midgut

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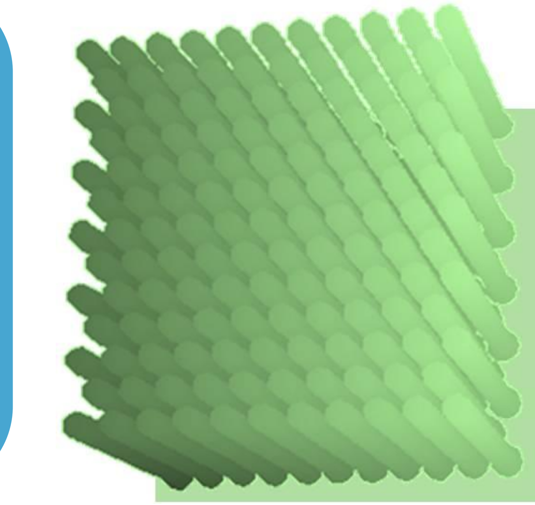

Abstract

Metabolism is the process by which energy obtained through food is used and stored, and for reasons unknown metabolism scales with body weight consistently across species. *Manduca sexta*, a type of caterpillar which grows to maturity in only 18 days and exhibits a 10,000-fold increase in weight, is an ideal organism for studying this scaling of metabolism. It has been suggested that the surface area of the caterpillar's midgut may play a crucial role in metabolic scaling. We present a model for midgut surface area which reflects the surface area contributions of long, thin, finger-like projections called microvilli, larger projections called villi, and folding of the midgut. We also investigate and generalize an interesting discovery related to the contribution of microvilli to midgut surface area. Surprisingly, one of the components of our model that determine the amount of surface area contributed by microvilli remains constant regardless of the dimensions of the microvilli. Finally, we discuss the implications of our model in the study of metabolic scaling, and we discuss some future research.

Introduction

Though scientists have shown that metabolic rate scales with body weight across species (Kleiber, 1932), it remains unclear *why* this occurs(Figure 1).

One possible explanation proposes a link between the amount of surface area available for energy-using processes and metabolic rate (Sernetz *et al*. 1985; West *et al*. 1999); the existence of such a link may explain the scaling of metabolism with body weight.

The current study is part of a larger project, the "Manduca InSTaRs (Interdisciplinary Science Training and Research)" project, which investigates metabolic scaling in a single, well-studied model organism: the *Manduca sexta* larva. These organisms are ideal for studying metabolism because while there is no marked change in behavior and morphology as the larva progress through five instars, they experience a 10,000-fold increase in weight in only 18 days (Goodman *et al.* 1985). These characteristics allow investigators to study a single larva over a wide range of sizes in a relatively short period of time.

Metabolic activity in *Manduca sexta* occurs in the highly folded midgut which contains two types of long fingerlike projections called villi and microvilli (Figure 2). We present a method for calculating midgut surface area that takes into account the surface area contributions of midgut folding, villi, and microvilli.

