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Special K: testing the potassium link between radioactive rubidium (^{86}Rb) turnover and metabolic rate

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ABSTRACT

The measurement of ^{86}Rb turnover recently has been suggested as a useful method for measuring field metabolic rate in small animals. We investigated a proposed mechanism of ^{86}Rb turnover, its analogy to K^+, by comparing the turnover of ^{86}Rb in a model insect, the rhinoceros beetle *Xylotrupes gideon*, fed a diet of plum jam or plum jam enriched with K^+ or Rb^+. The turnover of ^{86}Rb in the beetles on the K^+ and the Rb^+ diets was higher than that for beetles on the jam diet ($F_{2,311}$=32.4; P=1.58×10^{-13}). We also exposed the beetles to different ambient temperatures to induce differences in metabolic rate ($\dot{V}_{CO_2}$) while feeding them the jam and K^+ diets. $\dot{V}_{CO_2}$ was higher at higher ambient temperature (T_a) for both jam ($F_{1,11}$=14.56; P=0.003) and K^+ ($F_{1,8}$=15.39; P=0.004) dietary groups, and the turnover of ^{86}Rb was higher at higher T_a for both jam ($F_{1,11}$=10.80; P=0.007) and K^+ ($F_{1,8}$=12.34; P=0.008) dietary groups. There was a significant relationship between ^{86}Rb turnover and $\dot{V}_{CO_2}$ for both the jam ($F_{1,11}$=35.00; P=1.0×10^{-3}) and the K^+ ($F_{1,8}$=64.33; P=4.3×10^{-5}) diets, but the relationship differed between the diets ($F_{1,19}$=14.07; P=0.001), with a higher ^{86}Rb turnover in beetles on the K^+-enriched than on the jam diet at all T_a. We conclude that ^{86}Rb turnover is related to K^+ metabolism, and that this is the mechanism of the relationship between ^{86}Rb turnover and $\dot{V}_{CO_2}$. Studies relating ^{86}Rb turnover to $\dot{V}_{CO_2}$ should maintain dietary [K^+] as close as possible to that of natural diets for the most accurate calibrations for free-ranging animals.

KEY WORDS: ^{86}Rb k_b, *Xylotrupes gideon*, Insect, Isotope turnover, Metabolic rate

INTRODUCTION

The metabolic rate of an animal is a measure of that animal's energy expenditure and reflects the cost of living (Hulbert and Else, 2004). The basal metabolic rate (BMR) is generally used to compare the metabolic rate of endotherms to each other, being the metabolic rate measured under defined conditions when an animal is inactive, post-absorptive and non-reproductive within the thermoneutral zone (Ricklefs et al., 1996; McNab, 1997). The standard metabolic rate (SMR) is measured under less stringent conditions when an animal is at rest, and is broadly considered to represent the minimal cost of living (Hulbert and Else, 2004). In insects there is no definition for BMR, and the SMR is usually used for comparative purposes (Quinlan and Gibbs, 2006). Metabolic rate can be readily measured in the laboratory by direct calorimetry, which involves measuring the heat produced by an animal (Kleiber, 1961; McLean and Tobin, 1987). More commonly used is indirect calorimetry, including flow-through respirometry, which measures the gas metabolism of an animal (either oxygen consumption, $\dot{V}_{O_2}$, or carbon dioxide production, $\dot{V}_{CO_2}$, or both) and is recognised as one of the easiest and most accurate approaches to the measurement of metabolism (Frappell, 2006). These are often used as proxies for true calorimetric representations of energy expenditure by virtue of relatively straightforward conversions to calorimetry, providing the respiratory exchange ratio (RER) or respiratory quotient (RQ) is known.

While laboratory measures of metabolic rate are informative in a comparative context (Brody and Procter, 1932; Kleiber, 1932; Elgar and Harvey, 1987; McNab, 1997), it is often difficult to extrapolate these measurements under controlled conditions to the cost of living in dynamic ecological scenarios. The utility of extrapolating metabolic rate data from captive animals to free-living animals has been questioned, especially when the latter experience different conditions such as food supply, quality of life and weather compared with laboratory conditions (Nagy, 1987). In contrast, the field metabolic rate (FMR) is measured in animals that are free-ranging in their natural environment (Ricklefs et al., 1996) and reflects the summary cost of living for that animal in its natural environment. The FMR includes the costs of BMR, SMR, thermoregulation, locomotion, feeding, predation avoidance, alertness, posture and digestion (Nagy, 1987). As FMR is measured over several days, the average daily rate of metabolism is often referred to as daily energy expenditure (DEE) or average daily metabolic rate (ADMR). DEE and ADMR are considered to be the most relevant measure of metabolism for ecological studies as they come nearest to the real measure of metabolic rate under natural conditions when an animal pursues all its natural functions in that environment.

