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Tests of Diet and Insulin-like Peptides on the Duration of the 5th Instar of Male Lubber Grasshoppers

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Introduction

Consuming fewer calories while maintaining adequate nutrition (i.e. calorie restriction), even if that diet is started late in life, rapidly increases longevity by slowing the aging process (Mair et al. 2003). The mechanisms underlying rapidly reduced mortality rate in response to late-onset calorie restriction are largely unknown. Calorie restriction is associated with lower insulin-like peptides (ILP) levels in many organisms (Tater et al. 2003). We have shown that calorie restriction throughout adulthood, and late-onset calorie restriction, increases longevity in female grasshoppers (Wells et al. 2005; Hatle et al. 2006). The exact role of ILP in the enhanced longevity due to late-onset calorie restriction is unclear. Consequently, there is a need for experiments in which insulin signaling is manipulated only late in life.

Insulin is the hormone secreted by the pancreas of vertebrates that aids in the membrane transport of glucose from the blood into the body cells (Marieb 2005). More generally, insulin directs the absorptive state. Typically, insulin levels increase upon feeding, at which time energy is available for cells to grow. In this role, insulin acts as a growth regulator and signals cells to divide. In lower organisms like insects, ILP are present and appear to act primarily as growth regulators, with little role in glucose metabolism (Ebberink et al. 1989). Previous research on fruit flies has shown that removal of insulin-producing cells causes developmental delays and growth retardation (Rulifson et al. 2002). Hence, we sought to develop an experimental system to test the role of insulin signaling in calorie restriction. The duration of the 5th instar (the stage prior to adulthood) is affected by diet level in juvenile male lubber grasshoppers (Hatle et al. 2003). Developmental events that are affected by calorie intake likely are affected by insulin signaling. To determine whether maturation to adult molt in grasshoppers can be affected by insulin signaling, we injected grasshoppers with an antibody to human insulin (anti-insulin) and measured developmental timing.

Methods

Selected grasshoppers were randomly assigned to one of three treatment groups. The first treatment group was a control group injected with anti-IgG antibody from day three until day eight while being fed *ad libitum*. This group was not predicted to experience lower levels of insulin in the hemolymph or delayed developmental timing. The second treatment group was fed *ad libitum* and received injections of anti-insulin from day three until day eight, which was predicted to bind and inactivate ILP present in the grasshoppers' hemolymph. Therefore, this group was predicted to demonstrate an increase in the duration of the 5th instar. Grasshoppers assigned to the third treatment group were fed a reduced diet (completely consumed) from day three until day eight and were then fed *ad libitum* from day eight until adult molt. This group was also predicted to experience an increase in the duration of the 5th instar.

Two experimental trials were performed with the only difference being that the treatment period for the second trial was extended from day one until day eight, as opposed to day three until day eight during the first experimental trial.

Results

The first and second experimental trials yielded very similar results. Average body mass (g) of the grasshoppers was reduced only by calorie restriction, and only during the period of reduced diet (Figures 1 and 2).

