© 2021. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2021) 224, jeb240010. doi:10.1242/jeb.240010

SHORT COMMUNICATION

High carbohydrate diet ingestion increases post-meal lipid synthesis and drives respiratory exchange ratios above 1

Stav Talal[1,*], Arianne Cease[2], Ruth Farington[1], Hector E. Medina[3], Julio Rojas[4] and Jon Harrison[1]

ABSTRACT

Locusts have been reported to elevate metabolic rate in response to high carbohydrate diets; this conclusion was based on metabolic rates calculated from CO_2 production, a common practice for insects. However, respiratory exchange ratio (RER, CO_2 production divided by O_2 consumption) can rise above 1 as a result of *de novo* lipid synthesis, providing an alternative possible explanation of the prior findings. We studied the relationship between macronutrient ingestion, RER and lipid synthesis using South American locusts (*Schistocerca cancellata*) reared on artificial diets varying in protein: carbohydrate (p:c) ratio. RER increased and rose above 1 as dietary p:c ratio decreased. Lipid accumulation rates were strongly positively correlated with dietary carbohydrate content and ingestion. RERs above 1 were only observed for animals without food in the respirometry chamber, suggesting that hormonal changes after a meal may drive lipid synthesis. *Schistocerca cancellata* does not elevate metabolic rate on low p:c diets; in fact, the opposite trend was observed.

KEY WORDS: Carbohydrates, *De novo* lipogenesis, Locusts, Macronutrients, Respiratory exchange ratio, Respiratory quotient

INTRODUCTION

Diets in nature vary tremendously in protein:carbohydrate (p:c) ratio, but we still lack a firm understanding of how animals cope physiologically with this variation. During the last 30 years, locusts and grasshoppers have become one of the most important models for testing behavioral and physiological responses to dietary variation (Behmer, 2009; Simpson and Raubenhimer, 2012). It has been well demonstrated that locusts feeding on diets low in p:c ratio synthesize more lipid (Simpson and Raubenheimer, 2001; Zanotto et al., 1993). Additionally, locusts fed on artificial diets with lower p:c ratio have been reported to increase their CO_2 production rate ($\dot{V}_{CO_2}$), leading to the suggestion that locusts exhibit ‘wastage respiration’ on low p:c diets as a way to get rid of excess ingested carbohydrates (Zanotto et al., 1993, 1997). However, in these prior studies, metabolic rate was inferred from $\dot{V}_{CO_2}$, leaving open the possibility of the alternative hypothesis that the elevated CO_2 emission rate observed for locusts consuming low p:c food occurred because of an increase in the respiratory exchange ratio [$\dot{V}_{CO_2}$ and O_2 consumption rate ($\dot{V}_{O_2}$)], RER.

RER is a dynamic parameter, widely used in organismal and clinical biology to indicate fuel usage and calculate metabolic rate (Gluck et al., 2011; Högberg et al., 2006; Longo et al., 2010). For starved animals, RER values of 1 or 0.7 occur when metabolism is completely fueled by carbohydrates or lipids, respectively. Catabolism of proteins yields a RER of 0.8–0.85 (Elia and Livesey, 1988; Kleiber, 1961; Livesey and Elia, 1988). While RER is thought to go no higher than 1 in starved animals, when food is accessible, RER can exceed 1. For example, hummingbirds showed diurnal fluctuation of RER between 0.7 and 1.3 (night and day, respectively), reaching a maximum during the day when they had access to ∼20% sugar water (Powers, 1991). When commercial pigs are fattened, RER values up to 1.34 have been measured (Jakobsen and Thorbekt, 1993). One-week-old Ross chickens (broilers) had RER values of ∼1.2 when feeding on commercial food (Geelissen et al., 2006), and force-fed geese had post-feeding RER values as high as 1.4 (Benedict and Lee, 1937). The primary hypothesis to explain high RER values is *de novo* lipid synthesis, and this hypothesis is supported by multiple theoretical models (Elia and Livesey, 1988; Ferrannini, 1988; Hellerstein et al., 1996; Hellerstein, 1999; Livesey and Elia, 1988). In rodents, RER routinely goes above 1 in the feeding nocturnal phase, and this has been shown to be associated with *de novo* lipid synthesis (Ono-Moore et al., 2020; Wahlig et al., 2012). An alternative hypothesis to explain a RER above 1 is the use of the pentose phosphate pathway to generate antioxidants (Levin et al., 2017).

Here, we tested whether high carbohydrate diets induce elevated metabolic rate in locusts, whether dietary p:c ratio affects RER, and whether lipid accumulation explains variation in RER in locusts. To do this, we examined the effect of dietary p:c ratio on $\dot{V}_{CO_2}$, $\dot{V}_{O_2}$, RER, carbohydrate consumption and lipid accumulation. We utilized the South American locust *Schistocerca cancellata*, as their large size facilitates such measurements, and a contemporary outbreak of this species meant that they were highly available in the field.

MATERIALS AND METHODS

South American locusts

Schistocerca cancellata (Serville 1838) is a South American locust species which is usually limited to a narrow breeding zone in Argentina. However, during massive outbreaks, they can cover up to four-million square kilometers, including 6 countries (Medina et al., 2017). The last outbreak started at the beginning of 2015, and was still continuing during 2020. They are highly polyphagous, but earlier research suggests field populations of 5th and 6th instar nymphs prefer carbohydrate-biased diets (Talal et al., 2020).

Ethics statement

No special collecting permit or animal care protocol was required for this work. Field efforts in Paraguay were supported by the SENAVE, Paraguay. USDA permit to import locusts: P526P-19-03892 (permit holder: Dr Arianne Cease).