There are several ways that DEE can be measured. Heart rate has been measured in free-living animals and, using correlations of heart rate with metabolism, converted to DEE (Bevan and Butler, 1992; Bevan et al., 1994; Bevan et al., 1995a). More often, stable isotope turnovers have been used, in what is known as the doubly labelled water method (DLW) (Lifson et al., 1955; Schoeller, 1988; Westerterp and Plasqui, 2004). The DLW method calculates CO_2 production by measuring the fractional turnover of isotopes of hydrogen (i.e. ^{2}H or ^{3}H) and oxygen (i.e. ^{18}O). The accuracy and precision of the DLW method make it ideal for studies in some free-living animals (Schoeller and Webb, 1984; Williams and Nagy, 1984; Williams, 1985; Nagy et al., 1990; Tiebout and Nagy, 1991; Bevan et al., 1995b; Speakman, 1998; Jones et al., 2009). A key aspect to the DLW method, however, is the requirement to sample body fluids from the subject animal, which has made its application to invertebrate systems difficult and limited (Buscarlet et al., 1978; King and Hadley, 1979; Yokota, 1979; Cooper, 1983; Wolf et al., 1996), and also to recapture the animals within the biological half-

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life of the isotopes, which is often prohibitively short in small animals (Speakman, 1997; Bradshaw and Bradshaw, 2007).

As an alternative to the measurement of stable isotope turnover, radio-isotopic turnovers have been used to measure FMR (Relman, 1956; Odum and Golley, 1963; Argoud et al., 1987). It has now been fairly well established that the turnover of rubidium-86 (^{86}Rb), a gamma and beta radio-emitter, correlates well with metabolic rate in vertebrates (Mishima and Odum, 1963; Fairbanks and Burch, 1968; Baker and Dunaway, 1975; Williamson, 1975; Peters et al., 1995; Peters, 1996; Bradshaw and Bradshaw, 2007; Tomlinson et al., 2013), and that there are some general patterns to this relationship that span all of the taxa studied so far (Tomlinson et al., 2013). Radio-isotope enrichment, particularly of gamma emitters, can be measured by whole-body counting, obviating the need for sub-sampling of the body pool (Bradshaw and Bradshaw, 2007). Furthermore, radio-isotope enrichment provides a simpler avenue for measuring FMR, and one that extends the recapture period far beyond that of DLW in small animals because the biological half-life of the isotopes is longer (Bradshaw and Bradshaw, 2007; Tomlinson et al., 2013). The method may be particularly applicable to measuring FMR in insects (Tomlinson et al., 2013).

Our lack of a quantified mechanism that links ^{86}Rb biological turnover (^{86}Rb k_b, where k_b is the biological elimination constant) to FMR is, however, a limitation of the technique (Tomlinson et al., 2013). It is hypothesised that the body handles Rb^+ similarly to the way it handles K^+ because Rb^+ is an alkali metal with a single electron in its outer orbital (group I), and appears in the periodic table below K^+ (Bradshaw and Bradshaw, 2007; Tomlinson et al., 2013). The measure of ^{86}Rb k_b is probably an indirect measure of K^+ turnover, and because [K^+] is tightly regulated in the body, the turnover may simply be a measure of K^+ intake. Peters et al. (Peters et al., 1995), however, presented evidence that ^{86}Rb k_b was not simply a measure of K^+ intake in lizards, because the relationship between ^{86}Rb k_b and metabolic rate remained even after accounting for food intake. The other possibility is that ^{86}Rb k_b is measuring metabolic rate via some mechanism that is related to K^+, such as ATP utilisation by the ubiquitous Na^+/K^+-ATPase in cell membranes that maintains high intracellular [K^+] and low intracellular [Na^+]. An increase in the activity of the Na^+/K^+-ATPase should be proportional to oxidative metabolism, and if Rb^+ turnover captures the change in activity of the pump, then the measure will be related to metabolic rate but may also be influenced by K^+ intake.

The purpose of the present study was twofold; firstly, to validate the use of ^{86}Rb k_b to measure the DEE of an insect (in this case, *Xylotrupes gideon* Guérin-Méneville 1830) to provide a proof of concept that the technique could be used to measure FMR in insect taxa; secondly, to test the effects of increased dietary potassium intake upon the association of ^{86}Rb k_b with $\dot{V}_{CO_2}$.

