



## Metabolic rate depression is induced by caloric restriction and correlates with rate of development and lifespan in a parthenogenetic insect

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### ABSTRACT

Caloric restriction (CR) extends lifespan in most animals, but the mechanisms underlying this phenomenon are the subject of much debate. We investigated the association between longevity and resting metabolic rate (RMR) in Indian stick insects (*Carausius morosus*) by (i) determining the appropriate scaling coefficient for calculating mass-corrected RMR of insects throughout development, (ii) quantifying the response of RMR to diet history, and (iii) correlating RMR in multiple life-history stages with adult and total lifespan. Over a range of body sizes, whole-body RMR (measured as oxygen consumption rate) scaled linearly with body mass. Mass-specific RMR decreased in response to CR, particularly when food was restricted during juvenile stages. With one exception, RMR of insects in different life-history stages matched current feeding level and was not substantially affected by intake history. Total lifespan was affected by intake, with insects that experienced CR early in development living longer than insects that were fed ad libitum. Although CR was associated with extended total lifespan and decreased RMR, it was also associated with shortened adult lifespan. Thus, we found limited evidence that decreased RMR plays a causative role in determining longevity. Instead, CR and decreased RMR were associated with slower progression through pre-reproductive life-history stages.

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### 1. Introduction

Caloric restriction (CR) without malnutrition has been shown to extend both mean and maximum lifespan in yeast, invertebrates, fish, and mammals (McCay et al., 1935; Weindruch and Walford, 1988; Masoro, 1995, 2002). The physiological mechanisms underlying this effect remain unclear but are thought to be associated with one or more of the following: (a) retardation of growth and development (McCay et al., 1935; Miller et al., 2002), (b) decreased adiposity (Berg and Simms, 1960; Muzumdar et al., 2008), (c) reduction of metabolic rate (Sacher, 1977), (d) attenuation of age-associated increases in oxidative damage (Sohal and Weindruch, 1996), or (e) increased stability of metabolic regulatory networks (Demetrius, 2004). All of these suggested mechanisms are based to some extent on the assumption that CR exerts its effects on lifespan through alterations in the rate, fate, efficiency, or robustness of an organism's metabolism.

A century ago, Rubner (1908) identified an inverse relationship between longevity and mass-specific metabolic rate in an interspecific evaluation of mammals. Subsequently, evidence that longevity in flies is influenced by temperature (Loeb and Northrop, 1916, 1917) led Pearl (1928) to develop the rate of living theory.

According to this theory, longevity is determined by the rate at which essential cellular components are depleted or damaged, which in turn correlates with metabolic rate. Harman (1956) later provided a mechanistic explanation for the rate of living theory by suggesting that the primary determinant of longevity is the accumulation of damage to lipids, proteins, and nucleic acids caused by the production of reactive oxygen species (ROS) via oxidative phosphorylation in metabolic reactions.

Harman's oxidative stress theory has received substantial empirical support from data indicating that CR attenuates the age-associated accumulation of damage to biomolecules caused by ROS, thereby decreasing oxidative stress and retarding the aging process (Yu, 1996; Gredilla et al., 2001). According to this argument, CR-induced lifespan extension is mediated by a reduction in metabolic rate that leads to decreased ROS production (Sohal and Weindruch, 1996; Ramsey et al., 2000). More recent evidence that ROS can have beneficial cellular effects by functioning in signaling pathways (Thannickal and Fanburg, 2000) has called into question the assumption that ROS production is inherently detrimental to survival. Regardless of these potential benefits, it is still generally believed that the cumulative effects of excessive cellular concentrations of ROS contribute to increased oxidative stress, which has been directly linked to the aging process. However, whether a reduction in oxidative stress during periods of CR results from the effects of diet on metabolic rate remains to be determined.

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Although a link has been established between metabolic rate and oxidative stress (Ji and Leichtweis, 1997; Packer, 1997; Selman et al., 2002), the role of differences in metabolic rate in extending the lifespan of calorically restricted animals is currently the subject of much controversy (Ramsey et al., 2000; Selman et al., 2005; Hunt et al., 2006). Metabolic rate is sometimes decreased by CR, especially when expressed on a whole-animal basis (DeLany et al., 1999; Ramsey et al., 2000; Even et al., 2001; Blanc et al., 2003). In most studies, however, metabolic rate remains unchanged or even increases in response to CR (McCarter et al., 1985; Houthoofd et al., 2002a,b, 2005; Hulbert et al., 2004; Selman et al., 2005) and is either uncorrelated or positively correlated with lifespan (Houthoofd et al., 2002a; Hulbert et al., 2004; Speakman et al., 2004). Furthermore, elevated metabolic rate does not always lead to decreased lifespan, as demonstrated by the positive effect of exercise on longevity (Lee et al., 1995).

