# Differential aminopeptidase expression in *Manduca sexta* larvae based on life stage and dietary protein concentration

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## Abstract

Protein digestion in animals is important for the development and maintenance of tissues. How does an animal extract protein from food and how does this process vary at different dietary protein concentrations? Protein from food is converted into peptides by endopeptidases and those peptides are degraded into amino acids by carboxypeptidases and aminopeptidases (APNs). This study focused on APNs in the digestive process of *Manduca sexta* larvae at different life stages (3rd versus 4th instar) and with different diets (high versus low protein). Midgut tissue was extracted from the caterpillars and the anterior, middle, and posterior regions were isolated. RNA was extracted and reverse transcribed into cDNA which was used to measure mRNA expression using quantitative Real Time PCR. Third instar caterpillars showed higher APN mRNA expression than 4th instar caterpillars. Since proteases are upregulated in earlier stages of life, younger larvae may need more protein for development than larvae at later stages of life. *M. sexta* fed low protein food showed higher APN mRNA expression than those on high protein diets in the anterior midgut but not in the posterior midgut. These data suggest that when the animal was placed on a low protein diet, it became more efficient at digesting protein. When there was an abundance of protein in the diet, lower APN expression may have been adequate for most of the protein needs. Protein is digested mostly in the anterior midgut, which is why APN expression may have been upregulated in the anterior rather than the posterior midgut.

## Introduction

In nature, animal size spans 21 orders of magnitude (West *et al*. 1997). As the body mass of an animal increases, the surface area of the animal increases by the square while volume increases by the cube (West *et al*. 1997). Body mass affects functions of bodily systems, including metabolism. If metabolic rate is a function of geometric size, it should be scaled to the 2/3 based on surface area to volume ratio. However, studies have found that most biological occurrences actually scale to the ¾. It has been proposed that metabolism scales to an increased rate due to the branching of linear systems in transporting materials throughout the body (West *et al.* 1997).

*Manduca sexta* is a great model organism for studying the effect of size on metabolic rate because it grows 10,000 fold over its larval stage (Greenlee and Harrison 2005). The midgut is the responsible organ for digesting and absorbing nutrients in the body (Wieczorek *et al.* 2009). **How does the expression of digestive enzymes and absorptive transporters change with body size?**

In addition to the metabolism question, we asked whether varying levels of protein in the diet affected the expression of enzymes in the gut. *M. sexta* larvae feed on tobacco leaves (Diamond *et al.* 2010), which fluctuate in protein level during the growing season (Kingsolver and Woods 1998). Decreased consumption and increased growth rate have been shown to result from a high protein diet (Kingsolver and Woods 1998). The effect of protein concentration on digestive and absorptive processes has not been studied. **How does dietary protein concentration affect the expression of digestive enzymes and absorptive transporters?**

Four midgut proteins were studied: Aminopeptidase 3 (APN3) and Aminopeptidase 4 (PepN) which are involved in the breakdown of proteins (Wang *et al*. 2005), a potassium-amino acid cotransporter (KAAT) which functions in absorption of amino acids into the body, V-ATPase subunit e, a vacuole-type proton pump which powers KAAT, and masBSC. All four enzymes play a role in the breakdown of proteins and uptake of amino acids into the caterpillar's body.

**We predict that as dietary protein concentration increases, there will be an increase in expression of enzymes involved in the digestion and absorption of protein to account for the extra protein consumed. We also expect to see an increase in digestive enzymes and absorptive transporters as the animal increases in size due to higher metabolic rates and lower surface area to volume ratios.**

## Results – Body size

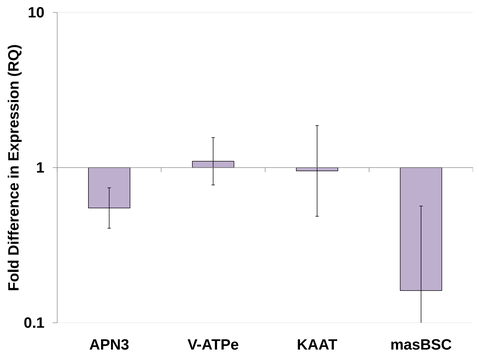



**Figure 1: APN3 expression decreased in the whole midgut from 3rd to 4th instar *Manduca sexta*.** Expression of APN3 was 2 fold higher in the 3rd instar than the 4th instar (n=9, p=0.093). No difference was seen in V-ATPase subunit e (n=9, p=0.797) or KAAT (n=9, p=0.946) expression in the different instars.