RESULTS AND DISCUSSION

Mass measurements

There was no significant change in *X. gideon* body mass during the experiments ($F_{2,24}$=0.196; P=0.824; N_{jam}=11, N_K=9, N_{Rb}=4). The mean (±s.e.) body mass was 3.84±0.43 g for the jam diet, 4.15±0.43 g for the K^+ diet and 4.47±0.4 3g for the Rb^+ diet.

The effect of dietary K^+ and Rb^+ on ^{86}Rb washout

There was a significant effect of diet on the exponential decay of ^{86}Rb over time ($F_{2,311}$=32.4; P=1.58×10^{-13}; N_{jam}=6, N_K=5, N_{Rb}=5). The jam diet had a significantly lower decay exponent than the K^+ (t_{12}=2.35; P=0.049) and Rb^+ (t_{12}=2.70; P=0.020) experimental diets, indicating a slower rate of decay. The K^+ and Rb^+ diets were not significantly different (t_{12}=0.469; P=0.886), and the biological decay of all three diets was faster than the physical decay of the ^{86}Rb radionuclide (Fig. 1). The exponential decay relationships were:

$$\%\ \text{Jam remaining} = 0.926e^{-0.106t}\ (F_{1,130}=177;\ P=2.20\times10^{-16}), \quad (1)$$

$$\%\ K^+\ \text{remaining} = 0.958e^{-0.203t}\ (F_{1,105}=927;\ P=2.20\times10^{-16}), \quad (2)$$

$$\%\ Rb^+\ \text{remaining} = 0.958e^{-0.234t}\ (F_{1,76}=99.4;\ P=1.90\times10^{-15}), \quad (3)$$

where t is the time since enrichment, and the statistics represent ANOVA of the ln-transformed linear regression. The ^{86}Rb counts were below the detection limit at approximately day 14 post-enrichment on the enriched diets, and between days 24 and 25 post-enrichment on the jam diet. There was natural attrition during these trials, especially on the Rb^+ experimental diet, and data from only 15 beetles were analysed.

The rapid biological decay of ^{86}Rb in *X. gideon* on all three diets indicates that ^{86}Rb was being excreted, possibly through the same excretory processes as K^+. The elimination of K^+ is a well-described aspect of insect iono-regulation (Schweikl et al., 1989; Wieczorek et al., 1989; Klein et al., 1991; Wieczorek et al., 1991; Maddrell and O'Donnell, 1992; Wieczorek, 1992; Zeiske, 1992; O'Donnell, 2008), and K^+ and Rb^+ are excreted through the same homeostatic processes (Ringer, 1884; Shoemaker et al., 1972; Lehninger, 1975; Metzler, 1977). The increased ^{86}Rb k_b in beetles on both the K^+ and Rb^+ experimental diets compared with the jam diet suggests that increases in K^+ intake increase the ^{86}Rb k_b. Furthermore, the turnover rates of both the K^+ and Rb^+ diet groups were the same, consistent with data reported by Fairbanks and Burch (Fairbanks and Burch, 1968), implying that, although ^{86}Rb is obviously analogous to Rb^+, it is also an analogue of K^+.

There was some variability around the general exponential decline (Fig. 1), where from day to day some measures tended to occasionally plateau, or sometimes increase at low levels of enrichment. This was probably a result of measurement variability, as small shifts in the posture of the beetles have higher proportional impacts on activity counts at low levels of enrichment. It is, however, also possible that the beetles managed to re-ingest some excreted ^{86}Rb isotope, and that their enrichment genuinely rose. Although this is a difficulty of using radioisotopes in contained and contaminated environments, it is unlikely to be a problem in the field, where excreted isotope should dissipate rapidly.

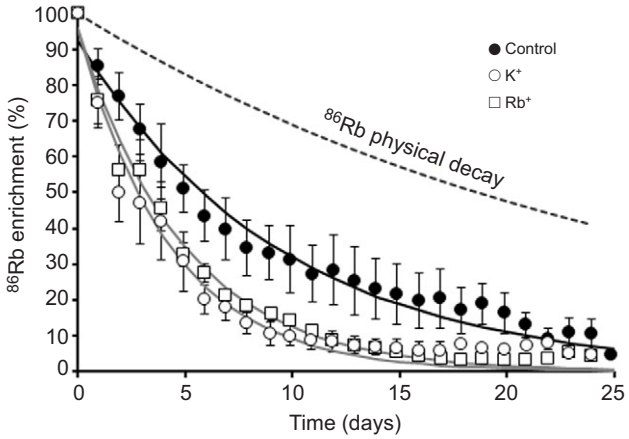

Fig. 1. ^{86}Rb washout curves of the *Xylotrupes gideon* control, K^+ and Rb^+ diet groups and the physical decay of ^{86}Rb over 25 days. Data are mean (±s.e.) percentage of initial enrichment, uncorrected for physical decay; lines are non-linear least squares models of exponential decay for each diet.