Much of the controversy about the effects of CR on metabolic rate results from disagreement over the most appropriate way to make intraspecific comparisons among individuals that differ in body size (Even et al., 2001; Speakman et al., 2002; Blanc et al., 2003; Poehlman, 2003; Selman et al., 2005; Ramsey and Hagopian, 2006). CR typically leads to altered body mass and/or tissue composition, and these alterations can directly affect metabolic expenditure (Speakman et al., 2002). As a result, comparisons of whole-animal metabolic rate among food-restricted and ad libitum-fed animals are only marginally informative. Strategies that correct for body size include dividing metabolic rate by body mass, by body mass raised to the 3/4 power, or by body mass raised to the 2/3 power. To account for differences in body composition, metabolic rate has also been divided by lean body mass (McCarter and Palmer, 1992; Blanc et al., 2003) and has been calculated using predictive equations based on the size of different organs or tissues (Greenberg and Boozer, 2000; Even et al., 2001; Selman et al., 2005). However, these corrections for intraspecific differences in metabolic mass are not typically validated over a wide range of body sizes in CR studies, thus exacerbating the uncertainty about the effect of CR on metabolic rate.

The purpose of this study was to investigate the association between aging and resting metabolic rate (RMR) in Indian stick insects (*Carausius morosus*). To achieve this goal, we first evaluated the mass dependence of RMR throughout development to allow for appropriate comparisons of RMR among insects that differed in size. We then quantified the response of RMR to different patterns of food intake and correlated adult and total lifespan with mass-specific RMR of individuals in multiple life-history stages. We studied *C. morosus* (Phasmatodea, Lonchodinae) because it is a relatively long-lived and hemimetabolous insect that reproduces via obligate apomictic parthenogenesis (Pijnacker, 1966), thereby permitting natural reproductive processes to occur while females are housed individually. Additionally, *C. morosus* consumes the same food throughout its lifetime, thus facilitating lifelong, quantitative dietary manipulations.

## 2. Materials and methods

### 2.1. Animal husbandry and feeding treatments

This study was conducted in a temperature-controlled quarantine facility in the Department of Zoology at the University of Florida. Lights within this facility were maintained on a 12:12 light:dark cycle. Female, parthenogenetic Indian stick insects (*Carausius morosus*) were housed individually from hatching to death in plastic cages (29.5 × 19 × 19 cm). Insects were fed discs cut from leaves of English ivy (*Hedera helix*) using biopsy punches (Miltex Instrument Co., Inc.) of different diameters for each life-

**Table 1**

Details of feeding and metabolic rate measurement methods in different life-history stages in *Carausius morosus*.

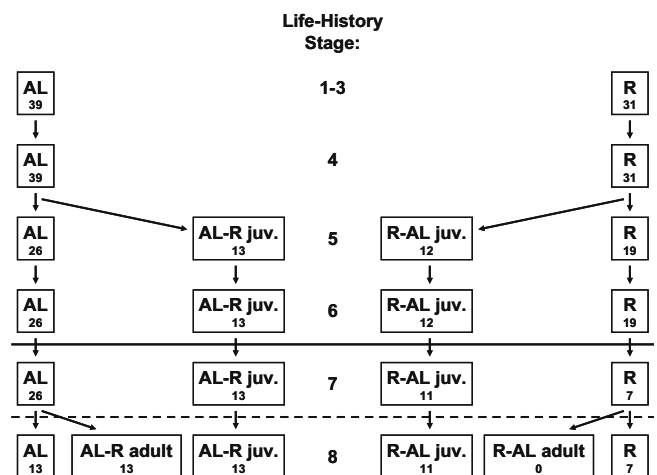
Life-history stage	Stage #	Leaf disc size (mm)	Approximate volume of metabolic chamber (mL)
Instar 1	1	2	–
Instar 2	2	3	–
Instar 3	3	4	–
Instar 4	4	5	20
Instar 5	5	6	40
Instar 6	6	8	50
Adult before first ov.	7	8	230
Adult after first ov.	8	8	230

Notes: Juvenile life-history stages are demarcated by ecdyses, and adult life-history stages are defined relative to the onset of reproductive activity. Leaf discs were cut from leaves of English ivy (*Hedera helix*). Metabolic chamber volumes are the volumes of each of a pair of identical glass jars connected by a U-tube manometer. Abbreviations: ov., oviposition.

history stage (Table 1). Drinking water was provided via daily misting of each cage.

