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Calorie restriction enhances longevity without reducing lifetime fecundity or glucose titers in female lubber grasshoppers

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Abstract

Calorie restriction, under eating while avoiding malnutrition, enhances longevity in many organisms, in part by delaying fecundity or lowering blood glucose. Calorie restriction begun at middle-age can also enhance longevity. We tested four diets on longevity of female lubber grasshoppers: *ad libitum* (free access to food), calorie restriction (60% or 71% of *ad libitum*), and delayed calorie restriction (60% after day 50). Constant calorie restriction increased longevity in grasshoppers by at least 66%. These diets lowered body mass, but it did not reduce lifetime fecundity or chronically lower blood glucose levels. Calorie restriction reduced the levels of stored protein after egg laying. Delayed calorie restriction also increased longevity (also by 66%), but because the body mass gain of this group did keep pace with *ad libitum* grasshoppers when they were fed identically, this result needs retesting. These extensions of lifespan likely are due to slowed aging.

Introduction

Calorie restriction, under eating while avoiding malnutrition, enhances longevity by slowing aging in yeast, worms, fruit flies, mice, and other organisms (Longo and Finch 2003; Tatar et al. 2003). Calorie restriction often acts in part by delaying or reducing allocation to reproduction, through a trade-off between early fecundity and longevity (Rose 1991). These reduced diets are associated with reduced body mass, lower insulin and blood glucose levels, production of stress proteins (i.e., heat shock proteins) (Arking 1998), and even reduced incidence of cancers (e.g., Pugh et al. 1999). The exact mechanism(s) by which calorie restriction delays aging is unclear, but minimizing accumulation of advanced glycation end-products appears to play a role (Arking 1998). Calorie restriction applied late in adult life can also enhance longevity, and these delayed calorie restriction regimes have recently been shown to reduce senescence within only a few days (Cao et al. 2001; Mair et al. 2003; Dhahbi et al. 2004). In addition, intermittent fasting (free access to food one day, only water the next day) in mice produces many of the physiological benefits of calorie restriction without limiting the cumulative number of calories consumed, but instead altering when those calories are consumed (Anson et al. 2003).

Invertebrates are model organisms for studying the mechanisms that can slow aging. Fruit flies (*Drosophila melanogaster*) are an especially widely used insect model for studying the genetics of aging (Hekimi and Guarente 2003; Longo and Finch 2003). While fruit flies are a superb experimental system for gene manipulation, they are too small for experiments in which individuals are bled and tracked throughout adult life.

Hence, we have begun a research program using the Eastern lubber grasshopper, *Romalea microptera*, to study longevity and aging.

Lubber grasshoppers are large insects (gravid females can reach 15 g). Compared to mice (the most common biomedical research model) they are inexpensive to collect and rear. In addition, the ~80 day adult lifespan of lubber grasshoppers is more manageable than the >2 year lifespan of mice. Previously, we have shown that greatly reduced diets (i.e., ~35% of *ad libitum* feeding) delay the laying of the first clutch (Hatle et al. 2004). The physiological underpinnings of reproduction, including peaks of juvenile hormone, ecdysteroids, and the egg-yolk precursor vitellogenin, are similarly delayed (Hatle et al. 2000; 2001; 2003). Through the reproduction vs. longevity trade-off, delayed reproduction could be predicted to be associated with greater longevity.

In the present experiment, we tested whether calorie restriction in adults would enhance longevity in lubber grasshoppers. In addition, we tested whether this (putative) increased longevity was associated with reduced fecundity, protein storage, or blood glucose levels.

Materials and Methods

Experimental animals and rearing

We collected juvenile *Romalea microptera* in Jacksonville, FL, USA, and reared them in the lab *en masse* at room temperature. They were fed Romaine lettuce *ad libitum*, and green beans and green onions occasionally. On the day an individual molted to adult, it was transferred to a 500 cc ventilated plastic container and a 14L:10D photoperiod with a corresponding 32ºC:24ºC thermocycle. We ran parallel experiments with both males and females, but because only 8 of 60 males had died when the experiment was terminated, we report data only for females.

Diet regimens

We tested five diet regimens on longevity: *ad libitum*, calorie restriction, delayed calorie restriction, intermittent fasting, and intermittent fasting control (Table 1). We had six, planned pairwise comparisons within the five diets: *ad libitium* vs. each of the other four diets, calorie restriction vs. delayed calorie restriction, and intermittent fasting vs. intermittent fasting control. Each day an individual was offered lettuce, it also received ~ five flakes of dry oatmeal. Each day an individual was not offered lettuce, the interior of its cage was misted with water.

Because the amount of food consumed by female lubber grasshoppers during adulthood is variable, we constantly adjusted the amount offered to the calorie restriction group, in response to the amount that was consumed by the *ad libitum* group. Each day, we measured the amount of dry lettuce consumed by the *ad libitum* group. Combining data from the previous 2 – 8 calendar days of the experiment, we calculated the average amount consumed per grasshopper per day for the *ad libitum* group. This average eaten by *ad libitum* grasshoppers was multiplied by 0.60 to obtain the amount of lettuce to offer each individual in the calorie restriction group. This same amount was also offered to the delayed calorie restriction group after day 50. Similarly, to determine the amount to offer individuals in the intermittent fasting control group, we calculated the average amount consumed per grasshopper per feeding day for the intermittent fasting group. This average eaten by intermittent fasting grasshoppers on the previous 1 – 4 feeding days was multiplied by 0.50 to obtain the daily amount of lettuce to offer each individual in the intermittent fasting control group.

Assignment to treatment groups

Individuals were assigned to the five feeding treatments by a counterbalanced method. This method of assignment was necessary because the amount consumed by some groups determined the amount of food to offer other groups, so data was needed from the determinant groups before the dependent groups could be started. Our sample size goal was 12 grasshoppers in each feeding treatment (i.e., 60). The first 18 females to molt to adult were assigned serially to the *ad libitum*, delayed calorie restriction, and intermittent fasting groups. The next 24 females to molt were assigned to the calorie restriction and the intermittent fasting control groups. Last, the next 18 females to molt were assigned serially to the *ad libitum*, delayed calorie restriction, and intermittent fasting groups. This assignment method allowed us to obtain data on the amounts to feed calorie restriction and intermittent fasting control groups, without greatly biasing our assignments to treatment groups. All the females molted to adult during a 12 day period (all but one within a 10 day period).