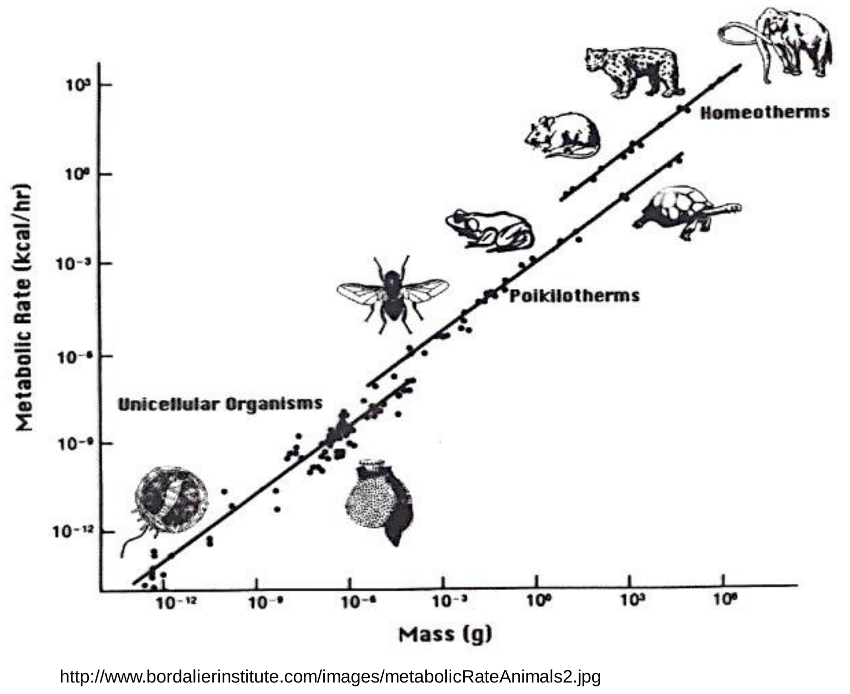

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Figure 1 Metabolic rate scales with body weight across species. Each line shown here has a slope of 0.75.

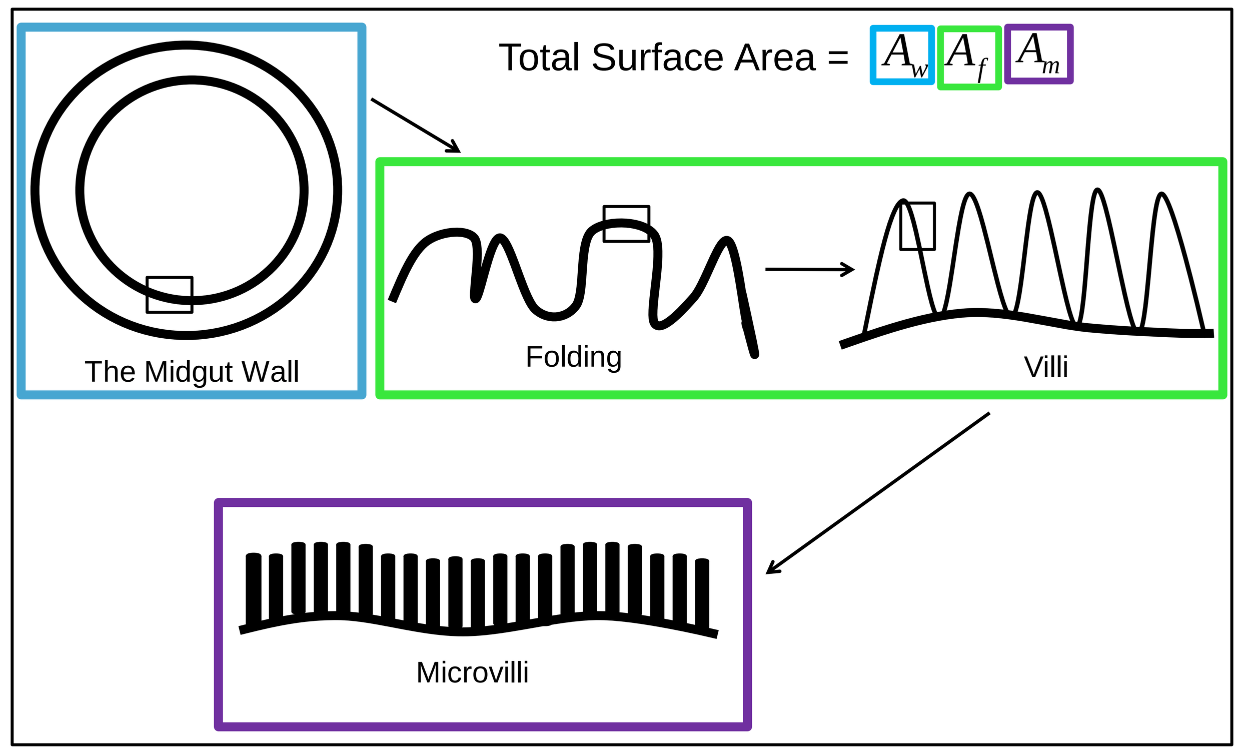


Figure 2 The surface area of the midgut is determined by the surface area of the midgut wall and surface area contributions of folding of the midgut, larger fingerlike structures called villi, and smaller fingerlike structures called microvilli. Colored boxes show which structure is associated with each term of the equation for total midgut surface area.