[1]School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA. [2]School of Life Sciences, School of Sustainability, Arizona State University, Tempe, AZ 85281, USA. [3]Dirección de Sanidad Vegetal - SENASA, Argentina. [4]Departamento de Campañas Fitosanitarias, Dirección de Protección Vegetal, SENAVE, Paraguay.

*Author for correspondence (stav.talal@gmail.com)

S.T., 0000-0003-1181-5291

Received 26 October 2020; Accepted 13 January 2021

Journal of Experimental Biology (2021) 224, jeb240010. doi:10.1242/jeb.240010

Animals and experimental design: field-based experiments

We collected *S. cancellata* 5th instar nymphs as they were marching in Gran Chaco, Paraguay, during April 2019. Details on collection, handling and lab rearing are provided in Talal et al., 2020; a brief description is provided here. Animals were brought to a SENAVE field lab and reared in group cages on locally collected grass (*Paspalum* sp.) for 3 days. Locusts were then provided with one of five different isocaloric artificial foods, which varied in protein and digestible carbohydrates (Raubenheimer and Simpson, 1993; Simpson and Abisgold, 1985): 7p:35c, 14p:28c, 21p:21c, 28p:14c and 35p:7c (% protein and % digestible carbohydrates, by dry mass), or were provided with both of the two most biased diets, 35p:7c and 7p:35c, to allow them to select their preferred p:c mixture. All the diets contained 54% cellulose and 4% vitamins and salts; proteins were provided as a mix of 3:1:1 casein:peptone: albumen, and carbohydrates were provided as a 1:1 mix of sucrose and dextrin. For 3–5 days before respirometry, nymphs were kept in groups of 15–20 individuals, in cages (20.3×20.3×20.3 cm) containing *ad libitum* amounts of their treatment diet and water tubes. During the respirometry measurements, locusts were not provided with food. Following respirometry, each individual was weighed using a portable scale (SLF103, Fisher Science Education, Waltham, MA, USA). In the field lab, the light:dark cycle was approximately 12 h:12 h, the temperature in the room averaged 32.2±1.9°C and relative humidity averaged 58.7±4.5% (means±s.d.), though humidity may have been higher in the locust chambers.

Animals and experimental design: lab experiments

In order to confirm the effect of different diets on energy metabolism, and to test whether metabolic responses differed during versus after food ingestion, we carried out respirometry under lab conditions with 6th (terminal) instar nymphs from a lab-reared population, with two food accessibility treatments (with or without food during respirometry). The lab experiments were conducted at Arizona State University (ASU) using 6th instar *S. cancellata* nymphs (two days after molt) from a population reared for nine generations in the lab from locusts collected from two locations in Argentina (Rio Cuarto, Córdoba, and Casa de Piedras, Catamarca). Lab rearing conditions were 30.0±0.5% relative humidity, 34.0±0.5°C during the day and 25.0±0.5°C during the night, under a 14 h:10 h light:dark photoperiod (supplementary radiant heat was supplied during the daytime by incandescent 40 W electric bulbs). During standard rearing, locusts were fed daily with wheat shoots, fresh lettuce leaves and wheat bran *ad libitum*. During the 5 days prior to respirometry, locusts were provided with one of three artificial diet treatments: 35p:7c, 21p:21c and 7p:35c. During this time, as for the field-based experiments, nymphs were reared in cages containing 15–20 individuals with *ad libitum* treatment diet and water. During the respirometry measurements, some locusts were provided with 0.3 g of their treatment diet and others had no food. Following respirometry, each individual was weighed.

Lipid accumulation and carbohydrate consumption

We measured lipid accumulation from the change in body lipid content over the course of the field experiment, using a different set of individuals. We used freshly caught 5th instar marching nymphs to measure initial lipid content. The nymphs were reared for 8 days on the two complementary diets (choice), or 6 days on one of the five no-choice diet treatments described above, and then killed by freezing and dried to a constant mass. We measured the macronutrient consumption by measuring the change in dry mass of the provided diets.

We used a chloroform extraction technique to measure lipid content (Loveridge, 1973), and calculated lipid accumulation (g) and accumulation rate (g day^{-1}) from the change in body lipid mass on each diet divided by number of days on the diet treatment. We measured carbohydrate consumption from the change in mass of dishes containing chemically defined artificial diets during the experiment.

Respirometry

We performed constant volume respirometry using a FoxBox field respirometry system (Sable Systems International, Las Vegas, NV, USA). The span of the oxygen analyzer was calibrated several times a day by flushing the system with dry, CO_2-free air for at least 20 min. The calibration of the CO_2 analyzer was carried out at the ASU lab, using pure nitrogen and two certified calibration tanks (252±1 and 1010±1 ppm of CO_2 balanced in nitrogen, factory certified). The respirometry chambers were 20 and 60 ml syringes (closed with a three-way valve) for the field studies and lab studies, respectively, because of the almost 3-fold difference in body mass between field-collected (∼0.5 g) and lab-reared (∼1.3 g) animals.

After inserting the nymph into the metabolic chamber, the chamber was flushed with dry, CO_2-free air for 1 min at a flow rate of 500 ml min^{-1}. The syringe was then sealed and placed at the rearing temperature for 50–70 min, after which 18 ml/45 ml (depending on the syringe size) of air was injected into a stream of dry, CO_2-free air, at a flow rate of 500 ml min^{-1}, which passed through a magnesium perchlorate column, CO_2 analyzer (FoxBox), an Ascarite®/silica gel column and then an oxygen analyzer (FoxBox). We corrected the metabolic chamber volume by subtracting the animal volume from it, which was calculated from animal mass assuming a density of 1. Baselining was repeated between individual measurements by passing dry CO_2-free air directly through the analyzers. Data collection and analysis were carried out using a UI-3 data acquisition interface and Expedata software (Sable Systems International).