Leaf discs were offered according to five treatment schedules (Fig. 1). Insects were offered either more leaf discs than they could consume within 24 h (ad libitum, AL) or a restricted number of discs (R) equal to 60% of the average daily mass-specific intake of continuously AL-fed insects in the same stage. Stages included each of six instars, adult prior to first oviposition, and adult after first oviposition (Table 1). Throughout the remainder of the text, abbreviations for any of the five specific treatment groups that we tested are italicized, whereas abbreviations for ad libitum or restricted diets at particular points in time are not italicized.

Intake of food-restricted insects was adjusted on a weekly basis to account both for increases in body mass during development and for age-dependent declines in consumption by stage 8 adult AL insects. We were unable to test a diet switch from restricted to ad libitum feeding at first oviposition (*R-AL adult*) because



**Fig. 1.** Schedule of diet treatments for *Carausius morosus* in 8 life-history stages (defined in Table 1). Insects were offered either more leaf discs than they could consume within 24 h (ad libitum, AL) or a restricted number of discs (R) equal to 60% of the average daily mass-specific intake of insects in group AL in the same life-history stage. Subsets of groups AL and R experienced diet switches from AL to R (AL-R) and from R to AL (R-AL), respectively, either on the first day of the fifth instar or at first oviposition. Rows of boxes correspond to different life-history stages. The solid horizontal line separates juvenile and adult life-history stages, and the dashed horizontal line separates pre-reproductive adults from reproductively active adults. Sample sizes for each treatment group during each life-history stage are indicated within each box. Abbreviations: AL, ad libitum; R, restricted; juv., juvenile. In the text, abbreviations for any of the five specific treatment groups that we tested are italicized, whereas abbreviations for ad libitum or restricted diets at particular points in time are not italicized.

survival to reproductive competence was extremely low for continuously food-restricted insects. To ensure a sufficient sample size in group *R*, all insects that were continuously food-restricted as juveniles were maintained on the restricted diet as adults.

The duration of each life-history stage was determined as the number of days between successive molts (for stages 4–6), between the terminal molt and the day of first oviposition (for stage 7), and between the day of first oviposition and death (for stage 8). Adult and total lifespan for each insect were determined as the number of days between the terminal molt and death or between hatching and death, respectively.

## 2.2. Metabolic chambers and oxygen consumption measurements

Resting metabolic rate (RMR) was measured as oxygen consumption rate in a closed system, manometric microrespirometer modified from Scholander (1950). Metabolic rates were measured 7–14 days after the beginning of each of stages 4–8 (Table 1) for each insect in all treatment groups. The metabolic chamber consisted of a pair of glass jars (the animal chamber and the control chamber) sealed with rubber stoppers connected to a U-tube manometer. The animal chamber contained soda lime with a color-change indicator of CO<sub>2</sub> saturation and a cotton pad moistened with deionized water to maintain ambient humidity at approximately 100% within the chamber. Thick, plastic mesh prevented the soda lime from contacting the insect and moistened pad. The control chamber contained only a moistened pad.

Insects were weighed, placed into the animal chamber, and allowed to acclimate to the chamber for 2.5 h. The metabolic chamber was then sealed. Carbon dioxide expired by the insect was absorbed by the soda lime, thereby deflecting the mineral oil in the U-tube manometer. The magnitude of this deflection was recorded after the animal had been sealed in the metabolic chamber for 3.5–6 h, depending on the size and stage of the insect. Room air was then injected into the animal chamber, and the magnitude of the deflection of the manometer in the opposite direction was recorded. In all cases, the volume of air injected was greater than the volume of oxygen consumed by the insect. The volume of oxygen consumed by the insect ( $V_{O_2}$ ) was estimated as  $V_{O_2} = V_{air} * d_{insect} / d_{air}$  where  $V_{air}$  is the volume of air injected,  $d_{insect}$  is the measured deflection of the oil in the manometer by the insect, and  $d_{air}$  is the measured deflection of the oil in the manometer by injected air.

Oxygen consumption rate ( $\dot{V}_{O_2}$ ) was calculated as  $V_{O_2} / t$  where  $t$  = time. Oxygen consumption rate was then converted to standard temperature and pressure (STP) using the equation  $\dot{V}_{O_2 \text{ at STP}} = \dot{V}_{O_2} * [273 / (273 + T_a)] * [(P_b - P_{H_2O}) / 760]$  where  $T_a$  is ambient temperature (°C),  $P_b$  is ambient barometric pressure (mm), and  $P_{H_2O}$  is the vapor pressure of water (mm). All measurements were made during daylight hours at an ambient air temperature of 23 °C, which was maintained in the quarantine room throughout the study. As *C. morosus* is nocturnal, daylight hours represented the resting phase for these insects.