Hemolymph samples

From one-half of the individuals, we collected hemolymph samples (see Hatle et al. 2000 for methods) for determination of total proteins and glucose. Samples were collected every 7 days, beginning at median day 15 (range 14 – 18). They were stored in hemolymph buffer (see Hatle et al. 2001) at -20ºC until analysis. Total hemolymph protein levels were determined using the Bradford (1976) assay with bovine serum albumin standards. Glucose levels were determined using a hexokinase glucose assay kit (Pointe Scientific, Canton, MI, USA), which measures the accumulation of NADH as glucose is converted to glucose-6-P and then gluconate-6-P. Because the hemolymph absorbed light at 340 nm (the wavelength used to measure NADH), we ran a blank with no hexokinase reagent for each sample. This method was validated by running a standard curve of glucose in samples with hemolymph, which after subtracting out the blank was nearly identical to a standard curve in buffer.

Results

Amounts eaten

Our “on-the-fly” methods were effective in implementing calorie restriction in the appropriate groups (Fig. 1). Through the median age at death for the *ad libitum* group (day 81), the calorie restricted group ate 58.8% of the lettuce eaten by the *ad libitum* group. The similar amounts eaten by the *ad libitum* and calorie restricted groups from days 22 – 29 and days 51 – 61 are due to the lag in our calculations of amounts eaten (see Diet Regimens in Materials and Methods). Similarly, the delayed calorie restriction group ate 99.8% of that eaten by the *ad libitum* group before the diet switch (days 0 – 50), but only 61.7% of that eaten by the *ad libitum* group after the diet switch (days 51 – 81).

Female lubber grasshoppers given free access to lettuce increase consumption from adult molt until day 11, decrease consumption until day 31, and then increase consumption again to day 41 (Fig. 1). This pattern likely reflects the egg production cycle of lubber grasshoppers. Females lay their first clutch at ~40 days, and then lay subsequent clutches every ~17 days.

Through the median age at death for the intermittent fasting group (also day 81), the intermittent fasting control group ate 88.7% of that eaten by the intermittent fasting group. This lower consumption by the intermittent fasting control group is due to the fact that they were offered the same amount that was consumed by the intermittent fasting group, and any food left remaining was not compensated for in subsequent days. One individual in particular in the intermittent fasting control group regularly left food uneaten. Finally, through day 81, the intermittent fasting control group ate 71.2% of that eaten by the *ad libitum* group.

Body masses

The calorie restricted (60%) diet reached a lower maximum body mass than the a*d libitum* group (Fig. 2). *Ad libitum* females attained their peak weight of 13.2 g at day 31 and maintained this weight until day 81 (the median age at death for the *ad libitum* group). Calorie restricted females attained their peak weight of 11.7 g at day 32 but then lost weight slowly until a nadir of 9.3 g at day 68. In contrast to the *ad libitum* group, delayed calorie restriction females attained a lower maximum body mass of 12.3 g at day 33, despite the fact that both groups were fed *ad libitum* until day 50. The delayed calorie restriction group then lost weight slowly until a nadir of 9.2 g at day 74. In fact, the delayed calorie restriction group had body mass similar to the calorie restriction group from day 35 until the end of the experiment.

The intermittent fasting and the intermittent fasting control groups had similar growth rates throughout the experiment. The body masses of the intermittent fasting group fluctuated an average of 1.56 ± 0.06 g daily, but their weights over several days were always similar to the intermittent fasting control group. When feeding and fasting days were mixed, intermittent fasting females attained their peak weight of 12.9 g at day 39, and then lost weight slowly until a nadir of 9.3 g at day 80. The intermittent fasting control group attained their peak weight of 12.1 g at day 31, and then lost weight slowly until nadirs of 10.3 g at day 43 and 9.8 g at day 59.

Survivorship

Median ages at death were compared using the Kaplan-Meier time-failure analysis (SAS Proc LifeTest; SAS Institute Inc., 1999). The median ages at death, in days, were: *ad libitum* – 83; calorie restricted (60%) – 135; delayed calorie restriction – 136; intermittent fasting – 83; intermittent fasting control (71%) – greater than 165. The medians were significantly different (Wilcoxon $\chi^2 = 22.5$; df = 4; $P = 0.0002$). We conducted the six, planned, pairwise post-tests indicated (Bonferroni-corrected $\alpha = 0.0083$): *ad libitium* vs. each of the other four diets, calorie restriction vs. delayed calorie restriction, and intermittent fasting vs. intermittent fasting control. Calorie restricted (60%; $\chi^2 = 1863$; $P << 0.001$), delayed calorie restriction ($\chi^2 = 1739$; $P << 0.001$), and intermittent fasting control (71%; $\chi^2 = 3096$; $P << 0.001$) groups all had much greater longevity than the *ad libitum* group (Fig. 3; pairwise Kaplan-Meier time-failure analyses; all df = 1). The intermittent fasting group also had slightly greater longevity than the *ad libitum* group ($\chi^2 = 349$; $P > 0.001$), largely because the maximum age of death was greater for the intermittent fasting group. The delayed calorie restriction group had statistically greater longevity than the calorie restriction (60%) group ($\chi^2 = 28$; $P < 0.001$), likely because there were more delayed calorie restriction individuals alive at the end of the experiment. Finally, the intermittent fasting control (71%) group had much greater longevity than the intermittent fasting group ($\chi^2 = 1404$; $P << 0.0001$).