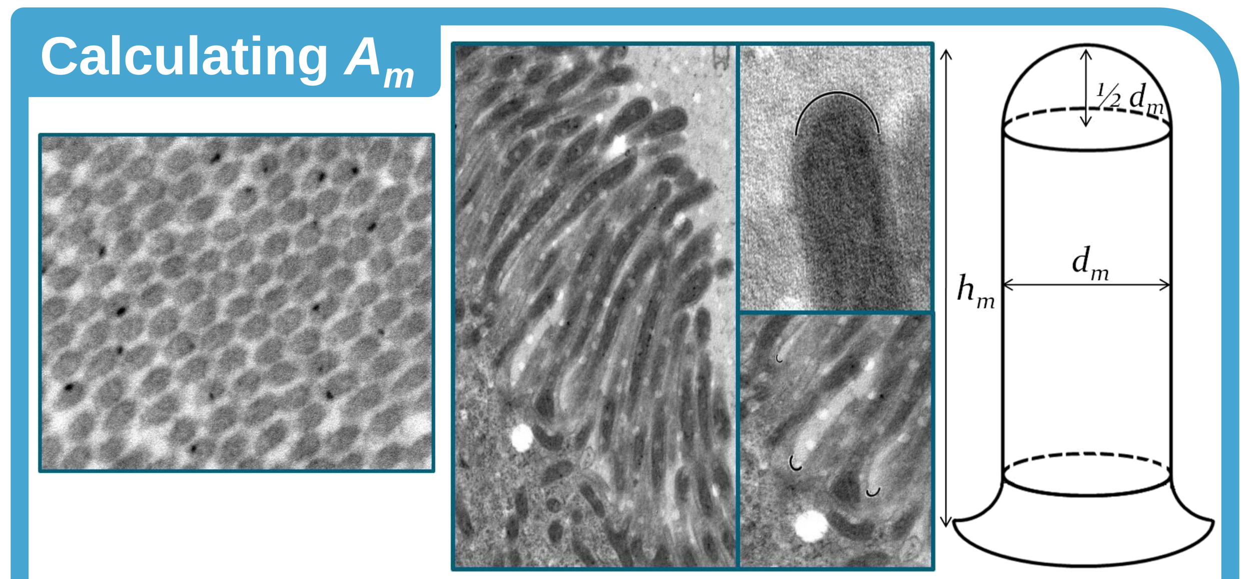
Total Surface Area

The total surface area of the midgut is given by the equation

Total Surface Area = $A_w A_f A_m$

where A_w is the surface area of the midgut wall without folding, villi, or microvilli, A_f is an amplification factor which accounts for the amount of surface area contributed by folding and villi, and A_m is another amplification factor which accounts for the amount of surface area contributed by microvilli (see Figure 2).

Calculating A_m



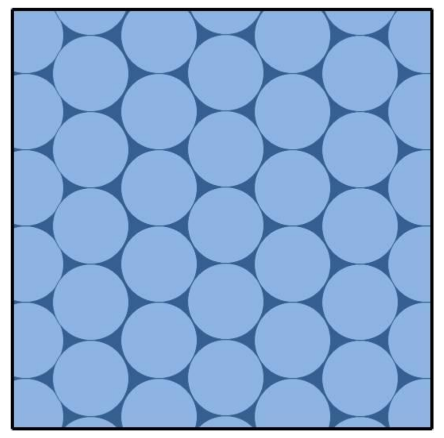
The pictures above all show microvilli. The first four are electron micrographs of microvilli in *Manduca sexta*, and the right-most is a microvillus in our model. From the left-most picture, note that the microvilli are hexagonally packed, that is, each microvillus has six equidistant neighbors, forming a hexagon. From the middle three pictures, note that the microvilli have rounded tops and curve into the midgut wall. The rounded top and curved base can be seen in the picture on the right. Note that d_m is the diameter of the microvillus and h_m is the height. Also, the number of microvilli in a particular region is N_m.

The surface area contribution of microvilli, A_m, which is the surface area of the microvilli that cover a 1 μm² region of midgut wall that is folded and covered with villi, is given by the equation

$$A_m = A_p + N_m(A_s + A_c + A_b).$$

The terms A_s, A_c, and A_b are respectively, the surface areas of the spherical top, cylindrical middle portion, and annular base of a single microvillus; all three are easily calculated. Multiplying by N_m gives the surface area of all of the microvilli in a 1μm² region of midgut. Next, we turn our attention to A_p, the area of the midgut wall not covered by microvilli.