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^{86}Rb k_b, temperature and metabolic rate

The rate of CO_2 production increased significantly with increasing ambient temperature (T_a) ($F_{1,19}$=30.1, P=2.71×10^{-5}; N_{jam}=5, N_K=4), and there was no effect of diet ($F_{1,19}$=0.031; P=0.862; Fig. 2A). At T_a=20°C the $\dot{V}_{CO_2}$ was 0.11±0.01 l day^{-1}, which increased to 0.15±0.01 l day^{-1} at 25°C and 0.18±0.01 l day^{-1} at 30°C on the jam diet. At T_a=20°C the $\dot{V}_{CO_2}$ was 0.11±5.0×10^{-4} l day^{-1}, which increased to 0.16±0.005 l day^{-1} at 25°C and 0.18±0.02 l day^{-1} at 30°C on the K$^+$ diet.

The ^{86}Rb k_b increased significantly with increasing T_a ($F_{1,19}$=19.4, P=3.03×10^{-4}; N_{jam}=5, N_K=4), and was significantly higher on the K$^+$-supplemented diet than on the jam diet at each T_a ($F_{1,19}$=73.2; P=6.10×10^{-8}; Fig. 2B). At T_a=20°C the ^{86}Rb k_b was 0.536±0.098 k_b g, which increased to 0.656±0.119 k_b g at 25°C and 0.964±0.078 k_b g at 30°C on the jam diet. At T_a=20°C the ^{86}Rb k_b was 1.22±0.108 k_b g, which increased to 2.14±0.129 k_b g at 25°C and 2.24±0.276 k_b g at 30°C on the K$^+$ diet.

Although the washout trials established a link between K$^+$ excretion and ^{86}Rb k_b, this is not informative of DEE, nor of any association between DEE and ^{86}Rb k_b. Consistent with our expectations for an ectotherm (Lighton, 1996; Komai, 1998; Zhou et al., 2000; Acar et al., 2001; Woodman et al., 2007; Gibbs and Hoshizaki, 2008), DEE increased with increasing T_a. Furthermore, consistent with the expectation that ^{86}Rb k_b should increase similarly to $\dot{V}_{CO_2}$ (Peters et al., 1995; Peters, 1996; Bradshaw and Bradshaw, 2007; Tomlinson et al., 2013), ^{86}Rb k_b also increased with increasing T_a.

As there was no difference in the $\dot{V}_{CO_2}$ of the beetles on the control diet and the K$^+$ experimental diet, it is apparent that additional K$^+$ intake had no effect on DEE. However, consistent with the washout trials, the relationship of ^{86}Rb k_b to T_a had a higher slope in the beetles receiving the K$^+$ diet to that in those receiving the jam diet. A more intuitive way of interpreting these data is that at the same T_a there was a higher ^{86}Rb k_b in the K$^+$ experimental diet group than in the jam diet group. As $\dot{V}_{CO_2}$ was not different between the two groups, presumably the effect was related to K$^+$ elimination, independent of the role of K$^+$ in what we measured as the metabolic rate ($\dot{V}_{CO_2}$).