Lifetime fecundity

Because early reproduction can trade-off with longevity, we tested the effects of diet on the age at first oviposition. Diet significantly affected age at first oviposition (Fig. 4; ANOVA; $F_{4,48} = 3.68$; $P = 0.011$), with intermittent fasting control (75%) females laying significantly younger than intermittent fasting females ($P = 0.001$). Figure 4 shows the ages at laying and masses of all clutches produced during the experiment (not only the first clutches). Mean ± SE ages at first oviposition in days were: *ad libitum* – 49.4±2.7; calorie restricted (60%) = 50.9±3.4; delayed calorie restriction = 43.1±1.8; intermittent fasting = 55.9±3.1; and intermittent fasting control (75%) = 45.8±1.8. No other planned pairwise comparison of ages at first oviposition was significantly different. In addition, diet did not affect the dry mass of the first clutch ($F_{4,48} = 0.70$; $P = 0.596$). Mean ± SE masses of the first clutch in dry mg were: *ad libitum* – 1180±140; calorie restricted (60%) = 1000±130; delayed calorie restriction = 1040±80; intermittent fasting = 1200±130; and intermittent fasting control (75%) = 1030±70.

Lubber grasshoppers have an obligate egg diapause as their overwintering stage. Because of this, the cumulative number of eggs laid by a female may be more important than the date at which these eggs are laid. Hence, for each female, we calculated the lifetime output of oviposited material, which as dry weight is mostly egg protein. These estimates of lifetime fecundity were compared among diets by ANOVA. Diet did not affect the overall production of egg mass (Fig. 5; $F_{4,57} = 1.32$; $P = 0.275$). Post-tests of planned comparisons showed that the intermittent fasting control (71%) group produced greater clutch mass than the *ad libitum* group ($P = 0.0449$). No other planned comparisons were significantly different.

Hemolymph protein profiles

Total hemolymph protein in lubber grasshoppers is a good estimate of the levels of three storage proteins, which together make up 70% of hemolymph protein. Hence, the total hemolymph protein is a good index of storage for reproduction (Hatle et al. 2001). We compared the total hemolymph protein levels across the five diets during the first six sampling dates (i.e., once weekly from median days 15 to 50) by MANOVA, with time as a dependent variable.

There were only minor differences in total hemolymph protein concentrations among diets on the same sampling day (Fig. 6). Sampling age strongly affected the hemolymph protein levels (Pallai's Trace; $F_{5,20} = 38.7$; $P < 0.0001$), as has been show previously (e.g., Hatle et al. 2001). There was a significant interaction of diet and sampling age ($F_{20,92} = 92.0$; $P = 0.036$), suggesting that the diets responded differently to the passage of time. Only on days 36 ($F_{4,28} = 3.97$; $P = 0.013$) and 43 ($F_{4,28} = 3.22$; $P = 0.029$) did diet affect hemolymph protein levels. Specifically, the calorie restricted (60%) group had lower hemolymph protein levels than the *ad libitum* group on days 36 ($P = 0.022$) and 43 ($P = 0.019$) and lower protein levels than the intermittent fasting group on day 36 ($P = 0.025$). No other comparisons of diets on a specific day were significantly different.

Hemolymph glucose profiles

There were no differences among diets in hemolymph glucose levels on any of the first six sampling days (all $F_{4,22} = 2.13$; all $P > 0.10$). Sampling age did not affect glucose levels (Pallai's Trace; $F_{5,14} = 1.9$; $P = 0.165$), and the interaction of diet and sampling age was not significant (Pallai's Trace; $F_{20,68} = 0.9$; $P = 0.576$).

Discussion

Calorie restriction during the entire adult period can enhance longevity in female lubber grasshoppers without reducing lifetime fecundity. This enhanced longevity can result from consuming either 60% or 71% of the calories consumed when given free access to food. Calorie restriction was associated with reduced body mass, but hemolymph levels of glucose were unchanged and levels of storage protein were changed little. This extension of lifespan likely is due to slowed aging.

Amounts eaten

Female lubbers reared on day-on / day-off intermittent fasting schedules, on their feeding days, ate ~140% of the lettuce eaten by *ad libitum* females on a typical day. In other words, the intermittent fasting group ultimately ate ~71% of the calories eaten by females with constant free access to food. In the wild, lubbers typically feed twice daily (DW Whitman, unpublished data). This suggests that lubber grasshoppers are able to compensate somewhat, but not completely, for days without food.

Body mass

Not surprisingly, female lubbers fed *ad libitum* reached greater maximum weight, and maintained greater weight, than lubbers on restricted diets (Fig. 3). However, this was also true even when *ad libitum* grasshoppers were compared to delayed calorie restriction grasshoppers before their diet was restricted at day 50. That is, two groups that consumed nearly identical diets did not gain body mass similarly. In fact, the delayed calorie restriction group had body masses more similar to the calorie restricted group from days 30 – 50. The *ad libitum* and delayed calorie restriction groups had identical rearing conditions as juveniles and similar exoskeletal sizes (measured as femur lengths; data not shown). The delayed calorie restriction group non-significantly tended to lay the first clutch earlier than the *ad libitum* group (Fig. 4). This anomaly in body mass confounds the result that delayed calorie restriction enhanced longevity. It may be that the delayed calorie restriction group was innately different from the *ad libitum* group, or it may be that the dietary treatment of delayed calorie restriction actually enhanced longevity. Additional data is needed to distinguish these possibilities.

Survivorship

Calorie restriction during the entire adult period clearly extended lifespan in female lubber grasshoppers, without reducing lifetime fecundity (Figs. 4 and 5). Median life spans of 60% and 71% calorie restriction grasshoppers were 62% and >98% longer than *ad libitum* grasshoppers, respectively. Calorie restriction enhances longevity in a broad range of organisms, including yeast, worms, fruit flies, and mice (Tatar et al. 2003). This life extension has been shown to result from slowed aging. Because of this wealth of previous data on calorie restriction slowing aging in other organisms, the most probable explanation for our results is that calorie restriction also slows aging in female lubber grasshoppers. The mechanism(s) by which calorie restriction delays aging has been the subject of intense investigation. We hope to test the roles of heat shock proteins and insulin-like molecules in slowing aging in lubber grasshoppers.