Using the lipid accumulation rate (g day^{-1}) measured for the field animals:

$$\text{Lipid accumulation rate} = \frac{\text{average 6 day lipid content} - \text{average initial lipid content}}{\text{no. days on diet}} \quad (1)$$

(where lipid content is in g and diet treatment was applied for 6 days) and the lipid ($C_{55}H_{102}O_6$) molecular weight 859.4 g mol^{-1}, we calculated the lipid synthesis rate (mol h^{-1}):

$$\text{Lipid } (C_{55}H_{102}O_6) \text{ synthesis rate} = \frac{\text{Lipid accumulation rate}}{859.4 \times 24} \quad (2)$$

(where lipid accumulation rate is in g day^{-1}, and the numerator is lipid molecular weight multiplied by the number of hours in a day). From the lipid synthesis rate, the stoichiometric equation for synthesizing dioleylpalmityltriglyceride (the most common triglyceride in animals) from glucose (Elia and Livesey, 1988):

$$12.93C_6H_{12}O_6 \rightarrow 1C_{55}H_{102}O_6 + 22.62CO_2 + 25.98H_2 \quad (3)$$

and the CO_2 production rate (mol h^{-1}):

$$CO_2 \text{production rate} = \text{lipid } (C_{55}H_{102}O_6) \text{synthesis rate} \times 22.62CO_2 \quad (4)$$

(where lipid synthesis rate is in mol h^{-1}), we calculated the

$\dot{V}_{CO_2}$ (l h^{-1}) attributable to lipogenesis:

$$\dot{V}_{CO_2}(\text{lipids}) = CO_2\text{production rate} \times 22.4, \quad (5)$$

where CO_2 production rate (in mol h^{-1}) is multiplied by the molar volume of a gas at standard temperature and pressure (22.4 l mol^{-1}). We subtracted this amount from the total measured $\dot{V}_{CO_2}$ to calculate a lipid synthesis-independent RER (Eqns 6 and 7).

$$\dot{V}_{CO_2}(\text{corrected}) = \dot{V}_{CO_2}(\text{measured}) - \dot{V}_{CO_2}(\text{lipids}), \quad (6)$$

$$\text{RER (corrected)} = \frac{\dot{V}_{CO_2}(\text{corrected})}{\dot{V}_{O_2}(\text{measured})}. \quad (7)$$

All raw data are available in Table S1.

Statistics

Statistical analyses were performed using SPSS 19.0 statistical software (IBM, Armonk, NY, USA). Prior to using any parametric analysis, data normality and homoscedasticity were confirmed. $\log_{10}$ transformation of RER yielded data that satisfied assumptions of parametric analysis. In order to compare the effect of different diets on $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$, we used one-way ANCOVA with mass as a covariate. We used one-way ANOVA to test diet effects on $\log_{10}$RER. Because we predicted a positive linear effect of dietary carbohydrate on gas exchange, we also used a general linear model (GLM) to test the effect of body mass and dietary carbohydrate content (%) on $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$. For the lab-reared locusts, we used a GLM, testing the effects of dietary carbohydrate content (%), body mass and food availability (scoring '0' when food was not available and '1' for available food) on $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$. We used a two-way ANOVA to test the effect of different diets and food availability on $\log_{10}$RER measured on the lab-reared locusts.

RESULTS AND DISCUSSION

For field-collected *S. cancellata*, diet treatments did not significantly affect $\dot{V}_{O_2}$ ($F_{5,86}$=1.41, P=0.23), and $\dot{V}_{CO_2}$ ($F_{5,86}$=0.88, P=0.50) when analyzed by ANCOVA (Fig. 1A). However, with a GLM, both the effects of body mass and dietary carbohydrate content (%) were highly significant for $\dot{V}_{O_2}$ (Fig. 1B: GLM corrected model: r^2=0.55, $F_{2,73}$=47.45, P<0.001; mass: $F_{1,73}$=82.49, P<0.001; dietary carbohydrate content: $F_{1,73}$=5.31, P=0.024); $\dot{V}_{CO_2}$ was only affected significantly by mass (Fig. 1B: GLM corrected model: r^2=0.51; $F_{2,73}$=40.66, P<0.001; mass: $F_{1,73}$=81.23, P<0.001; dietary carbohydrate content: $F_{1,73}$=2.17, P=0.145). The measured RER increased strongly as dietary p:c ratio decreased, with RER rising from a mean of 0.88 on the most protein-biased diets to a mean of 1.15 on the most carbohydrate-biased diet (one-way ANOVA: $F_{5,86}$=22.55, P<0.001; Fig. 1C). Individuals given a choice of two diets had RER values of ∼1.05, significantly higher than those of the 28p:14c and 35p:7c treatment groups, but not significantly different from the other three treatment groups (Bonferroni *post hoc* tests; Fig. 1C). The corrected RER values (see Materials and Methods) were below 1 for all diet treatments (Fig. 1C).

For the lab-reared *S. cancellata*, $\dot{V}_{O_2}$ was affected by mass, food availability and the percentage of dietary carbohydrates (GLM corrected model: r^2=0.51, $F_{3,89}$=30.94, P<0.001; mass: $F_{1,89}$=66.29, P<0.001; food availability: $F_{1,89}$=4.38, P=0.039; dietary carbohydrate content: $F_{1,89}$=18.32, P<0.001; Fig. 2A). As in the field, the $\dot{V}_{CO_2}$ of lab-reared locusts was affected only by mass (GLM corrected model: r^2=0.335, $F_{3,89}$=14.96, P<0.001; mass: $F_{1,89}$=40.73, P<0.001; food availability: $F_{1,89}$=1.67, P=0.200; dietary carbohydrate content: $F_{1,89}$=1.75, P<0.189) (Fig. 2B). The RER was significantly affected by food availability ($F_{1,86}$=11.22, P=0.001) and diet treatment ($F_{2,86}$=26.33, P<0.001), as well as by the interactions of these two factors ($F_{2,86}$=36.07, P<0.001).