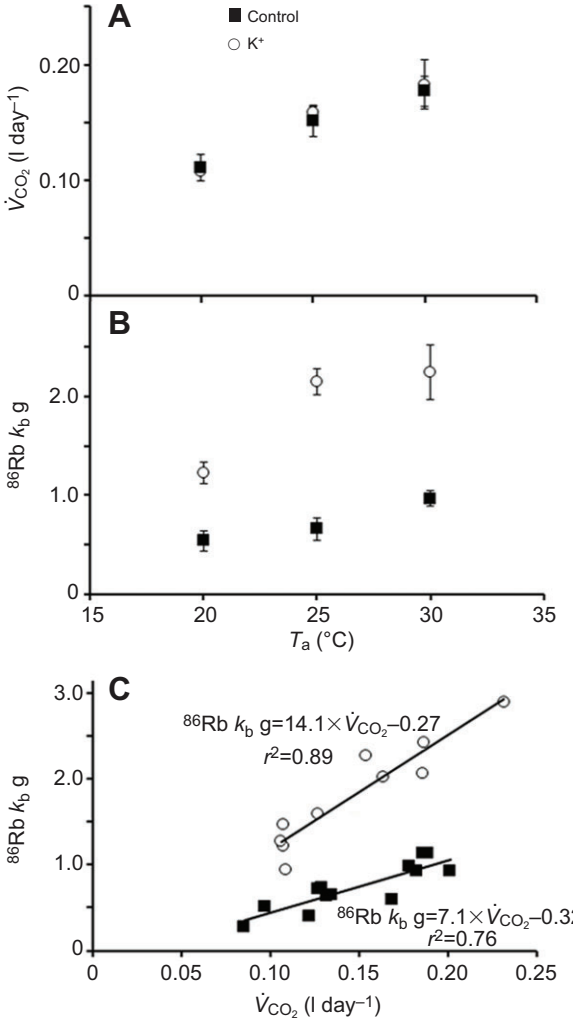

Fig. 2. Interrelationships of isotopic and indirect calorimetric measures of metabolic rate. The effect of ambient temperature (T_a) on $\dot{V}_{CO_2}$ (A), and ^{86}Rb k_b g (B). (C) The relationship between $\dot{V}_{CO_2}$ and ^{86}Rb k_b g for K$^+$ and control diet. Linear trendlines are shown.

The effect of metabolic rate on ^{86}Rb washout

There was a significant linear relationship between $\dot{V}_{CO_2}$ and ^{86}Rb k_b for both the jam ($F_{1,11}$=35.00; P=1.0×10^{-3}) and the K$^+$ diets ($F_{1,8}$=64.33; P=4.3×10^{-5}). The relationship had a significantly higher slope, resulting in higher ^{86}Rb k_b for a given $\dot{V}_{CO_2}$, on the K$^+$-enriched diet compared with the jam diet ($F_{1,19}$=215.8; P=7.95×10^{-12}; Fig. 2C).

We have shown that ^{86}Rb k_b is related to metabolic rate in an insect, and that the measure is changed when dietary [K$^+$] intake is altered. Buscarlet et al. (Buscarlet et al., 1974) stated that the biological half-life of radioisotopes in insects obeys the same laws as those regulating metabolism, and good correlations between metabolic rate and ^{86}Rb k_b have been reported in vertebrates (Peters et al., 1995; Peters, 1996; Bradshaw and Bradshaw, 2007; Tomlinson et al., 2013). The results for the control jam diet were not congruent with a previous meta-analysis of ^{86}Rb k_b for ectotherms (Tomlinson et al., 2013) (Fig. 3), registering at a lower elevation, with lower ^{86}Rb k_b at a given $\dot{V}_{CO_2}$. The data for *X. gideon* on the K$^+$ experimental diet, however, were congruent with those previous findings (Fig. 3). Deviations from the established pattern could be an artefact of elevated K$^+$ elimination to maintain ionic balance on the higher [K$^+$] diet, but this interpretation was not supported by the meta-analysis, where the K$^+$-enriched diet group conformed to expectations. This raises two possibilities: either our jam diet had a lower [K$^+$] than is normal in the natural diet of *X.*

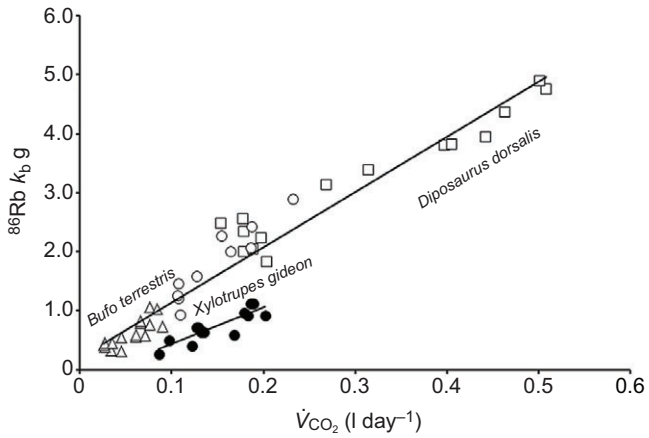

Fig. 3. Results of a meta-analysis of ^{86}Rb biological turnover versus CO_2 production in a range of ectothermic species. See Tomlinson et al. (Tomlinson et al., 2013) and references therein. The results for *X. gideon* on the jam diet (filled circles) were not consistent with the established relationship; the results for those beetles on the K$^+$ diet (open circles) were consistent with these relationships.