A_p and its Consequences



Consider the portion of the midgut not covered by microvilli, the area of which is A_p. This is the dark blue region in the picture on the right. The light blue circles are the portions of the midgut wall that are covered by microvilli bases. One way to calculate A_p is

$$A_p = 1 - N_m(\text{Area of 1 circle}).$$

Note that we can calculate the area of one circle from the size of a microvillus. We subtract the area of the N_m circles from 1 because A_p gives us the surface area of the portion of a region of midgut with area 1 μm² that is not covered with microvilli. When we plug in the microvilli size parameters and obtain the area of 1 circle, surprisingly, we get that

$$A_p = 1 - \frac{\sqrt{3}}{6}\pi \ ,$$

a constant. This means that A_p does not depend on microvilli size or the number of microvilli in our region. It turns out, that for any region of midgut, A_p depends only upon the area of the region. We can write this statement about the surface area of the space between the microvilli on a region of midgut wall as a theorem about circles in a planar region with finite area. We will need the fact that microvilli are hexagonally packed.

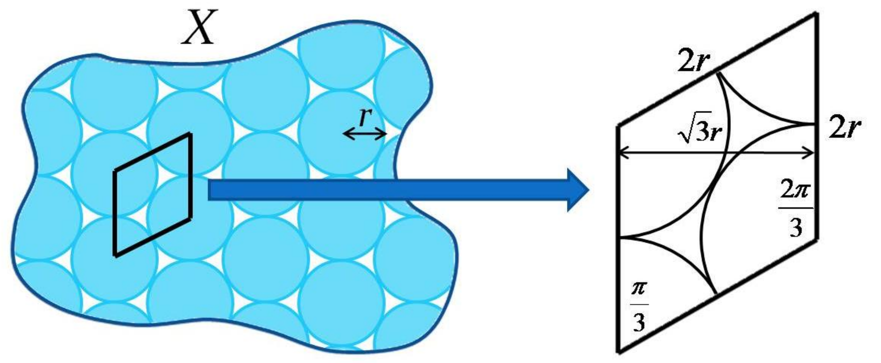
Theorem

Let X be a subset of $\mathbb{R}^2$ with finite area. If X contains $n \geq 1$ hexagonally packed circles, each of radius $r > 0$, $N \subset X$ is the set of these circles, and $N \neq X$ then

$$r = \frac{1}{\sqrt{n}}\sqrt{\frac{A(X)}{2\sqrt{3}}} \quad \text{and} \quad A(N^C) = A(X) - \pi\frac{A(X)}{2\sqrt{3}}.$$

Proof

Let X be a planar region with finite area that contains $n \geq 1$ hexagonally packed circles each with radius r. Connect the centers of four neighboring circles to form a rhombus of side length $2r$.



Note that this rhombus has area $2\sqrt{3}r^2$ and that it contains the equivalent of 1 circle. Also note that X has area $A(X)$ and contains n circles. Since both the rhombus and X contain hexagonally packed circles each of radius r, the ratio of the number of circles in the rhombus to the area of the rhombus is the same as the ratio of the number of circles in X to the area of X. That is,

$$\frac{1}{2\sqrt{3}r^2} = \frac{n}{A(X)} \quad \Rightarrow A(X) = 2\sqrt{3}nr^2 \quad \Rightarrow r = \frac{1}{\sqrt{n}}\sqrt{\frac{A(X)}{2\sqrt{3}}} \ .$$

Now we need to show that

$$A(N^C) = A(X) - \pi\frac{A(X)}{2\sqrt{3}}.$$

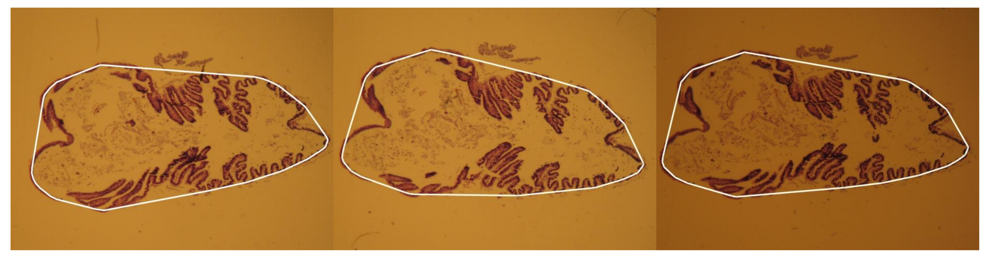
Recall that N is the set of all of the circles in X. This means $A(N)$ is simply the area of n circles. Using this fact and plugging in the value for r which we obtained above gives