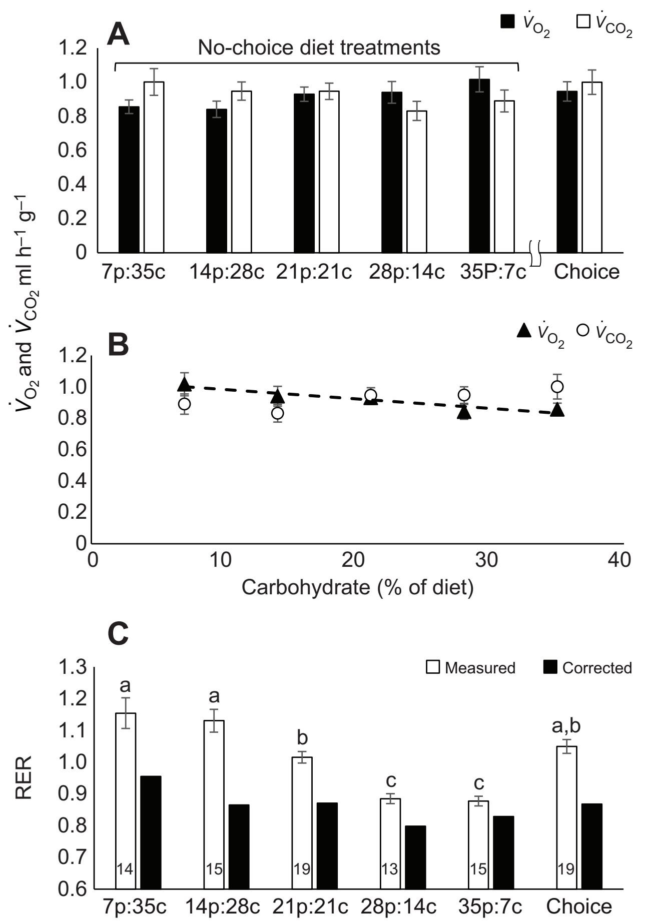

Fig. 1. Gas exchange responses of field-collected *Schistocerca cancellata* fed for 3–5 days on artificial diets containing different protein: carbohydrate (p:c) ratios. (A) Mass-corrected oxygen consumption rate ($\dot{V}_{O_2}$) and carbon dioxide production rate ($\dot{V}_{CO_2}$) were not significantly affected by the ingested macronutrient content when diet was treated as categorical variable (ANCOVA). (B) General linear model (GLM) analysis on locusts from the no-choice diet treatment group showed a significant effect of dietary carbohydrate content on $\dot{V}_{O_2}$ but not $\dot{V}_{CO_2}$. (C) Respiratory exchange ratio (RER) increased as the p:c ratio decreased, with RER significantly above 1 when carbohydrate-biased foods were ingested. On all diet treatments, the lipid synthesis-corrected RER values were below 1. Groups with similar letters did not differ significantly (Bonferroni *post hoc* tests, P<0.05), and the number inside the bars indicates the number of individuals in each treatment group. The data are presented as means±s.e.m.

Mean lipid accumulation rate was highly correlated with the mean carbohydrate consumption rate of each diet treatment group (Fig. 3A). The slope, which is the efficiency of conversion of dietary carbohydrates to lipid stores, was ∼17%. In addition, lipid accumulation rate was highly correlated with RER across diet treatment groups (Fig. 3B).

Our data do not support the 'wastage respiration hypothesis' for high carbohydrate diets of Zanotto et al. (1997). In contrast, we show that, for *S. cancellata*, $\dot{V}_{O_2}$ falls as the p:c ratio drops (Figs 1 and 2). Our results are consistent with other studies that have found higher metabolic rate (elevated $\dot{V}_{O_2}$) on diets higher in protein content, including research on shrimps (Taboada et al., 1998), birds (MacLeod and Dabutha, 1997), fish (Jobling and Davies, 1980) and humans (Johnston et al., 2002), likely due to the energetic costs of