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gideon, or the previous published literature was based on animals receiving K^+-enriched diets. While the [K^+] of our K^+ experimental diet was similar to the 1.81±0.14 mg g^{-1} reported by the USDA (USDA, 2004) for naturally occurring levels of K^+ in fruit, the [K^+] of jam was substantially lower (0.75 mg g^{-1}). This suggests that our jam diet provided less than the normal dietary K^+ for the beetles, while our K^+ experimental diet in fact represents a more accurate control. Under such a scenario our data would be consistent with previous findings (Fig. 3). The dietary K^+ has not previously been controlled or reported in other studies. Such a confounding of comparative interpretation strengthens our recommendation that, although ^{86}Rb k_b can be used to infer FMR in a broad range of taxa, dietary K^+ should be carefully controlled in any laboratory validations of the technique to mimic the [K^+] that a species will be consuming in the wild.

The mechanism of association between ^{86}Rb k_b and DEE has been speculated to be the substitution of $^{86}Rb^+$ for K^+ by the Na^+/K^+-ATPase that transports K^+ into, and Na^+ out of, cells in an active process that consumes ATP (Peters et al., 1995; Peters, 1996; Bradshaw and Bradshaw, 2007; Tomlinson et al., 2013). Peters et al. (Peters et al., 1995) extensively explored the possibilities that ^{86}Rb k_b and ^{22}Na k_b were co-correlated with both the metabolic rate and the dietary intake of the ions, and managed to separate the effectors for each isotope using statistical methods. While ^{22}Na k_b was mostly influenced by food intake, ^{86}Rb k_b was most strongly associated with DEE (Peters et al., 1995). Following the conclusions of Peters et al. (Peters et al., 1995), with some inferences from our data presented here, we speculate that, after the bolus of initial enrichment, the Na^+/K^+-ATPase transports most of the injected $^{86}Rb^+$ into cells, thus entering an intracellular pool. The movement of K^+ (and $^{86}Rb^+$) out of the cell should be directly proportional to the activity of the Na^+/K^+-ATPase, as intracellular K^+ is regulated. As the activity of the Na^+/K^+-ATPase comprises a large component of DEE (Withers, 1992), the rate of this exchange should increase or decrease proportionally with metabolic rate. An increase in dietary K^+ will result in an increase in the excretion of K^+ (and $^{86}Rb^+$), and it is the extracellular fluid that is filtered by the Malpighian tubule system to produce excreta. The mechanism suggested is that $^{86}Rb^+$ is released from the cell at a rate that is proportional to metabolic rate, and the rate of excretion of K^+ (and $^{86}Rb^+$) from the extracellular fluid is proportional to K^+ intake. When $^{86}Rb^+$ leaves the cell, some of it will be excreted, while some will return to the intracellular fluid via the Na^+/K^+-ATPase. If the rate of excretion of K^+ (and $^{86}Rb^+$) from the extracellular fluid increases when K^+ intake increases, then the balance of $^{86}Rb^+$ exchange between the intracellular and extracellular pools is altered. While the transfer of $^{86}Rb^+$ from the intracellular to the extracellular pool remains constant, more of that $^{86}Rb^+$ will be excreted from the extracellular pool, reducing the probability of a given molecule of $^{86}Rb^+$ being transported back to the intracellular pool via Na^+/K^+-ATPase, and increasing its probability of being excreted. While there is a constant movement of ^{86}Rb from the intracellular to the extracellular fluid in proportion to metabolic rate, the excretion of ^{86}Rb from the extracellular fluid increases when K^+ intake increases. The association of ^{22}Na with food intake probably results from ^{22}Na being maintained mostly in the extracellular body pool that is regulated in response to ionic intake with food, and is only tangentially influenced by the Na^+/K^+-ATPase and metabolic rate.

Conclusion

FMR is a useful ecological measurement because it quantifies the energetic link between a species and its environment. It can be used to understand the impacts of temperature and climate (Anderson and Jetz, 2005), reproduction (Fyhn et al., 2001; Bevan et al., 2002; Jodice et al., 2003), ecosystem productivity (Bradshaw and Bradshaw, 1999; Bradshaw and Bradshaw, 2007; Bradshaw et al., 2007), dispersal (Clusella Trullas et al., 2006) and anthropogenic disturbance upon a species of interest. Although the DLW method has broad support and application for measuring FMR, the technique has theoretical and practical limits, where some species and habitats are not suitable for the technique (for review, see Tomlinson et al., 2013). The biological turnover of radioactive ^{86}Rb has been proposed as an alternative that avoids some of the limitations of DLW, and may be applicable to taxa that are unsuitable for DLW (Peters et al., 1995; Peters, 1996; Bradshaw and Bradshaw, 2007; Tomlinson et al., 2013). A problem with using ^{86}Rb k_b as a measure of an animal's metabolism is a lack of understanding of the mechanistic link between metabolism and the turnover of ^{86}Rb (Bradshaw and Bradshaw, 2007; Tomlinson et al., 2013). One suggested mechanistic pathway is that Rb^+ turnover is related to K^+ turnover, and we here provide some evidence to support that contention.