## 2.3. Statistical analyses

Least-squares linear regression of log-transformed data was used to test the relationship between resting metabolic rate ( $\dot{V}_{O_2 \text{ at STP}}$ ) and body mass ( $M$ ) for insects that were either continuously ad libitum-fed (*AL* and *AL-R adult*,  $n = 26$ ) or continuously food-restricted (*R*,  $n = 7$ ) during each of stages 4–7. Only data for insects that survived, successfully oviposited, and were maintained on a single diet treatment prior to the onset of reproductive activity were included in these analyses. We did not include data for stage 8 in the regression analyses because body mass during the reproductive lifespan was affected by egg load. The significance

of regressions and regression coefficients was tested by analysis of variance.

Because the slope of the regressions for continuously ad libitum-fed and continuously food-restricted insects did not differ significantly from 1.0, mass-specific metabolic rates were calculated as  $\dot{V}_{O_2 \text{ at STP}} / M$ , where  $M$  is body mass immediately prior to placement of the insect into the metabolic chamber. Body masses, adult and total lifespans, and mass-specific metabolic rates within each life-history stage were tested for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) and transformed, if necessary, to meet these assumptions. Data that met these assumptions were analyzed using analysis of variance (ANOVA). Significant treatment effects were then explored further using Tukey's honestly significant difference (HSD) post hoc test. Data that did not meet the assumptions for parametric analysis were analyzed using a Kruskal–Wallis test followed by pairwise Mann–Whitney U tests with a Bonferroni correction for multiple comparisons. Mass-specific metabolic rates for all insects that survived and successfully oviposited ( $n = 57$ ) were also analyzed using repeated measures ANOVA with Tukey's HSD post hoc test. Spearman's rank correlation test was used to test the strength of the relationships among mass-specific metabolic rates in each stage, duration of each stage, and adult and total lifespan.

Within life-history stages, body size and metabolic rate data for all insects that were maintained on a restricted or ad libitum diet prior to a diet switch were pooled together. Reported sample sizes for juveniles include only those insects that survived through the end of the sixth instar, and reported sample sizes for adults include only those insects that successfully oviposited. Sample sizes of group *R* decreased for adults because 12 females in this group failed to oviposit, and data for insects that failed to become reproductively active were excluded from the analysis of adult metabolic rates. One insect in group *R-AL adult* progressed through an unexpected supernumerary instar before the terminal ecdysis, so all data for this insect beyond stage 6 were excluded from further analysis. Data were analyzed using SPSS, Release 11.0.0 (SPSS, Inc.) and are reported as means  $\pm$  standard error with  $\alpha = 0.05$ .

## 3. Results

### 3.1. Determining the mass exponential scaling coefficient

Body mass differed significantly among treatment groups for all life-history stages in which metabolic rate was determined (Fig. 2). As a result, analysis of covariance (ANCOVA) could not be used to evaluate size-corrected metabolic rates because the assumption that covariate values have similar distributions and ranges for all treatment groups (Quinn and Keough, 2002) was violated. Instead, we determined the appropriate scaling coefficient for calculating mass-specific metabolic rates. To do so, we plotted RMR against body mass for all insects that were continuously food-restricted ( $n = 7$ ) and for all insects that were continuously ad libitum-fed ( $n = 26$ ) prior to first oviposition.

We found that whole-body RMR ( $\dot{V}_{O_2 \text{ at STP}}$ ,  $\mu\text{l h}^{-1}$ ) scaled allometrically with body mass ( $M$ , g) according to the following equations (Fig. 3):

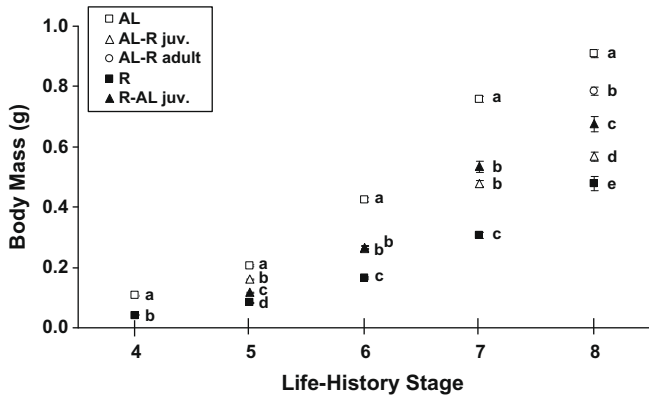
Continuously AL insects :

$$\dot{V}_{O_2 \text{ at STP}} = 374.1M^{0.975} \quad (n = 104, r^2 = 0.981, p < 0.0001)$$