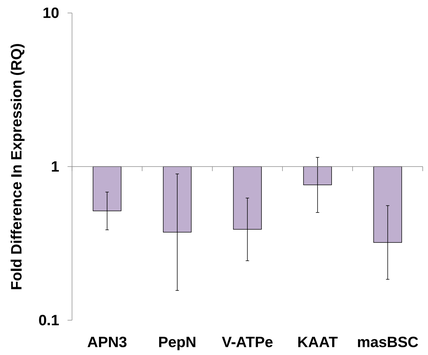



**Figure 2: APN3 expression decreased in the posterior midgut from 3rd to 4th instar *Manduca sexta*.** Expression of APN3 was 2 fold higher in 3rd instar versus 4th instar caterpillars (n=10, p=0.057). No difference was seen in expression of V-ATPase subunit e (n=10, p=0.085) or KAAT (n=10, p=0.527) between 3rd and 4th instar.

## Results – Dietary protein

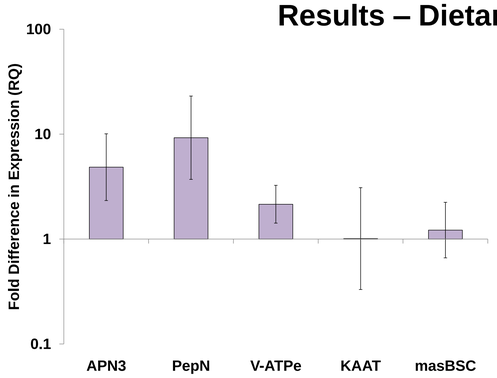
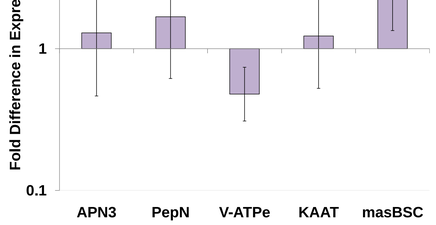



**Figure 3: APN3 and PepN expression was higher in the anterior midgut of *Manduca sexta* reared on low protein diets compared to those reared on high protein diets.** APN 3 was 4.8 fold higher in the larvae fed low protein than the measured expression in those reared on high protein (n=9, p=0.042). PepN also showed higher expression in the low protein treatment than the high protein treatment (9.2 fold increase, n=9, p=0.051).

**Figure 4: No difference in expression was observed in the posterior midgut of *Manduca sexta* caterpillars reared on high and low protein diets.** Expression of APN3 was 1.3 fold higher in caterpillars reared on a low protein diet compared to those reared on a high protein diet (n=10, p=0.408). PepN showed a similar trend: expression was 1.7 higher in the low protein diet than the high protein diet (n=10, p=0.488).




## Methods

*Manduca sexta* (California Biological) were reared at 27 °C on a 16L:8D photoperiod. *M. sexta* were randomly assigned to a high protein diet (36 g Casein) or a low protein diet (12 g Casein) and also time of dissection was randomly assigned to 3rd, 4th, or 5th instar. Food was changed every 3 days. Twenty four hours after a molt, midguts from 3rd, 4th, and 5th instar caterpillars were removed. Anterior, middle, and posterior regions of the midgut were isolated and stored at -80 °C. Total RNA was extracted from tissues using the RNA-STAT 60 reagent (Tel-Test) and quantified using nanospectroscopy. RNA was rid of Genomic DNA using the TURBO DNA-free kit (Ambion). Five µg DNA-free Total RNA was reverse transcribed into cDNA with random hexamers using the Taqman Reverse Transcription kit (Applied Biosystems). Primers were designed on Primer Express software (Applied Biosystems), were synthesized (Operon), and optimized. Quantitative real-time PCR reactions (SYBR Green, Applied Biosystems) were performed in triplicate on a 96-well microtiter plate using the relative quantification ΔΔCt method on an ABI prism 7500 sequence detection system.

## Conclusions

Body size: Only Aminopeptidase 3 showed a difference in expression as a function of body size while the other genes examined showed no difference (Figures 1 and 2). APN3 was downregulated in the 4th instar larvae indicating that protein digestion might be more important in the growth and development of larvae at earlier life stages.

Dietary protein: Aminopeptidase expression was up-regulated only in the anterior midgut (Figure 3) by low protein diets, perhaps as a means to balance protein and carbohydrate intake.

## Acknowledgements

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## References

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