Measurement of ^{86}Rb k_b is a cheap, non-invasive and practical technique to measure DEE and FMR in small vertebrates and insects. We found that the ^{86}Rb k_b was influenced by dietary levels of K^+ and Rb^+, but that changes in the intake of these ions did not affect $\dot{V}_{CO_2}$. Although there were significant relationships between $\dot{V}_{CO_2}$ and ^{86}Rb k_b, the relationship was different in beetles on the different diets, presumably as a result of the influence of K^+ elimination upon ^{86}Rb k_b. These data support the likelihood that ^{86}Rb k_b is related to DEE and FMR via the action of the ubiquitous Na^+/K^+-ATPase. Future calibrations of ^{86}Rb k_b with $\dot{V}_{CO_2}$ will need to control [K^+] intake to natural levels in order to maximise the accuracy of the relationship between ^{86}Rb k_b and DEE to make it ecologically relevant as a measure of FMR.

MATERIALS AND METHODS

Animal source and maintenance

We tested the association between ^{86}Rb k_b and the dietary intake of K^+ using rhinoceros beetles (*X. gideon*, Coleoptera; Scarabidae; Dynastinae), native to India, south-east Asia, Papua New Guinea, Australia, the British Solomon Islands and the New Hebrides (Bedford, 1975). They are regarded as pests of coconut palm plantations in the southern regions of Asia and several regions of the Pacific (Zelazny and Alfiler, 1987). These insects are approximately 4 g mass, and thus large enough to enrich with radioisotope by injection, and also available through a pet trade in Australia. Mature beetles (15 male and 16 female) were purchased in two separate shipments from the Australian Insect Farm (Innisfail, QLD, Australia), shipped overnight to the U.W.A. Crawley campus in Western Australia, and maintained for 15 days prior to the beginning of experiments. The beetles were maintained in Perspex tanks (25 cm height×15 cm width×30 cm length) kept in a laboratory room near windows. The laboratory was maintained at 23.5±0.1°C constantly. The beetles were kept on a bed of organic mulch (Richgro Ezi-Wet Organic Mulch, Australia) and fed an *ad libitum* control diet of mashed peaches (Ardmona, Australia) and commercially available plum jam (IXL, Australia).

The beetles were assigned randomly to three treatment groups. The beetles on the jam diet received fresh mashed peaches and plum jam daily. The other groups received the same diet, except that the plum jam was enriched with 0.53 g of either KCl (Merck) or RbCl (BDH Chemicals Ltd) added to 375 ml of plum jam. The final [K^+] of the jam diet was 0.35 mg g^{-1}, while the K^+ and Rb^+ diets were enriched to 1.42 mg g^{-1}, as measured by flame photometry. All other elements of the diet were unchanged, and water and food were provided *ad libitum*. Throughout the experiments, the beetles were weighed daily to monitor their condition using

a balance (Sartorius) with a precision of 0.1 mg. In cases where body mass declined by more than 10%, the food quantity offered was increased.

Isotope enrichment and counting protocols

This research was conducted under UWA Radiation Safety Approval 09/07/01.

At the beginning of each experiment, each beetle was injected with 0.1 ml of a 0.05 MBq ^{86}RbCl solution (Perkin Elmer) in sterile saline (0.09% NaCl, Intravenous Infusion BP, Baxter). Dilutions were made in a sterile, disposable 1 ml syringe (Livingstone, Australia), and administered via a 27G needle (Terumo, Japan). The beetles were injected through the ventral integument between abdominal segments one and two. It is presumed that the injectate entered directly into the haemocoel. Following the injections, the beetles were returned to their respective tanks where they remained for a 24 h equilibration period until the experimental measurements began.

Whole-body radioactivity counts during the washout study were made using a portable gamma counter (PSR8, Nucleonics, UK) with a 50×50 mm sodium iodide crystal, while counts during the respirometry trial were made using an AmpTek GammaRad V (AmpTek, USA) gamma counter with a 76×76 mm sodium iodide crystal. During the counting procedure, the beetles were enclosed in a 50 mm diameter plastic vial and restrained from moving by a styrofoam plug the same diameter as the vial placed above the beetle. To reduce the effects of external sources of radiation, the vial was enclosed within a cylindrical lead shield. Each time the ^{86}Rb remaining in a beetle was measured, three 60 s background counts were recorded. Following the background counts, a minimum of three 60 s counts were taken with the beetle in the chamber. Each set of counts was averaged, and only accepted when the variation between the three counts was less than 2%. The averaged background count was subtracted from the averaged radioactivity count of each beetle.