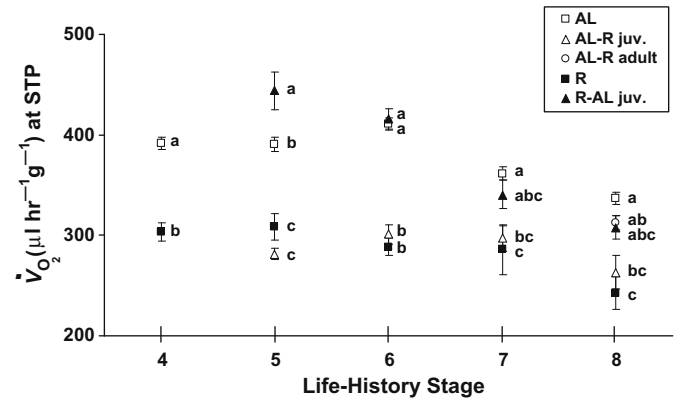
Continuously R insects :

$$\dot{V}_{O_2 \text{ at STP}} = 254.1M^{0.921} \quad (n = 28, r^2 = 0.938, p < 0.0001)$$

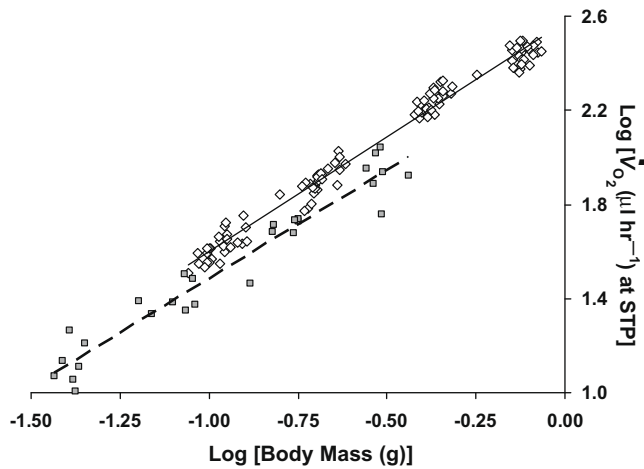
However, neither of the scaling coefficients was significantly different from 1.0 (ANOVA,  $p > 0.05$  for both treatments), so the



**Fig. 2.** Body mass ( $\bar{x} \pm SE$ ) of insects 7–14 days after the beginning of each of five life-history stages (defined in Table 1). Body masses were determined immediately prior to placing insects in metabolic chambers. Treatment groups are defined in Fig. 1. Different letters indicate values that are significantly different ( $p < 0.05$ ) among treatment groups within stages according to parametric analysis (ANOVA and Tukey's HSD post hoc test) or nonparametric analysis (Kruskal–Wallis test and pairwise Mann–Whitney U tests with a Bonferroni correction for multiple comparisons), where appropriate.



**Fig. 4.** Mass-specific resting metabolic rate (RMR) 7–14 days after the beginning of each of five life-history stages (defined in Table 1). RMR was measured as oxygen consumption rate ( $\dot{V}_{O_2}$ ) at standard temperature and pressure (STP). Treatment groups and abbreviations are defined in Fig. 1, and data analysis is the same as in Fig. 2. Different letters indicate values that are significantly different ( $p < 0.05$ ) among treatment groups within stages. Each point represents  $\bar{x} \pm SE$ .



**Fig. 3.** Relationship between resting metabolic rate (RMR) and body mass of insects that were continuously food-restricted (shaded squares, broken line,  $n = 7$  insects) or continuously ad libitum-fed (open diamonds, solid line,  $n = 26$  insects) during each of stages 4–7 (defined in Table 1). RMR was measured as oxygen consumption rate ( $\dot{V}_{O_2}$ ) at standard temperature and pressure (STP). Trend lines are least-squares linear regressions (see text for equations) with slopes not significantly different from 1.0 (analyses of variance,  $p > 0.05$  for both slopes).

relationship between RMR and body mass is assumed to be linear and can be described using the following equations:

Continuously AL insects :

$$\dot{V}_{O_2} \text{ at STP} = 355.7M + 9.52 \quad (n = 104, r^2 = 0.965, p < 0.0001)$$

Continuously R insects :

$$\dot{V}_{O_2} \text{ at STP} = 270.6M + 3.59 \quad (n = 28, r^2 = 0.885, p < 0.0001)$$

### 3.2. Response of RMR to intake history

Using a scaling coefficient of 1.0, we found significant differences in mass-specific RMR ( $\mu\text{l h}^{-1} \text{g}^{-1}$ ) among treatment groups in each of stages 4–8 (Fig. 4). In all cases, metabolic rate of continuously AL insects was significantly higher than that of continuously R insects. Insects that were switched from AL to R as

juveniles had metabolic rates comparable to those of continuously R insects, whereas insects that were switched from R to AL as juveniles had metabolic rates higher than those of continuously AL insects but only in stage 5.