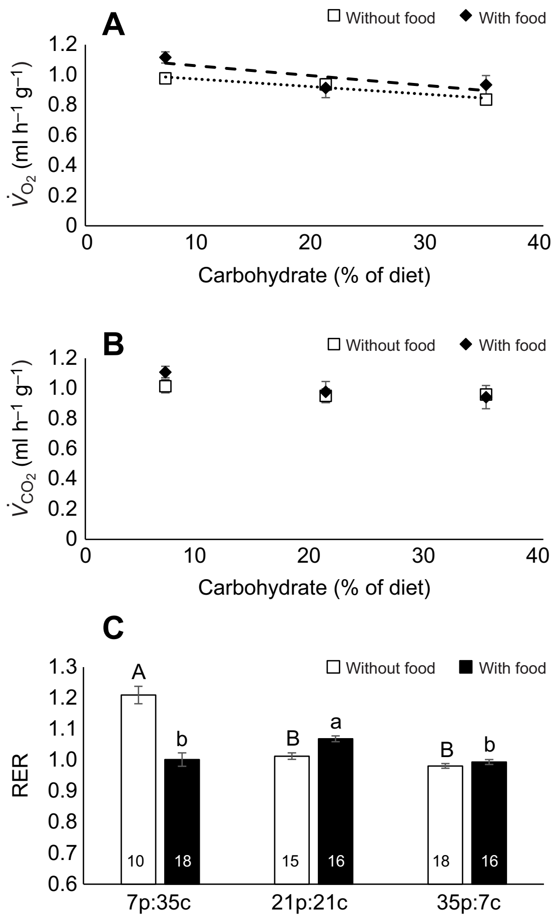

Fig. 2. Gas exchange responses of lab-reared *S. cancellata* fed for 5 days on artificial diets containing different p:c ratios. (A,B) GLM showed a significant effect of individual mass, food availability and dietary carbohydrate content on $\dot{V}_{O_2}$ (A), whereas $\dot{V}_{CO_2}$ was affected only by individual mass (B). (C) Two-way ANOVA showed that RER was affected by diet treatment, food availability and the diet×availability interaction (for statistics, see Results). Groups with similar uppercase/lowercase letters did not differ significantly (Bonferroni *post hoc* tests, P<0.05), and the number inside the bars indicates the number of individuals in each treatment group. The data are presented as means±s.e.m.

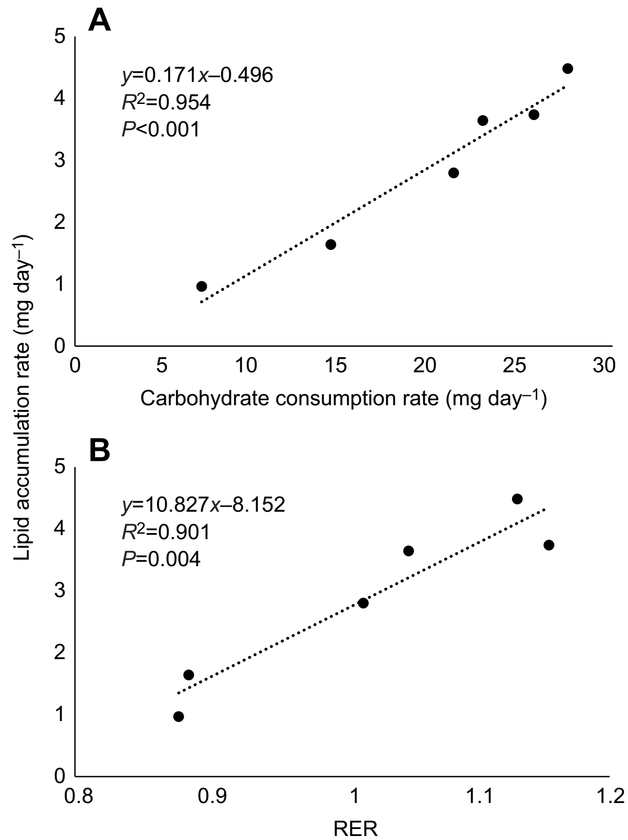

Fig. 3. Mean lipid accumulation rate for field-collected *S. cancellata*. The mean lipid accumulation rate over 8 days was well predicted by the mean carbohydrate consumption rate (A) and the mean RER (B).

increased gluconeogenesis from amino acids when dietary p:c ratio is high (Veldhorst et al., 2009; Williamson et al., 1971). Additionally, having food available during respirometry elevated $\dot{V}_{O_2}$ (Fig. 2A). This could be the effect of increased metabolism by digestion, absorption and assimilation processes (McCue, 2006).

Consumption of low p:c diets is associated with *de novo* synthesis of lipid from carbohydrate, driving RER above 1 in *S. cancellata*. The effects of diet on RER were similar for the unfed field-caught and lab-reared locusts (Figs 1 and 2), though we only measured lipid accumulation in the field-caught locusts. The elevated RER during lipid synthesis from carbohydrate occurs as a result of CO_2 release as pyruvate is converted to acetyl COA, as well as through activation of the pentose phosphate pathway (Schulz, 1978; Vagelos, 1971). The pentose phosphate pathway is activated during lipid synthesis to produce NADPH, which is required for fatty acid elongation (Elia and Livesey, 1988). Our results do not refute Levin et al.'s (2017) hypothesis that RER above 1 can be caused by activation of the pentose phosphate pathway in order to generate antioxidants. However, our results suggest that any study documenting RER above 1 should test for lipid synthesis as an explanatory factor.

When the quantity of lipids synthesized from carbohydrate can be estimated (as in this study), it is possible to calculate a RER corrected for lipid synthesis, that should represent the RER associated with catabolism, using Eqn 1. The lipid synthesis-corrected RER was ∼0.96 on the highest carbohydrate diet, and decreased to near 0.83 on the highest protein diets (Fig. 1C), reasonable values that suggest that locusts catabolize primarily carbohydrate when eating carbohydrate-rich diets and protein when eating protein-rich diets.

RER values only rose above 1 when food was not available, suggesting hormonal stimulation of lipid synthesis occurs after meal cessation. Our results showing that RER rose when food was not available contrast somewhat with results for rodents, in which RER falls when food is not consumed (Hou et al., 2019; Wahlig et al., 2012). However, the time frame studied and the temporal pattern of feeding differ in locusts and rodents. Locusts can consume food throughout the day and night if conditions are right, alternating multiple minutes of consumption and 'rest' during which food is processed (Simpson, 1990). Regardless of diet, locusts with artificial food in the respirometer (all of which ingested food for at least part of the meal) had a RER near 1, with RER only rising strongly above 1 on the high carbohydrate food when no food was available (Fig. 2). Perhaps *de novo* lipid synthesis is not appreciably activated until after cessation of feeding; alternatively, during feeding, much of the metabolic rate may be due to carbohydrate oxidation by the muscles required for feeding. The hormonal control of lipid synthesis and its timing relative to ingestion remain poorly known. In mammals, high lipid synthesis is triggered by post-feeding insulin secretion (Moustaïd et al., 1996; Smith and Kahn, 2016). While insulin/insulin like growth factor (IGF) occur in insects, there is insufficient evidence to indicate whether insulin-related peptides have similar effects in insects (Badisco et al., 2013; Nässel and Vanden Broeck, 2016). In *Rhodnius prolixus*, hemolymph lipids and carbohydrates rise following RNAi injection against IGF transcript, suggesting inhibition of lipid and glycogen synthesis in the fat body (Defferrari et al., 2016). However, injection of insulin-like peptide into *Bombyx mori* larvae did not affect the lipid stores in hemolymph or fat body (Kawabe et al., 2019).