Intermittent fasting without reduced cumulative consumption in mice has been shown to reduce insulin and glucose titers and improve resistance to neural injury, all physiological responses that have been linked to calorie restriction (Anson et al. 2003). The intermittent fasting and intermittent fasting control groups in the present paper were designed to test for possible effects of an infrequent feeding schedule (separate from the cumulative amount consumed) on survival and glucose titers in female lubber grasshoppers. We observed greater mortality in the intermittent fasting group than in the intermittent fasting control (71%) group. This may indicate that intermittent fasting nullifies the life extension of calorie restriction. There is a wealth of data indicating that calorie restriction enhances longevity by slowing aging in other organisms, but little (if any) data indicating that intermittent fasting effects aging separately from calorie restriction in other organisms. Hence, it is premature to conclude that intermittent fasting affects aging in lubber grasshoppers merely from survivorship curves. Our results may indicate an aging-independent source of mortality in intermittent fasting grasshoppers, such as increased disease susceptibility or periodic dehydration stress. While we find the possibility of intermittent fasting acting on aging mechanisms intriguing, more data is needed to support this conclusion.

Lifetime fecundity

In the wild, lubber grasshoppers are univoltine (i.e., one generation per year). That is, they hatch in the spring, become adults in early summer, lay eggs in late summer, and overwinter as eggs. In the lab, eggs have an obligate 4 month diapause (Chladny and Whitman 1997; Stauffer and Whitman 1997). Our anecdotal observations suggest that eggs laid within a month of each other, incubated at ~15ºC for several months, and then warmed to room temperature, seem to hatch at similar times (JD Hatle, personal observations). While we have no direct evidence that date of laying is not related to hatching date in the wild, the egg diapause of lubbers suggests that the number of eggs a female lays in her lifetime may be more important to contributing to the next generation than the age at which they are laid. Certainly, living longer in the wild (to survive to lay more clutches) is more difficult than surviving in the lab, because of predation and disease. That said, lubber grasshoppers have an impressive array of chemical and behavioral defenses (Whitman 1990; Hatle et al. 2002), and mark-recapture studies suggest survival in the wild is high (DW Whitman, unpublished data). Hence, the cumulative, lifetime mass of eggs laid (independent of the time at which these eggs were laid) is a reasonable measure of lifetime fecundity for lubber grasshoppers.

Lifetime fecundity did not vary among diets (Fig. 5). Among our six, planned, pairwise comparisons, only *ad libitum* vs. intermittent fasting control (71%) was statistically different (P = 0.045), with intermittent fasting control grasshoppers laying somewhat more eggs than *ad libitum* grasshoppers. It may be that the enhanced longevity of calorie restriction also leads to increased fecundity. At the very least, it seems that *ad libitum* feeding is not necessarily the only route to maximizing fecundity for female lubber grasshoppers.

Hemolymph total protein and glucose levels

Grasshoppers and other phytophagous insects are protein limited during egg production. During the first half of egg production, females ingest large quantities of food (see *ad libitum* at day 10 in Fig. 1) to store protein. Levels of three hemolymph storage proteins also increase during the first 20 days of adulthood, then fall to intermediate levels at oviposition around 35 – 40 days (Hatle et al. 2001). In previous work, we have show that females on an extremely limiting diet (~25-40% of *ad libitum*) have lower levels of storage proteins (Hatle et al. 2004). In this study, we are able to address whether 60% or 71% calorie restricted diets, which enhance longevity but do not decrease lifetime fecundity, lower protein storage in the hemolymph.

Calorie restricted (60%) females had lower levels of hemolymph storage proteins than *ad libitum* females only at days 36 and 43. Producing a clutch of eggs requires an enormous investment of protein by females. A full clutch is 1100 dry mg (10 – 15% of the mass of a female) and is largely protein. Hence, the most likely explanation for the decrease in protein levels in calorie restricted (60%) females is that hemolymph protein stores were depleted for producing the clutch. In contrast, *ad libitum* females maintained high hemolymph protein levels when producing the first clutch. Intermittent fasting control (71%) females showed a similar, but non-significant, trend as calorie restricted (60%) females.

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Table 1. Diet treatments used in this experiment.

Diet treatment	Romaine lettuce offered
Ad libitum	Constant, free access to lettuce
Calorie restriction	60% of that consumed by the *ad libitum* group
Delayed calorie restriction	Constant, free access to lettuce for the first 50 days of adulthood, then the calorie restriction diet from day 51 until death
Intermittent fasting	Free access to food one day, then only water the next day, and so on
Intermittent fasting control	The same cumulative amount of lettuce consumed by the intermittent fasting group, but fed daily

Figure captions

Figure 1. Lettuce consumed by female lubber grasshoppers on five diet treatments. Diet treatments were effective in manipulating the amount of lettuce consumed. We offered the *ad libitum* group free access to lettuce, collected uneaten lettuce, and determined the amount consumed daily. The calorie restricted group was offered meals equivalent to 60% of the lettuce consumed by the *ad libitum* group. The delayed calorie restriction group was offered free access to lettuce the first 50 days, then fed identically to the calorie restricted group from day 51 until death. The intermittent fasting group was offered free access to lettuce one day, only water the next day, and so on. Each day, the intermittent fasting control group was offered meals of 50% of the lettuce consumed by the intermittent fasting group on feeding days. This intermittent fasting control diet resulted in meals of about 71% of the lettuce consumed by the *ad libitum* group. The plot line for each group ends at the median age at death for that group.

Figure 2. Body masses of female lubber grasshoppers maintained on five diet treatments. The delayed calorie restriction group began on a reduced diet at 50 days. See text for further descriptions of diets. The *ad libitum* group reached a higher maximum weight than both the calorie restricted (60%) and the delayed calorie restriction groups. The intermittent fasting and intermittent fasting control (= 71% of *ad libitum*) groups had similar body masses throughout the experiment. The plot line for each group ends at the median age at death for that group.

Figure 3. Survivorship of female lubber grasshoppers kept on five diet treatments. The delayed calorie restriction group began on a reduced diet at 50 days. See text for further

descriptions of diets. The calorie restricted (60%), delayed calorie restriction, and intermittent fasting control (71%) groups all had greater longevity than the *ad libitum* group. In contrast, the intermittent fasting group had similar longevity to the *ad libitum* group. The plot line for each group ends at the median age at death for that group.

Figure 4. Cumulative oviposited material (largely eggs) by female lubber grasshoppers on five diet treatments. See text for description of diets.