In adults, metabolic rates of insects that experienced a diet switch tended to be intermediate between the metabolic rates of continuously AL and continuously R insects. Repeated measures ANOVA revealed a significant effect of time on RMR as well as an interaction between time and diet treatment, with R and AL-R juv. insects differing from AL, AL-R juv., and AL-R adult insects in the effect of life-history stage on RMR (Table 2).

### 3.3. Correlations among RMR, adult lifespan, and total lifespan

Diet treatment significantly affected both adult and total lifespan (Table 3). Adult and total lifespan were positively correlated, and significant positive correlations also existed among the mass-specific metabolic rate measurements for individual insects in stages 5–8 (Table 4).

Mass-specific metabolic rate in stages 5 and 6 was positively correlated with adult lifespan, whereas mass-specific metabolic rate in stage 4 was negatively correlated with total lifespan. Total lifespan was positively correlated with the duration of each of stages 4–7, and adult lifespan was positively correlated with the duration of stage 8. Metabolic rate was negatively correlated with the duration of each of stages 4–7 and positively correlated with the duration of stage 8 (Table 4).

**Table 2**

Repeated measures analysis of variance results for mass-specific resting metabolic rate (RMR) measured in 57 insects from five different treatment groups in life-history stages 4–8.

Source of variation	df	SS	F	p
<i>Mass-specific RMR</i>				
Between subjects effects				
Group	4	$3.16 \times 10^5$	44.05	<0.0001
Error	52	$9.33 \times 10^4$		
Within subjects effects				
Time	4	$2.19 \times 10^5$	33.81	<0.0001
Group*Time	16	$2.07 \times 10^5$	8.01	<0.0001
Error (Time)	208	$3.36 \times 10^5$		

Notes: See Table 1 for an explanation of life-history stages and Fig. 1 for an explanation of the five treatment groups tested.

**Table 3**  
Adult and total lifespan ( $\bar{x} \pm SE$ ) of insects in each of five treatment groups.

Treatment group	Adult lifespan (days)	Total lifespan (days)
AL	157.4 $\pm$ 8.7 <sup>d</sup>	255.3 $\pm$ 8.7 <sup>c</sup>
AL-R juv.	90.8 $\pm$ 3.6 <sup>ab</sup>	222.1 $\pm$ 3.8 <sup>b</sup>
AL-R adult	82.6 $\pm$ 1.9 <sup>a</sup>	179.1 $\pm$ 2.5 <sup>a</sup>
R	105.0 $\pm$ 5.7 <sup>bc</sup>	352.4 $\pm$ 5.9 <sup>d</sup>
R-AL juv.	140.5 $\pm$ 12.2 <sup>cd</sup>	326.7 $\pm$ 10.8 <sup>d</sup>

Notes: Adult lifespan is defined as the number of days between the terminal molt and death, and total lifespan is defined as the number of days between hatching and death. Different letters within columns indicate values that are significantly different ( $p < 0.05$ ) among treatment groups. Sample sizes, treatment groups, and abbreviations are the same as in Fig. 1. Statistical analyses are the same as in Fig. 2.

#### 4. Discussion

Early observations that ambient temperature influences lifespan in flies (Loeb and Northrop, 1916, 1917) led to the hypothesis that caloric restriction (CR) exerts its beneficial effects on longevity by lowering metabolic rate (Sacher, 1977). Although evidence supporting the claim that CR is associated with decreased metabolic rate has been reported (DeLany et al., 1999; Ramsey et al., 2000; Even et al., 2001; Blanc et al., 2003), most intraspecific studies have identified either no correlation or a positive correlation between metabolic rate and lifespan (Houthoofd et al., 2002a; Hulbert et al., 2004; Speakman et al., 2004). As a result, the “reduction of metabolic rate hypothesis” explaining the lifespan-extending effects of CR has lost favor in recent years.

However, controversy about the associations between caloric restriction, metabolic rate, and longevity still exists. Much of this controversy stems from disagreement about the most appropriate statistical method for correcting measurements of energy expenditure for differences in body size (Even et al., 2001; Speakman et al., 2002; Blanc et al., 2003; Poehlman, 2003; Selman et al., 2005; Ramsey and Hagopian, 2006), as CR typically causes changes in either body mass or body composition (Speakman et al., 2002). Unfortunately, most studies of CR and metabolic rate have not included empirical intraspecific assessments of the relationship between body size and metabolic rate. Instead, the scaling factor that is used to account for differences in body size in many studies is often inferred from interspecific comparisons.