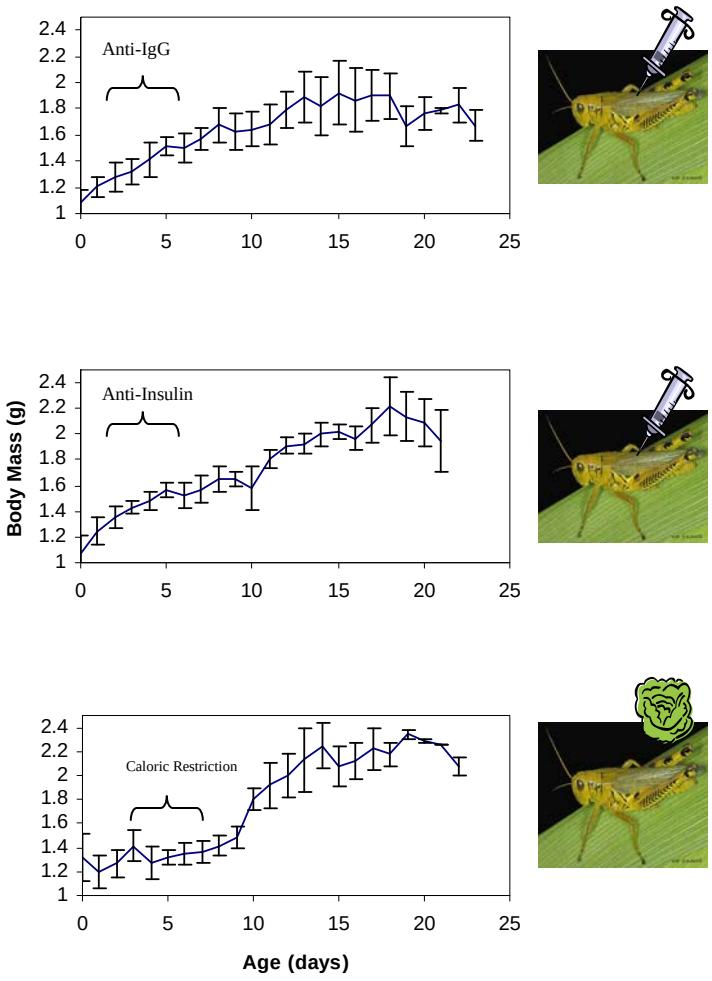

Figure 1 Body mass and duration of 5th instar for first trial

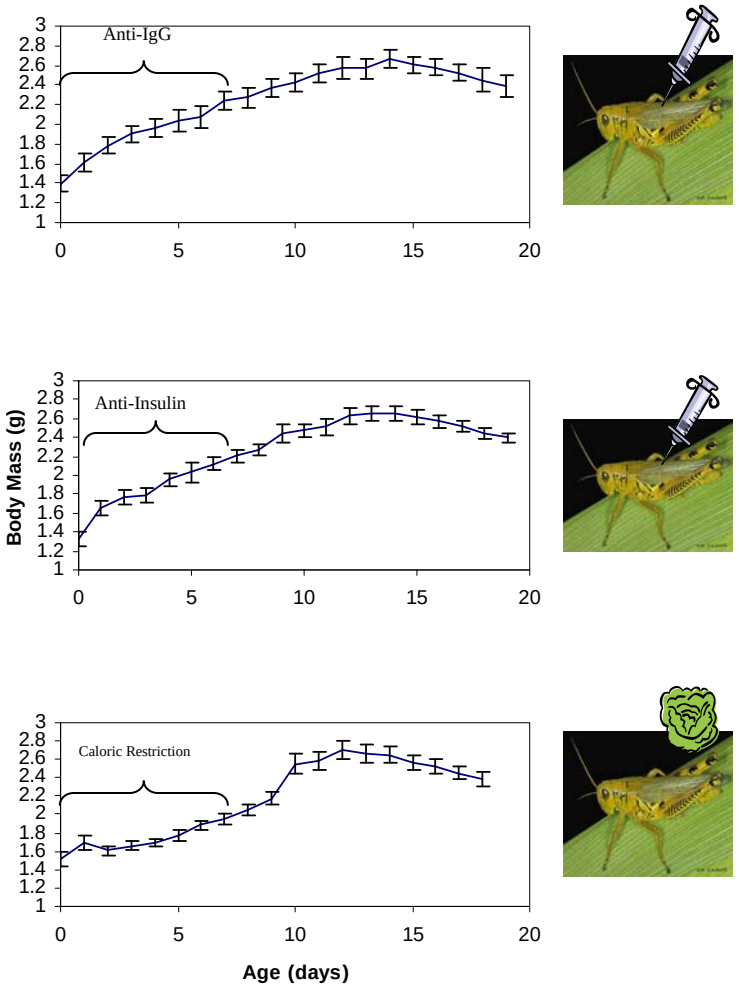

Figure 2 Body mass and duration of 5th instar for second trial

The grasshoppers in the anti-IgG treatment groups did not exhibit any significant growth or developmental timing effects, as predicted. Contrary to prediction, the anti-insulin treatment groups showed no significant change in the duration of the 5th instar. The grasshoppers in the caloric restriction treatment groups also demonstrated no significant change in the duration of the 5th instar (in contrast to a similar experiment by Hatle et al. 2003), but did exhibit lower body masses during the treatment period than grasshoppers in the other treatment groups.

The average age at adult molt was also calculated and compared amongst the three different treatment groups. The anti-insulin and caloric restriction treatment groups were predicted to have higher average ages at adult molt than the anti-IgG control group.

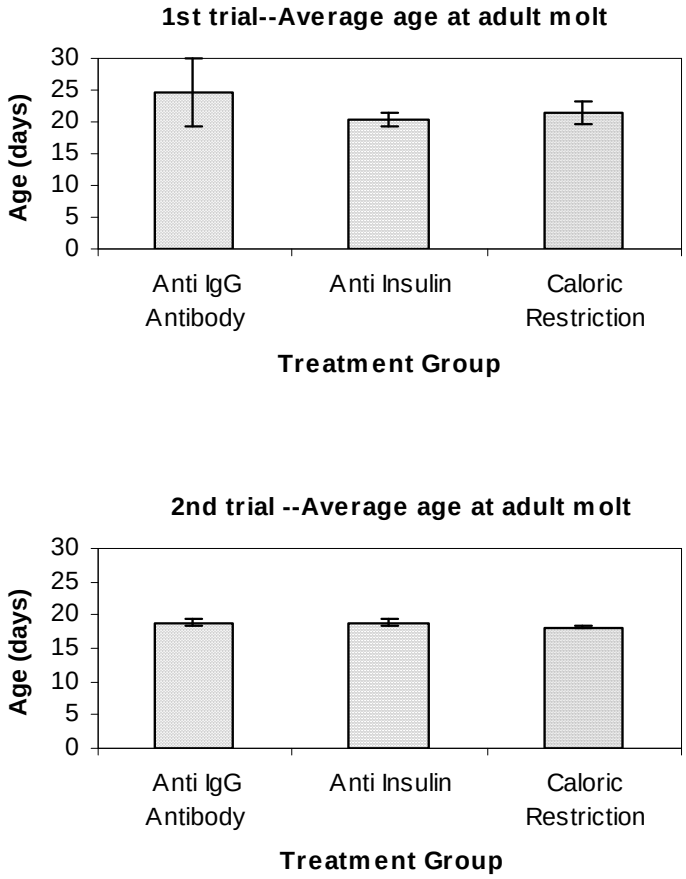

Figure 3 Average age at adult molt for treatment groups during first and second experimental trials