RER values are routinely used to calculate metabolic rate (Lighton, 2008); however, our study suggests that current conversions may lead to considerable errors for feeding/growing animals and for insects catabolizing substantial protein. Many studies of insect respiration report only $\dot{V}_{CO_2}$ and calculate metabolic rate based on assumed RER. It is also relatively common for insect physiologists to measure metabolic rate for non-starved individuals, given that starvation is not a normal situation for a herbivorous insect such as a locust. Here, we demonstrated that the RER value is highly variable and is affected by dietary macronutrient ratio, and that metabolic rate calculated only from $\dot{V}_{CO_2}$ may be misleading. Also, the standard conversion from oxygen to joules or calories assumes no protein catabolism. For vertebrates, it is relatively common to calculate metabolic rate based on non-protein RER, which is usually estimated from measurements of nitrogenous waste (Elia and Livesey, 1988; Ferrannini, 1988; Livesey and Elia, 1988; Simonson and DeFronzo, 1990). The equations that exist to do this are based on mammalian models that assume that the major nitrogenous end-product is urea (Gessaman and Nagy, 1988). However, insects excrete urates, allantoin and ammonia, the ratio of which can depend on hydration state and diet (Harrison, 1995; Harrison and Kennedy, 1994; Zanotto et al., 1993). No calculations exist in the literature to estimate metabolic rate from non-protein RER for animals with such diverse excretory products. In mammals, it has been estimated that the error in metabolic rate from not including protein catabolism is only 1–2% if protein metabolism is ∼12% of the total metabolism (Ferrannini, 1988; Gessaman and Nagy, 1988; Kaiyala et al., 2019; Simonson and DeFronzo, 1990). However, it is plausible that the contribution of protein catabolism to total metabolism is much greater on very high p:c diets, such as may occur with predaceous insects, which could lead to much higher error.

Importantly, in order to use these common equations for metabolic rate calculation, two assumptions must not be violated, which, surprisingly, are not familiar to many physiologists. First, because indirect calorimetry estimates heat production based on nutrient combustion and respirometry gases, it assumes that $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ values are produced/consumed only by oxidation. However, CO_2 is also produced when *de novo* lipid synthesis occurs (Eqn 1). Second, all excretory nitrogen products are assumed to be produced by protein/amino acid deamination for oxidation (Simonson and DeFronzo, 1990). However, on high p:c diets, nitrogenous waste may be produced to support gluconeogenesis from amino acids, which could be relatively high when animals are fed on high protein/low carbohydrate diets (Veldhorst et al., 2009; Williamson et al., 1971). Clearly, further research is required to develop appropriate conversion factors to calculate metabolic rate from gas exchange for growing animals, and for animals such as insects that utilize a diversity of nitrogenous waste products. Careful measurement of RER under various conditions, combined with assessment of metabolic pathway flux and/or direct calorimetry will be required.

Acknowledgements
We want to thank Jacob Youngblood for his essential help in the field and field animal maintenance, as well as technical assistance during our field trip to Paraguay. Many thanks to Kelly O'Meara and Rick Overson for organizing and coordinating our research trip to Paraguay. We want to thank SENAVE for providing us with accommodation in Chaco, Paraguay, as well as field lab conditions for nutritional experiments. Many thanks to Marco Antonio Sosa Rolon who provided any help that we needed throughout our research in Paraguay. We also want to thank Eduardo Trumper and Fernando Copa for providing their essential knowledge of this locust species. Many thanks to Fredy Colque, Ricardo Oyols, Cesar Espinoza Vilca and Milton Cortez, our SENASAG-Bolivian collaborators for helping us locate locust bands near the Paraguay–Bolivia border and who took part in field experiments and plant and locust collections.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: S.T., A.C., H.E.M., J.R., J.H.; Software: S.T.; Validation: S.T., A.C., R.F., J.H.; Formal analysis: S.T., R.F.; Investigation: S.T., A.C., R.F., H.E.M., J.R., J.H.; Writing - original draft: S.T., A.C., J.H.; Project administration: A.C., J.H.

Funding
This work was supported by National Science Foundation (NSF) IOS-1826848 and United States - Israel Binational Agricultural Research and Development Fund (BARD) FI-575-2018 grants.

Supplementary information
Supplementary information available online at https://jeb.biologists.org/lookup/doi/10.1242/jeb.240010.supplemental

References

Badisco, L., Van Wielendaele, P. and Vanden Broeck, J. (2013). Eat to reproduce: a key role for the insulin signaling pathway in adult insects. *Front. Physiol.* **4**, 202. doi:10.3389/fphys.2013.00202

Behmer, S. T. (2009). Insect herbivore nutrient regulation. *Annu. Rev. Entomol.* **54**, 165-187. doi:10.1146/annurev.ento.54.110807.090537

Benedict, F. G. and Lee, R. C. (1937). *Lipogenesis in the Animal Body, with Special Reference to the Physiology of the Goose*, 232 pp. Carnegie Inst. Washingt. Pub.