Gamma emission counts were corrected for the physical decay of the isotope by dividing the count by the radioactive decay exponent:

$$\text{Corrected biological decay} = C_t / [\mathrm{e}^{(-0.693 / 18.66)\,t}], \quad (4)$$

where C_t is the radioactivity count and t is the time from injection at which the counts were made each day. The biological elimination constant for each beetle (k_b) was calculated as:

$$k_b = \frac{\left(\ln(C_1) - \ln(C_2)\right)}{t_2 - t_1}, \quad (5)$$

where C_1 and C_2 are the first and last radioactivity counts for each beetle, respectively, and t_1 and t_2 are the times that C_1 and C_2 were measured.

^{86}Rb washout trials

To test whether increased dietary intake of K^+ and Rb^+ changed the rate of biological elimination of ^{86}Rb, we measured and compared the rate of decay over time (henceforth referred to as the washout). After the injection of ^{86}Rb, the beetles were measured daily for at least 26 days until the enrichment reached background levels in each beetle. Each day the percentage of the isotope remaining in each beetle was calculated with reference to its initial enrichment.

^{86}Rb turnover, temperature and metabolic rate

Following the washout trial, where no difference was found in ^{86}Rb washout between the K^+ and Rb^+ diets (see Results and Discussion), the Rb^+ experimental group was removed from the next phase of experimentation. A four-channel flow-through respirometry system was constructed following Withers (Withers, 2001). Compressed air was passed through a mass flow controller (Aarlborg DFC-17, USA) at a rate of 200 ml min^{-1} (ambient temperature and pressure dry). The air was passed into a cylindrical glass chamber (85 mm length×70 mm diameter) bedded with organic mulch, in which the beetle was housed for the duration of the experiment. The excurrent airstream was dried with Drierite (anhydrous calcium sulphate, W. A. Hammond Company Ltd, USA), used in line with recommendations by White et al. (White et al., 2006) to minimise CO_2 absorption, and passed through a Qubit S151 infrared CO_2 analyser (Qubit systems Inc., USA). Each respirometry system was maintained at constant nominal temperatures (T_a) of 20, 25 or 30°C. Analog data signals from all the equipment were interfaced to a computer via a DataQ 710 data acquisition board, and collected every minute using a custom-written Visual Basic version 6.0 data acquisition program. A minimum 12 h baseline was run before and after each respirometry trial, recording the background [CO_2] of a blank chamber including the mulch bedding. Using $\dot{V}_{CO_2}$ as the sole measure of metabolic rate depends on the assumption that the RER does not change from treatment to treatment, but as this usually changes in response to the proportional representation of dietary lipids and carbohydrates (Withers, 1992), this is probably a safe assumption with the controlled diets presented here.

The beetles were weighed prior to respirometry trials, and their radioactivity counted. The beetles were then exposed to each temperature in a random order for 2 days. During the respirometry trials, the beetles were weighed daily, and their radioactivity counted. While the beetles were removed from the chamber, the respirometry systems were allowed to return to baseline. While they were in the respirometry chamber, the beetles were provided with their experimental diet and water *ad libitum*.

Statistical analyses

Body mass was compared between diets and over time using a two-way repeated measures ANOVA. Exponential effects of time on ^{86}Rb decay nested by diet were tested by ANOVA with Tukey *post hoc* tests to compare the different diets. Non-linear least squares models were used to characterise the ^{86}Rb exponential decay relationships of each diet over time. The significance of these relationships was tested by linear regression using ln-transformed decay. Following the discussion by Warton and Hui (Warton and Hui, 2011), the percentage of corrected biological decay was not arcsin transformed prior to parametric analysis. Linear mixed effects models were used to characterise the relationships between $\dot{V}_{CO_2}$ and T_a, and between ^{86}Rb k_b and T_a, nested within dietary treatments. Reduced major axis regression was used to characterise the relationships between ^{86}Rb k_b and $\dot{V}_{CO_2}$ within dietary groups. These relationships were compared for slope and intercept between experimental groups using ANCOVA. Statistical analyses were performed in R version 2.15.2 using the packages 'car', 'nlme', 'multcomp' and 'biology' (Logan, 2010). Data are presented as means ± s.e.

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Competing interests

The authors declare no competing financial interests.

Author contributions

All authors made substantial and equivalent contribution to the conception, design and execution of the experiments. P.D.M. was a research training student co-supervised by S.K.M. and S.T.

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