For the first experimental trial, the average age of the grasshoppers at adult molt between the three different treatment groups was not significantly different (Figure 3). The results from the second experimental trial were similar to the first as there was also no significance difference between the ages at adult molt for each of the treatment groups.

The average percent weight gain was also measured for each treatment group in the first and second experimental trials (Figure 4).

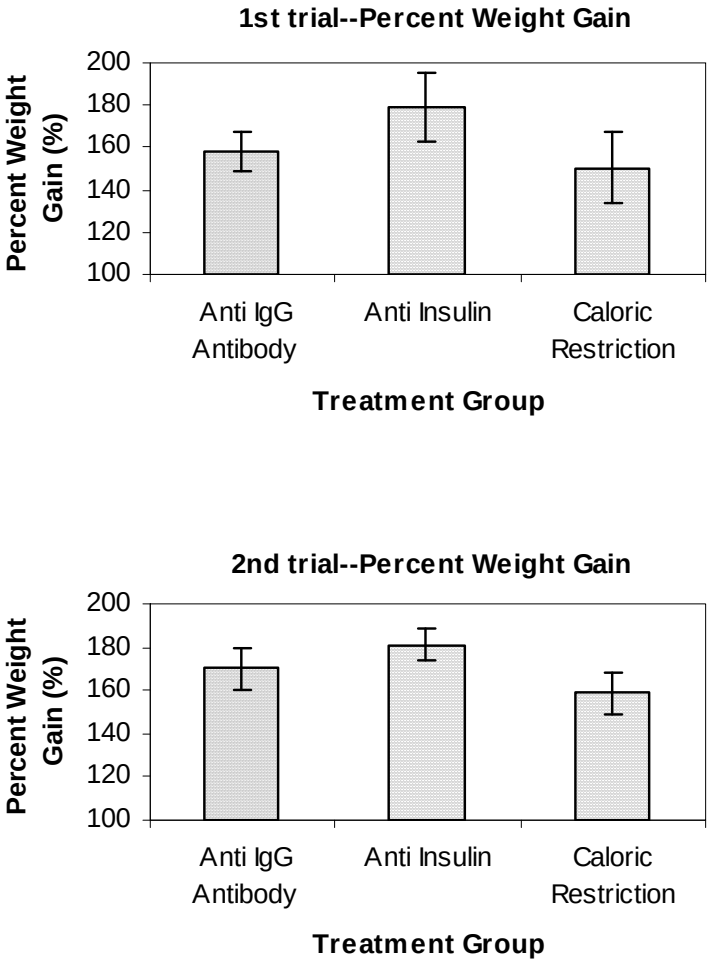

Figure 4 Average percent weight gain for treatment groups during first and second experimental trials

In both experimental trials, the anti-insulin treatment group had non-significantly higher weight gain, whereas, the caloric restriction treatment group has non-significantly lower weight gain. For both experimental trials, the percent weight gain between all treatment groups was not significantly different.

Discussion and Conclusion

There were no significant differences in durations of the 5^{th} instar among anti-IgG (control) injected, anti-insulin injected, or calorie restriction grasshoppers. These data suggest that our anti-insulin treatments did not delay development. However, caution is warranted in concluding that insulin-like signaling molecules play no role in grasshopper development. It may be that our anti-insulin, developed to vertebrate insulin, did not bind the insulin-like molecule of a distantly related insect. Nevertheless, we failed in our attempt to develop an experimental system to manipulate insulin signaling in grasshoppers.

The percent weight gain results showed more promising data. During caloric restriction, percent weight gain was slightly lower for the caloric restriction group than the control and anti-insulin groups, although not statistically significant. This could indicate a delay of growth due to restricted diet. However, immediately following calorie restriction, the percent weight gain rapidly increased. This, in conjunction with the inability to lengthen the 5^{th} instar, suggests our calorie restriction treatment did not delay development. We used identical methods to Hatle et al. (2003), in which development to adult was delayed by a reduced diet. It is unclear why the protocol of Trial 2 failed to replicate the result of delaying development. This species of grasshopper has population variation in the physiological regulation of developmental timing (Hatle et al. 2004). Perhaps the grasshoppers from Jacksonville used in this experiment require less nutrition than the animals used by Hatle et al. (2003) from a colony founded with Miami grasshoppers.

Performing more extensive tests, using different formulations of anti-insulin and more extreme levels of calorie restriction, may be useful for developing a system to manipulate the insulin signaling of grasshoppers. This could lead to research on the effects of caloric restriction and modified insulin signaling on longevity.

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