Defferrari, M. S., Orchard, I. and Lange, A. B. (2016). An Insulin-Like Growth Factor in Rhodnius prolixus is involved in post-feeding nutrient balance and growth. *Front. Neurosci.* **10**, 566. doi:10.3389/fnins.2016.00566

Elia, M. and Livesey, G. (1988). Theory and validity of indirect calorimetry during net lipid synthesis. *Am. J. Clin. Nutr.* **47**, 591-607. doi:10.1093/ajcn/47.4.591

Ferrannini, E. (1988). The theoretical bases of indirect calorimetry: a review. *Metabolism* **37**, 287-301. doi:10.1016/0026-0495(88)90110-2

Geelissen, S. M. E., Swennen, Q., Van Der Geyten, S., Kühn, E. R., Kaiya, H., Kangawa, K., Decuypere, E., Buyse, J. and Darras, V. M. (2006). Peripheral ghrelin reduces food intake and respiratory quotient in chicken. *Domest. Anim. Endocrinol.* **30**, 108-116. doi:10.1016/j.domaniend.2005.06.005

Gessaman, J. A. and Nagy, K. A. (1988). Energy metabolism: errors in gas-exchange conversion factors. *Physiol. Zool.* **61**, 507-513. doi:10.1086/physzool.61.6.30156159

Gluck, M. E., Venti, C. A., Salbe, A. D., Votruba, S. B. and Krakoff, J. (2011). Higher 24-h respiratory quotient and higher spontaneous physical activity in nighttime eaters. *Obesity* **19**, 319-323. doi:10.1038/oby.2010.206

Harrison, J. F. (1995). Nitrogen metabolism and excretion in locusts. In *Nitrogen Metabolism and Excretion* (ed. P. J. Walsh and R. Wright), pp. 119-131. Boca Raton, FL: CRC Press.

Harrison, J. F. and Kennedy, M. J. (1994). In vivo studies of the acid-base physiology of grasshoppers: the effect of feeding state on acid-base and nitrogen Excretion. *Physiol. Zool.* **67**, 120-141. doi:10.1086/physzool.67.1.30163838

Hellerstein, M. K. (1999). De novo lipogenesis in humans: metabolic and regulatory aspects. *Eur. J. Clin. Nutr.* **53**, s53-s65. doi:10.1038/sj.ejcn.1600744

Hellerstein, M. K., Schwarz, J.-M. and Neese, R. A. (1996). Regulation of hepatic de novo lipogenesis in humans. *Annu. Rev. Nutr.* **16**, 523-557. doi:10.1146/annurev.nu.16.070196.002515

Högberg, H., Engblom, L., Ekdahl, Å., Lidell, V., Walum, E. and Alberts, P. (2006). Temperature dependence of O2 consumption; opposite effects of leptin and etomoxir on respiratory quotient in mice. *Obesity* **14**, 673-682. doi:10.1038/oby.2006.76

Hou, T., Su, W., Guo, Z. and Gong, M. C. (2019). A novel diabetic mouse model for real-time monitoring of clock gene oscillation and blood pressure circadian rhythm. *J. Biol. Rhythms* **34**, 51-68. doi:10.1177/0748730418803719

Jakobsen, K. and Thorbekt, G. (1993). The respiratory quotient in relation to fat deposition in fattening-growing pigs. *Br. J. Nutr.* **69**, 333-343. doi:10.1079/BJN19930037

Jobling, M. and Davies, P. S. (1980). Effects of feeding on metabolic rate, and the Specific Dynamic Action in plaice, Pleuronectes platessa L. *J. Fish Biol.* **16**, 629-638. doi:10.1111/j.1095-8649.1980.tb03742.x

Johnston, C. S., Day, C. S. and Swan, P. D. (2002). Postprandial thermogenesis is increased 100% on a high-protein, low-fat diet versus a high-carbohydrate, low-fat diet in healthy, young women. *J. Am. Coll. Nutr.* **21**, 55-61. doi:10.1080/07315724.2002.10719194

Kaiyala, K. J., Wisse, B. E. and Lighton, J. R. B. (2019). Validation of an equation for energy expenditure that does not require the respiratory quotient. *PLoS ONE* **14**, 1-15. doi:10.1371/journal.pone.0211585

Kawabe, Y., Waterson, H. and Mizoguchi, A. (2019). Bombyxin (bombyx insulin-like peptide) increases the respiration rate through facilitation of carbohydrate catabolism in Bombyx mori. *Front. Endocrinol. (Lausanne).* **10**, 150. doi:10.3389/fendo.2019.00150

Kleiber, M. (1961). *The Fire of Life: An Introduction to Animal Energetics*. New York and London: John Wiley and Sons,Inc.

Levin, E., Lopez-Martinez, G., Fane, B. and Davidowitz, G. (2017). Hawkmoths use nectar sugar to reduce oxidative damage from flight. *Science (80-.).* **355**, 733-735. doi:10.1126/science.aah4634

Lighton, J. R. B. (2008). *Measuring Metabolic Rates: A Manual for Scientists*. New York: Oxford University Press.

Livesey, G. and Elia, M. (1988). Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: Evaluation of errors with special reference to the detailed composition of fuels. *Am. J. Clin. Nutr.* **47**, 608-628. doi:10.1093/ajcn/47.4.608

Longo, K. A., Charoenthongtrakul, S., Giuliana, D. J., Govek, E. K., McDonagh, T., DiStefano, P. S. and Geddes, B. J. (2010). The 24-h respiratory quotient predicts energy intake and changes in body mass. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **298**, 747-754. doi:10.1152/ajpregu.00476.2009

Loveridge, J. P. (1973). Age and the changes in water and fat content of adult laboratory-reared Locusta migratoria migratorioides R and F. *Rhod. J. Agric. Res.* **11**, 131-143.