$$A(N^C) = A(X) - A(N) = A(X) - n\pi r^2 = A(X) - n\pi\left(\frac{1}{\sqrt{n}}\sqrt{\frac{A(X)}{2\sqrt{3}}}\right)^2$$

$$= A(X) - n\pi\left(\frac{1}{n}\right)\left(\frac{A(X)}{2\sqrt{3}}\right) = A(X) - \frac{\pi}{2\sqrt{3}}A(X) \ .$$

Finding A_w

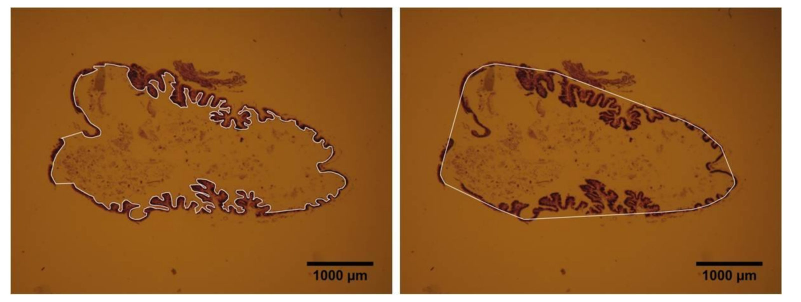
Now we find the surface area of the midgut wall without the contribution of folding, villi, or microvilli. The images that follow are sequential slices of the midgut in a *Manduca sexta* larva. Large amounts of folding and a large number of villi are seen on the midgut wall in these pictures. To get an idea of what the midgut wall looks like without folding and villi, we consider the smallest possible convex, polygonal region that contains all of the interior of the midgut. This polygon is shown in the images below.



We can stack the sequential midgut wall polygons and connect them to form a surface. The surface area of this surface gives us an estimate of A_w, the surface area of the midgut wall with no folding, villi, or microvilli.

Finding A_f

Finding the amplification factor for villi and folding is a bit more difficult since villi and folding vary quite a bit from organism to organism. One possible way to find A_f is to take slices of the midgut and make two perimeter measurements on each slice. The first is the perimeter of the midgut wall including folding and villi. This is the length of the white curve in the picture on the left. The second is the perimeter of the midgut wall without villi, folding, and microvilli. This is the perimeter of the polygon defined above and shown in white in the image on the right. We then divide the first measurement by the second, which gives us A_f.



However, this method is time consuming, as we must measure the actual perimeter for quite a few slices for each section of the midgut of each organism. Finding a way to determine A_f using the numbers or sizes of folds and villi is a project for future research.

Future Work

- Obtain more microvilli parameter measurements and measures of villi and folding contributions to surface area from the pictures we have
- Develop a better way to obtain the villi and folding amplification factor
- Obtain more pictures
- Construct functions for microvilli parameters, A_m, A_f, and A_w, that depend on body weight, age, metabolic rate, etc
- Examine the relationships between calculated midgut surface area and metabolic rate

References

- Goodman, W. G.; Carlson, R. O.; Nelson, K.L. (1985) Analysis of larval and pupal development in the tobacco hornworm (Lepidoptera: Sphingidae), *Manduca sexta*. Ann. Entomol. Soc. Am. 78:70-80.
- Kleiber, M. (1932) "Body size and metabolism. Hilgardia 6: 315-353. (Cited in *Moses et. al*., 2008).
- Sernetz, M.; Gelleri, B.; Hofmann, J. (1985) The organism as bioreactor – interpretation of the reduction law of metabolism in terms of heterogeneous catalysis and fractal structure. J. Theo. Biol. 117: 209-230.
- West, G. B.; Brown, J. H.; Enquist, B. J. (1999) The fourth dimension of life: fractal geometry and allometric scaling of organisms. Science 284: 1677-1679.

Acknowledgements

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