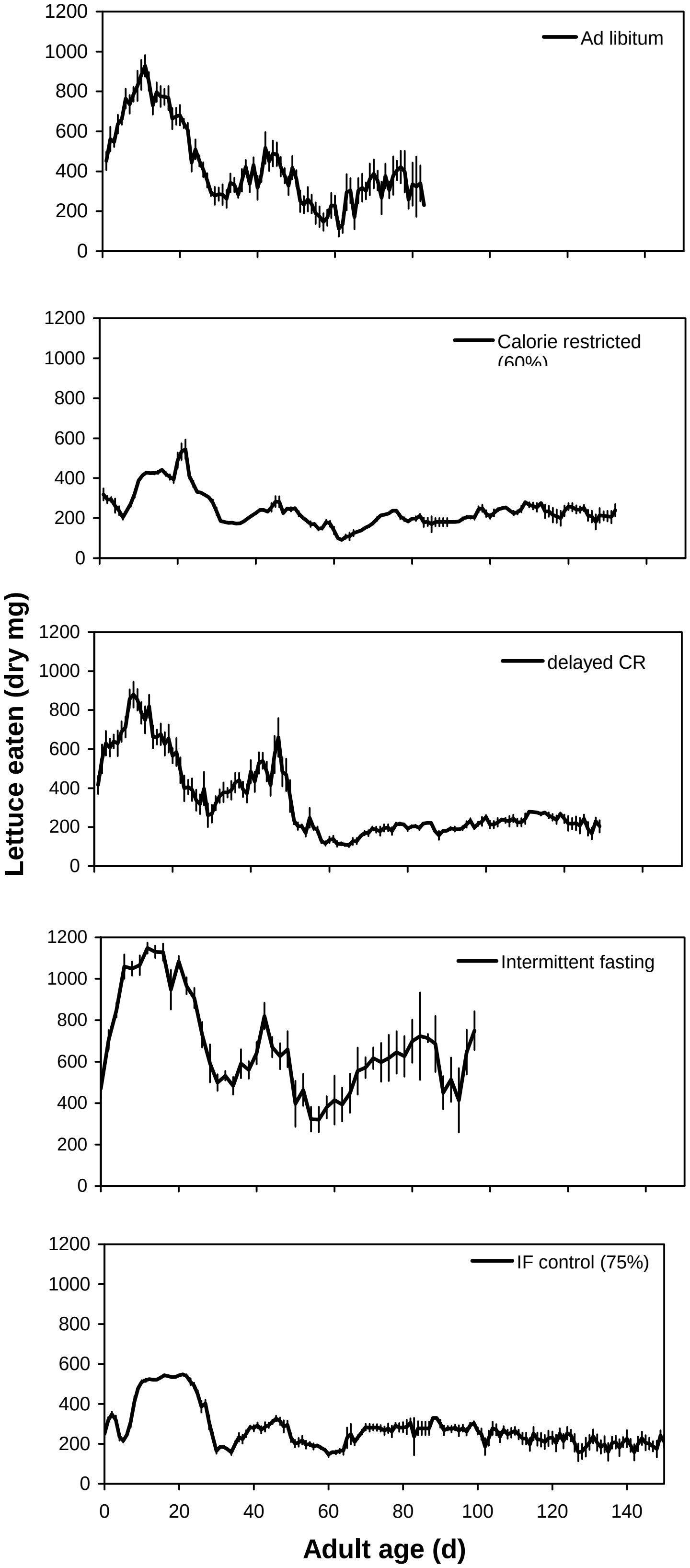
Figure 5. Lifetime fecundity, estimated as cumulative dry mass of oviposited materials, in female lubber grasshoppers reared on five diet treatments. Ad lib = *ad libitum*, CR = calorie restricted (60%), delay CR = delayed calorie restriction, IF = intermittent fasting, and IF control = intermittent fasting control (71%). See text for description of diets. The intermittent fasting control group had marginally great lifetime fecundity than the *ad libitum* group. All other comparisons of lifetime fecundity were not significantly different.

Figure 6. Total hemolymph protein levels in female grasshoppers kept on five different diets. See text for descriptions of diets. Total hemolymph proteins serve as an estimate of protein storage. Data are plotted as median, chronological ages (see Hemolymph samples in Methods for details). Diets differed little in hemolymph protein levels. The calorie restricted (60%) group had lower protein levels than the *ad libitum* group on days 36 (P = 0.022) and 430 (P = 0.019) and lower protein levels than the intermittent fasting group on day 36 (P = 0.025). No other comparisons of diets on a specific day were significantly different.

Figure 7. Hemolymph glucose levels in female lubber grasshoppers on five different diets. See text for descriptions of diets. Data are plotted as median, chronological ages

(see Hemolymph samples in Methods for details). Diets did not differ in hemolymph glucose levels at any age.