MacLeod, M. G. and Dabutha, L. A. (1997). Diet selection by Japanese quail (Coturnix coturnix japonica) in relation to ambient temperature and metabolic rate. *Br. Poult. Sci.* **38**, 586-589. doi:10.1080/00071669708418040

McCue, M. D. (2006). Specific dynamic action: a century of investigation. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **144**, 381-394. doi:10.1016/j.cbpa.2006.03.011

Medina, H. E., Cease, A. J. and Trumper, E. V. (2017). The resurgence of the South American locust (Schistocerca cancellata). *Metaleptea* **37**, 17-21.

Moustaïd, N., Jones, B. H. and Taylor, J. W. (1996). Insulin increases lipogenic enzyme activity in human adipocytes in primary culture. *J. Nutr.* **126**, 865-870. doi:10.1093/jn/126.4.865

Nässel, D. R. and Vanden Broeck, J. (2016). Insulin/IGF signaling in Drosophila and other insects: Factors that regulate production, release and post-release action of the insulin-like peptides. *Cell. Mol. Life Sci.* **73**, 271-290. doi:10.1007/s00018-015-2063-3

Ono-Moore, K. D., Rutkowsky, J. M., Pearson, N. A., Williams, D. K., Grobe, J. L., Tolentino, T., Lloyd, K. C. K. and Adams, S. H. (2020). Coupling of energy intake and energy expenditure across a temperature spectrum: impact of diet-induced obesity in mice. *Am. J. Physiol. Metab.* **319**, E472-E484. doi:10.1152/ajpendo.00041.2020

Powers, D. R. (1991). Diurnal variation in mass, metabolic rate, and respiratory quotient in Anna's and Costa's hummingbirds. *Physiol. Zool.* **64**, 850-870. doi:10.1086/physzool.64.3.30158211

Raubenheimer, D. and Simpson, S. J. (1993). The geometry of compensatory feeding in the locust. *Anim. Behav.* **45**, 953-964. doi:10.1006/anbe.1993.1114

Schulz, A. R. (1978). Simulation of energy metabolism in the simple-stomached animal. *Br. J. Nutr.* **39**, 235-254. doi:10.1079/BJN19780034

Simonson, D. C. and DeFronzo, R. A. (1990). Indirect calorimetry: methodological and interpretative problems. *Am. J. Physiol. Endocrinol. Metab.* **258**, E399-E412. doi:10.1152/ajpendo.1990.258.3.E399

Simpson, S. J. (1990). The pattern of feeding. In *The Biology of Grasshoppers* (ed. R. F. Chapman and A. Joern), pp. 73-104. New York: John Wiley and Sons.

Simpson, S. J. and Abisgold, J. D. (1985). Compensation by locusts for changes in dietary nutrients: behavioural mechanisms. *Physiol. Entomol.* **10**, 443-452. doi:10.1111/j.1365-3032.1985.tb00066.x

Simpson, S. J. and Raubenheimer, D. (2001). The geometric analysis of nutrient-allelochemical interactions: a case study using locusts. *Ecology* **82**, 422-439. doi:10.1890/0012-9658(2001)082[0422:TGAONA]2.0.CO;2

Simpson, S. J. and Raubenhimer, D. (2012). *The Nature of Nutrition. A Unifying Framework from Animal Adaptation to Human Obisity*. Princeton University Press.

Smith, U. and Kahn, B. B. (2016). Adipose tissue regulates insulin sensitivity: role of adipogenesis, de novo lipogenesis and novel lipids. *J. Intern. Med.* **280**, 465-475. doi:10.1111/joim.12540

Taboada, G., Gaxiola, G., Garcia, T., Pedroza, R., Sanchez, A., Soto, L. A. and Rosas, C. (1998). Oxygen consumption and ammonia-N excretion related to protein requirements for growth of white shrimp, Penaeus setiferus (L.), juveniles. *Aquac. Res.* **29**, 823-833. doi:10.1111/j.1365-2109.1998.tb01108.x

Talal, S., Cease, A. J., Youngblood, J. P., Farington, R., Trumper, E. V., Medina, H. E., Rojas, J. E., Fernando Copa, A. and Harrison, J. F. (2020). Plant carbohydrate content limits performance and lipid accumulation of an outbreaking herbivore. *Proc. R. Soc. B* **287**, 20202500. doi:10.1098/rspb.2020.2500

Vagelos, P. R. (1971). *Regulation of Fatty Acid Biosynthesis*. Academic Press, Inc.

Veldhorst, M. A. B., Westerterp-Plantenga, M. S. and Westerterp, K. R. (2009). Gluconeogenesis and energy expenditure after a high-protein, carbohydrate-free diet. *Am. J. Clin. Nutr.* **90**, 519-526. doi:10.3945/ajcn.2009.27834

Wahlig, J. L., Bales, E. S., Jackman, M. R., Johnson, G. C., McManaman, J. L. and MacLean, P. S. (2012). Impact of high-fat diet and obesity on energy balance and fuel utilization during the metabolic challenge of lactation. *Obesity* **20**, 65-75. doi:10.1038/oby.2011.196

Williamson, J. R., Jákob, A. and Scholz, R. (1971). Energy cost of gluconeogenesis in rat liver. *Metabolism* **20**, 13-26. doi:10.1016/0026-0495(71)90056-4

Zanotto, F. P., Simpson, S. J. and Raubenheimer, D. (1993). The regulation of growth by locusts through post-ingestive compensation for variation in the levels of dietary protein and carbohydrate. *Physiol. Entomol.* **18**, 425-434. doi:10.1111/j.1365-3032.1993.tb00617.x

Zanotto, F., Gouveia, S., Simpson, S. J. and Calder, D. (1997). Nutritional homeostasis in locusts: is there a mechanism for increased energy expenditure during carbohydrate overfeeding? *J. Exp. Biol.* **200**, 2437-2448.