Our first objective in this study was to determine empirically the most suitable scaling coefficient for calculating mass-corrected RMR of insects that differed in body size. To do so, we evaluated the relationship between metabolic rate and body size over a range of body masses spanning more than an order of magnitude. As a general rule, metabolic rate should increase with body mass according to the equation  $y = a \cdot m^b$  where  $y$  is whole-animal metabolic rate,  $a$  is a proportionality coefficient,  $m$  is body mass, and  $b$  is the mass exponential scaling coefficient (or metabolic coefficient). The value for  $b$  is often assumed to be either 0.75 (Kleiber, 1932) or

0.67 (Heusner, 1982). However, our data showed that over a wide range of body sizes in both continuously ad libitum-fed (AL) and continuously food-restricted (R) insects, the value for  $b$  was not significantly different from 1.0.

It follows, then, that metabolic rate in Indian stick insects scales linearly with body mass. This study is not the first to report an isometric relationship between metabolic rate and body size. Intraspecific evaluations of metabolic rate in numerous other species have revealed that the value for  $b$  often approaches or even exceeds 1.0, particularly in larvae or juveniles (reviewed in Glazier, 2005). Apol et al. (2008) suggested that the discrepancy between these findings and the expected allometric scaling coefficient of 0.67 or 0.75 (which is derived largely from interspecific relationships) may reflect evolutionary optimization of physiological networks within but not across species.

Empirically determining the metabolic coefficient in *C. morosus* allowed us to evaluate appropriately the effects of different patterns of intake on RMR. To our knowledge, this study is the first to elucidate the responses of metabolic rate to different intake histories in individual insects through a developmental time series. In general, CR induced decreases in mass-specific RMR relative to ad libitum-fed insects, particularly during juvenile life-history stages. These results stand in stark contrast to those of others (Cooper et al., 2004; Hulbert et al., 2004) who found no effect of CR on metabolic rate in insects. However, both of these studies entailed dietary manipulations and metabolic rate measurements of adult flies, and only one (Hulbert et al., 2004) quantified metabolic rate of individuals. Perhaps this disagreement among studies reflects species-specific differences in metabolic responses to food restriction.

Alternatively, the disagreement may derive from methodological differences in the protocols used to impose a food restriction. As in our study, flies in the Cooper study (Cooper et al., 2004) experienced a quantitative food restriction whereby known, lesser amounts of food of comparable quality were given to CR flies than to AL flies. However, flies were group-fed, and intake was not adjusted with time to compensate for age-dependent decreases in consumption of AL flies. Conversely, flies in the Hulbert study (Hulbert et al., 2004) were individually maintained but experienced a qualitative food restriction whereby ad libitum amounts of food of lower quality were given to CR flies than to AL flies.

Mass-specific RMR of insects that experienced a diet switch from ad libitum to restricted feeding as juveniles (*AL-R juv.*) declined to *R* levels within two weeks of the diet switch. These lower metabolic rates persisted throughout life, indicating that the decrease from *AL* levels was not merely a transient effect of altered body composition during the initial weeks of food restriction. On the other hand, insects that were switched from a restricted to an ad libitum diet as juveniles (*R-AL juv.*) over-compensated for this diet switch and demonstrated metabolic rates higher than those of *AL* insects, but only in the stage in which the diet switch occurred.

**Table 4**

Spearman's rank correlations ( $\rho$ ) for mass-specific metabolic rate (MSMR) in each of five life-history stages, duration of each stage, and adult and total lifespan for individual insects.

Variable	Total Lifespan	MSMR Stage 4	MSMR Stage 5	MSMR Stage 6	MSMR Stage 7	MSMR Stage 8	Stage 4 Duration	Stage 5 Duration	Stage 6 Duration	Stage 7 Duration	Stage 8 Duration
Adult Lifespan	<b>0.697**</b>	-0.207	<b>0.328*</b>	<b>0.285*</b>	0.190	0.245	0.222	-0.168	-0.113	-0.112	<b>0.744**</b>
Total Lifespan		<b>-0.447**</b>	0.109	-0.094	-0.123	-0.179	<b>0.690**</b>	<b>0.406**</b>	<b>0.487**</b>	<b>0.459**</b>	0.173
MSMR Stage 4			-0.020	0.134	0.051	0.153	<b>-0.617**</b>				
MSMR Stage 5				<b>0.670**</b>	<b>0.357**</b>	<b>0.367**</b>		<b>-0.612**</b>			
MSMR Stage 6					<b>0.475**</b>	<b>0.538**</b>			<b>-0.791**</b>		
MSMR Stage 7						<b>0.424**</b>				<b>-0.513**</b>	
MSMR Stage 8											<b>0.615**</b>

Notes: Life-history stages are defined in Table 1. Sample size:  $n = 70$  for correlations involving data collected in juveniles and  $n = 57$  for correlations involving lifespan data or data collected in adults. Significant correlations are indicated in bold. Asterisks indicate level of significance (\*  $p < 0.05$ , \*\*  $p < 0.01$ ).