Figure 1


Ad libitum
Calorie restricted (60%)
delayed CR
Intermittent fasting
IF control (75%)
Lettuce eaten (dry mg)
Adult age (d)
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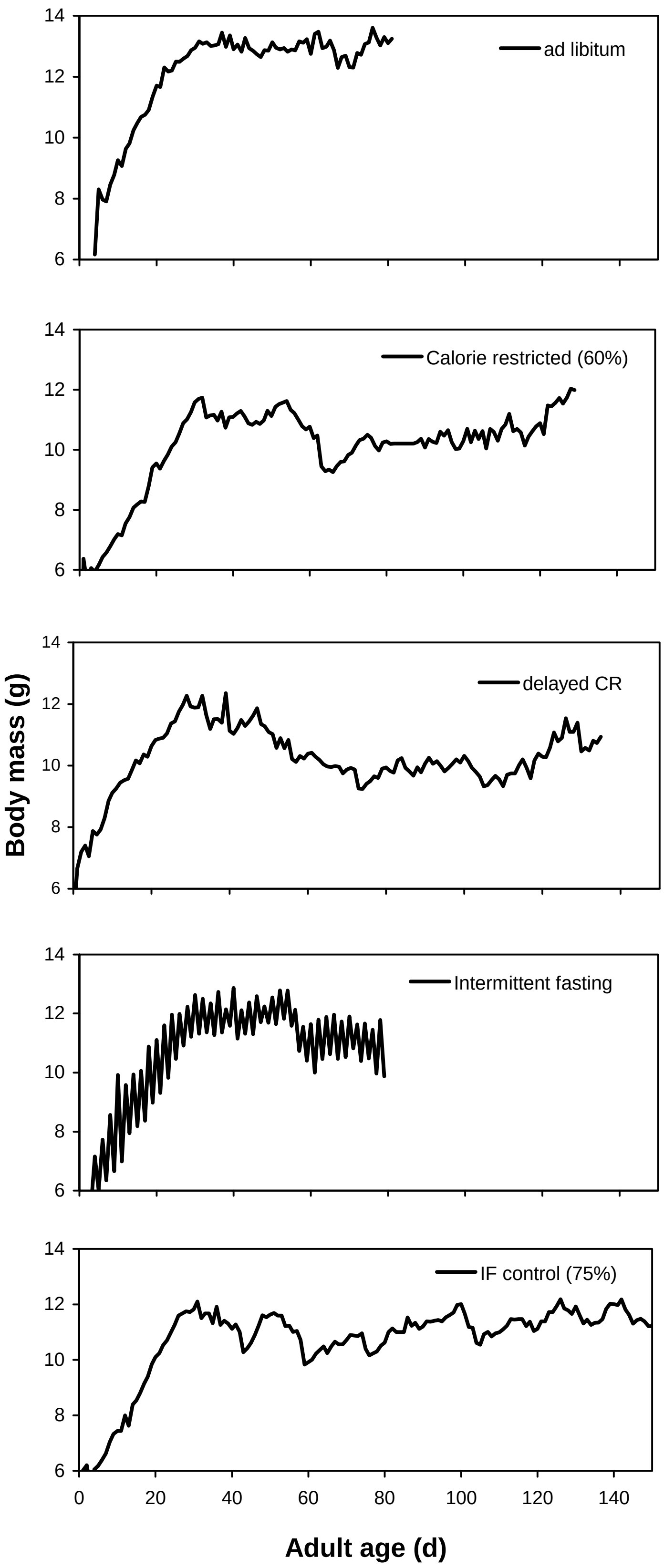
200
400
600
800
1000
1200
0
20
40
60
80
100
120
140

Figure 2


ad libitum
Calorie restricted (60%)
delayed CR
Intermittent fasting
IF control (75%)
Body mass (g)
Adult age (d)
6
8
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14
0
20
40
60
80
100
120
140

Figure 3

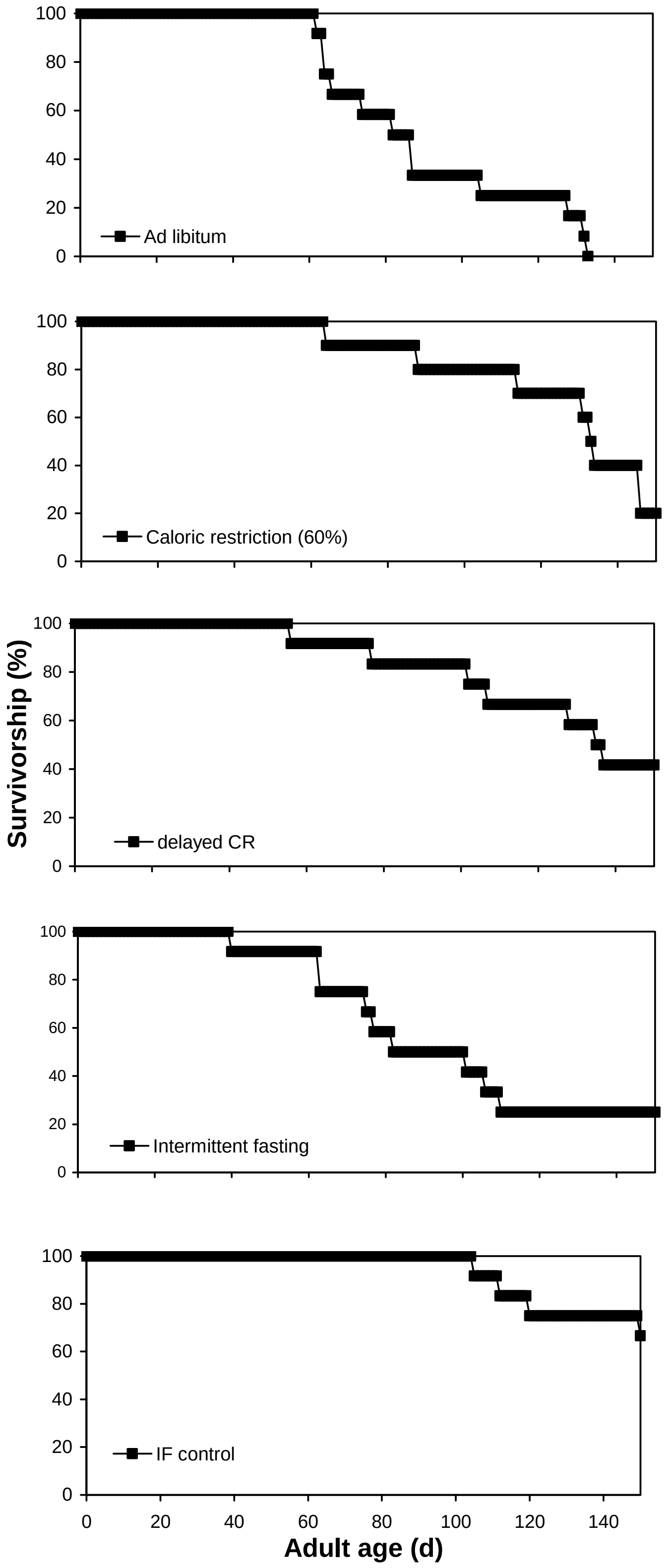

100
80
60
40
20
0
Ad libitum
100
80
60
40
20
0
Caloric restriction (60%)
Survivorship (%)
100
80
60
40
20
0
delayed CR
100
80
60
40
20
0
Intermittent fasting
100
80
60
40
20
0
IF control
0
20
40
60
80
100
120
140
Adult age (d)