Metabolic rate appears to be more sensitive to intake during juvenile stages than during adult stages, as it did not significantly decrease in insects that experienced a diet switch after first oviposition (*AL-R adult*). Alternatively, the energetically expensive tasks of vitellogenesis and oogenesis may have masked any effects of CR on RMR in adults after first oviposition. In addition, our metabolic rate data for reproductively active adults may not represent true estimates of resting mass-specific energy expenditure because of the potential confounding influence of egg load on body mass.

After we quantified the effects of different patterns of food intake on RMR, our final objective was to correlate RMR in multiple life-history stages with adult and total lifespan. Although intake history modulated both metabolic rate and longevity, the association between these responses was not straightforward. We found significant differences in the mass-specific metabolic rates of insects that differed in adult and total lifespan, but metabolic rates were only weakly and often positively correlated with longevity. This finding corroborates the prevailing sentiment that the longevity-enhancing effects of CR are most likely not mediated by decreased energy expenditure (Masoro, 2005). In fact, our results do not contradict the recent suggestion that lifespan may be determined more by the robustness and stability of metabolic pathways than by metabolic rate (Demetrius, 2004; Olshansky and Rattan, 2005; Braeckman et al., 2006).

The strength and direction of the association between metabolic rate and lifespan depended on whether longevity was calculated as adult or total lifespan. Metabolic rate during the final two instars was weakly and positively correlated with adult lifespan but uncorrelated with total lifespan, whereas metabolic rate during the fourth instar was weakly and negatively correlated with total lifespan but uncorrelated with adult lifespan. These discrepancies can be reconciled by considering the effects of CR on rate of development. The decreases in metabolic rate induced by CR were strongly and consistently correlated with the duration of each life-history stage. Insects fed ad libitum had higher metabolic rates, progressed through the last three instars more rapidly, and became reproductively active as adults sooner than food-restricted insects. As a result, the longevity-enhancing effects of lifelong CR in *C. morosus* appear to be mediated directly by a reduction in rate of development rather than by a reduction in metabolic rate.

In our study, progression through each of life-history stages 4–7 (encompassing the final three instars and pre-reproductive adult stage) was retarded by CR, regardless of whether CR was first imposed at hatch or during development. This finding lends support to the controversial “retardation of growth and development hypothesis” (McCay et al., 1935; Miller et al., 2002) used to explain CR-induced lifespan extension. However, insects in our study that experienced CR at any time during life died sooner after becoming reproductively active and thus had shortened overall adult lifespans than insects that were continuously fed ad libitum. This result is at odds with the overwhelming majority of studies on CR and aging. In most cases, both mean and maximum lifespan are extended by CR (McCay et al., 1935; Weindruch and Walford, 1988; Masoro, 1995, 2002), with enhanced longevity after a switch to restricted feeding resulting from decreased instantaneous risk of mortality (Mair et al., 2003) and/or from a slowing of the subsequent rate of accumulation of aging-related damage (Pletcher et al., 2000).

The discrepancy between our study and others may result from the fact that food restriction protocols in insects, unlike in rodents, typically entail reductions in food quality rather than quantity (Partridge et al., 2005). In rare instances, insects have been subjected to quantitative food restriction (Boggs and Ross, 1993; Carey et al., 2002; Cooper et al., 2004; this study), but in all cases this form of CR was associated with shortened rather than extended adult lifespan. Had our food restriction protocol permitted ad libi-

tum consumption of lower quality food, it is possible that adult lifespan would have been extended rather than shortened by CR. Therefore, using a different feeding protocol might have changed the direction and strength of the correlations we observed between metabolic rate and longevity.

One of the limitations inherent to the use of RMR as an estimate of total caloric expenditure is that RMR accounts for only a fraction of the total daily energy budget of free-living, active animals. For example, RMR in small mammals has been shown to represent roughly 30% (range: 14–63%) of daily energy expenditure (Speakman, 2000). Accordingly, the effects of CR on the overall energy budget of an animal cannot be reliably extrapolated from its effects on RMR (Speakman et al., 2002). Thus, despite minimal support for a causal relationship among CR, RMR, and lifespan, metabolic responses of the total energy budget to CR may be at least partly responsible for retarding the aging process in some species. Indeed, evidence from the current study suggests that the lifespan-extending properties of early-onset CR result from retardation of growth and development that is, in turn, correlated with alterations in metabolic expenditure.

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