Figure 4

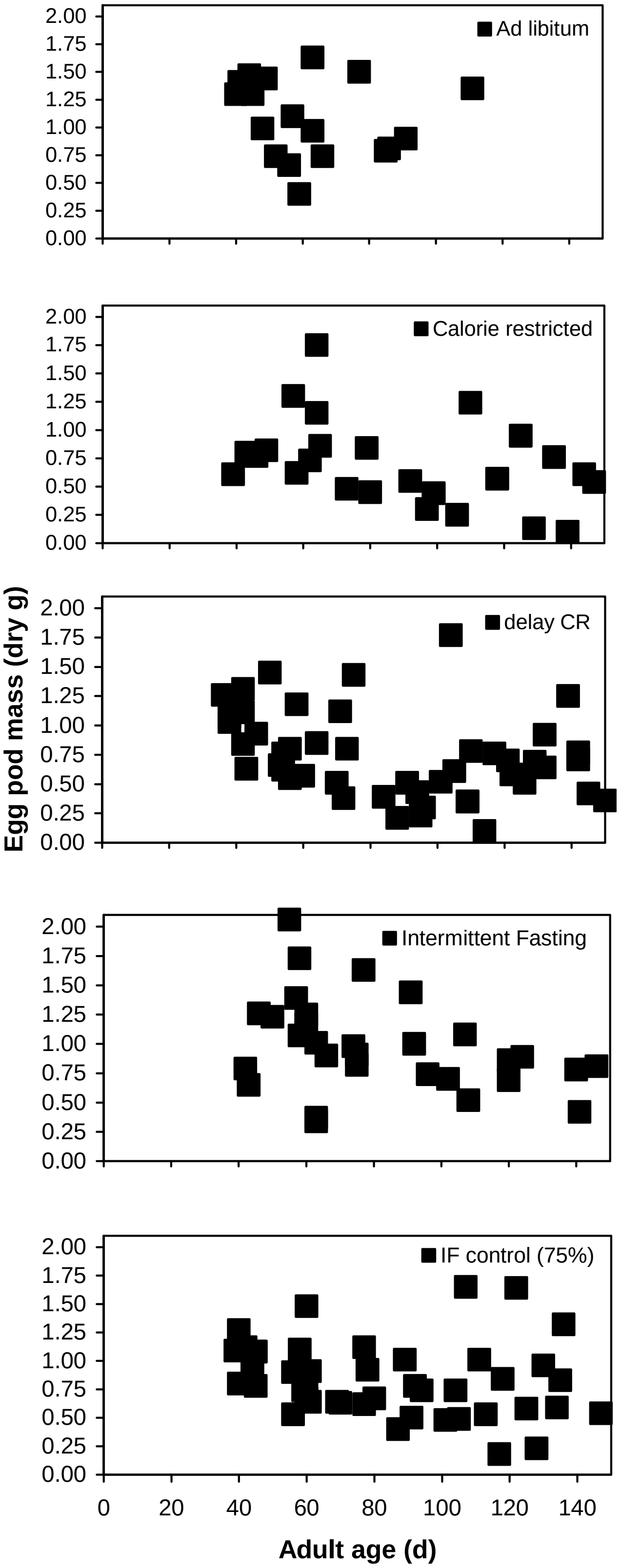
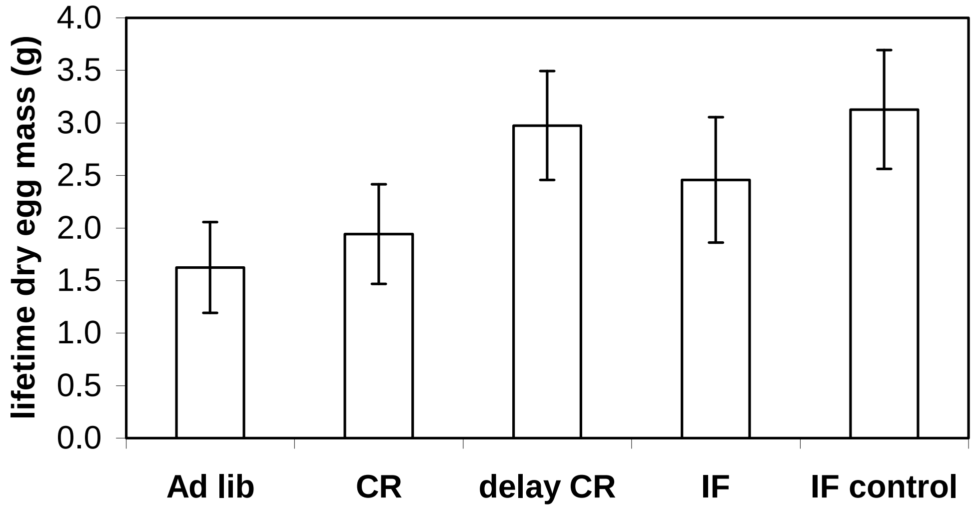

■ Ad libitum
■ Calorie restricted
■ delay CR
■ Intermittent Fasting
■ IF control (75%)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Egg pod mass (dry g)
0
20
40
60
80
100
120
140
Adult age (d)

Figure 5



4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
lifetime dry egg mass (g)
Ad lib
CR
delay CR
IF
IF control

Figure 6

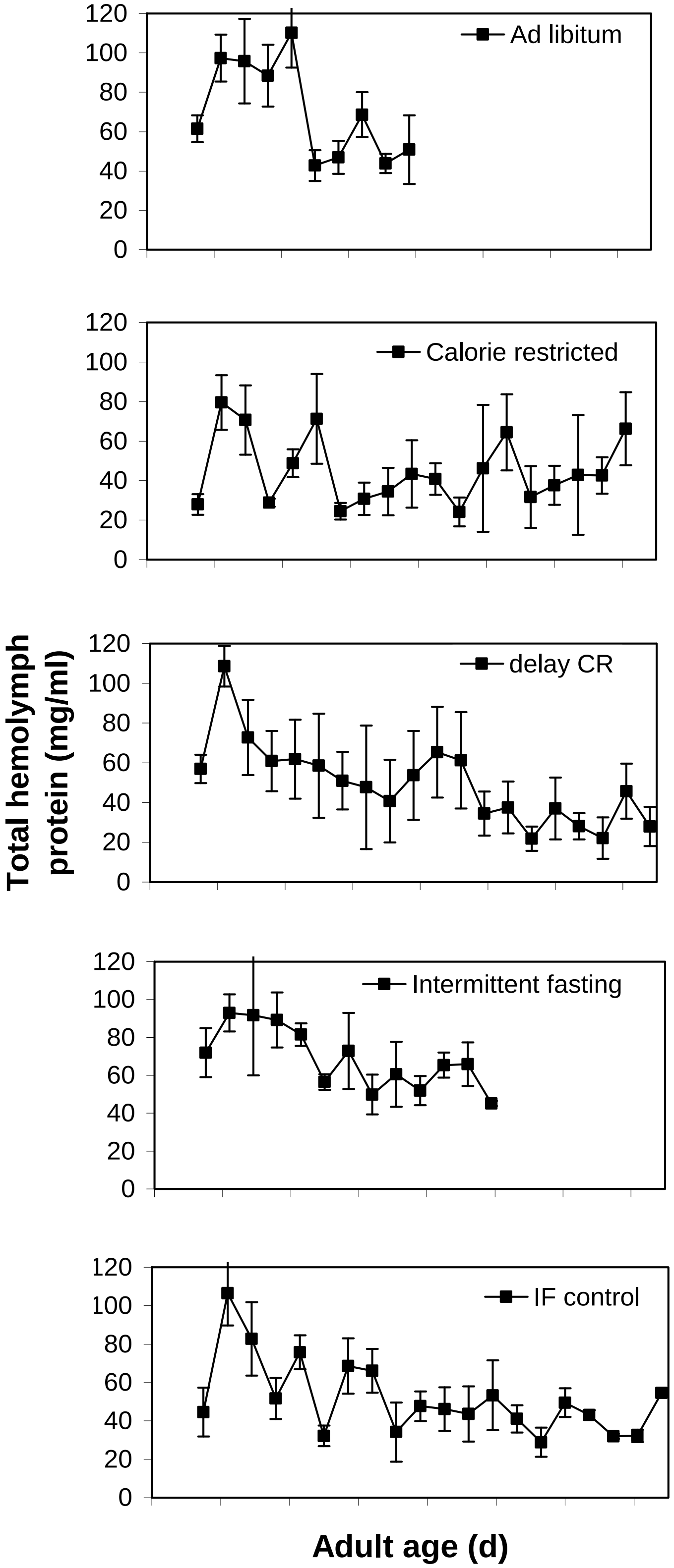
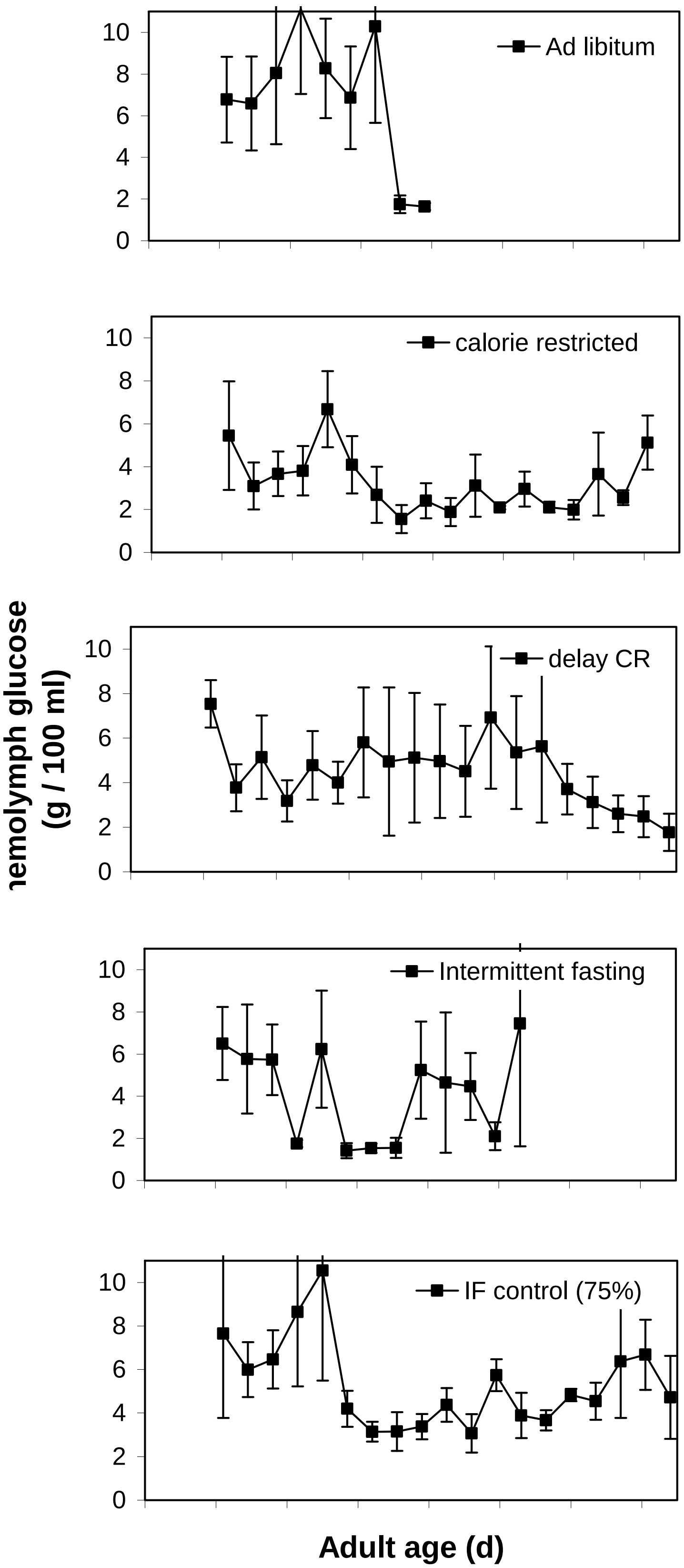

Ad libitum
Calorie restricted
delay CR
Intermittent fasting
IF control
0
20
40
60
80
100
120
Total hemolymph protein (mg/ml)
Adult age (d)

Figure 7.


Ad libitum
calorie restricted
delay CR
Intermittent fasting
IF control (75%)
0
2
4
6
8
10
hemolymph glucose
(g / 100 ml)
Adult age (d)