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Review

Body size, energy metabolism and lifespan

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Summary

Bigger animals live longer. The scaling exponent for the relationship between lifespan and body mass is between 0.15 and 0.3. Bigger animals also expend more energy, and the scaling exponent for the relationship of resting metabolic rate (RMR) to body mass lies somewhere between 0.66 and 0.8. Mass-specific RMR therefore scales with a corresponding exponent between –0.2 and –0.33. Because the exponents for mass-specific RMR are close to the exponents for lifespan, but have opposite signs, their product (the mass-specific expenditure of energy per lifespan) is independent of body mass (exponent between –0.08 and 0.08). This means that across species a gram of tissue on average expends about the same amount of energy before it dies regardless of whether that tissue is located in a shrew, a cow, an elephant or a whale. This fact led to the notion that ageing and lifespan are processes regulated by energy metabolism rates and that elevating metabolism will be associated with premature mortality – the rate of living theory.

The free-radical theory of ageing provides a potential mechanism that links metabolism to ageing phenomena, since oxygen free radicals are formed as a by-product of oxidative phosphorylation. Despite this potential synergy in these theoretical approaches, the free-radical theory has grown in stature while the rate of living theory has fallen into disrepute. This is primarily because comparisons made across classes (for example, between birds and mammals) do not conform to the expectations, and even within classes there is substantial interspecific variability in the mass-specific expenditure of energy per lifespan. Using interspecific data to test the rate of living hypothesis is, however, confused by several major problems. For example, appeals that the resultant lifetime expenditure of energy per gram of tissue is 'too variable' depend on the biological significance rather than the statistical significance of the variation observed. Moreover, maximum lifespan is not a good marker of ageing and RMR is not a good measure of total energy metabolism. Analysis of residual lifespan against residual RMR reveals no significant relationship. However, this is still based on RMR.

A novel comparison using daily energy expenditure (DEE), rather than BMR, suggests that lifetime expenditure of energy per gram of tissue is NOT independent of body mass, and that tissue in smaller animals expends more energy before expiring than tissue in larger animals. Some of the residual variation in this relationship in mammals is explained by ambient temperature. In addition there is a significant negative relationship between residual lifespan and residual daily energy expenditure in mammals. A potentially much better model to explore the links of body size, metabolism and ageing is to examine the intraspecific links. These studies have generated some data that support the original rate of living theory and other data that conflict. In particular several studies have shown that manipulating animals to expend more or less energy generate the expected effects on lifespan (particularly when the subjects are ectotherms). However, smaller individuals with higher rates of metabolism live longer than their slower, larger conspecifics.

An addition to these confused observations has been the recent suggestion that under some circumstances we might expect mitochondria to produce fewer free radicals when metabolism is higher – particularly when they are uncoupled. These new ideas concerning the manner in which mitochondria generate free radicals as a function of metabolism shed some light on the complexity of observations linking body size, metabolism and lifespan.

Key words: ageing, rate of living theory, free radical, oxidative stress.

Introduction

Historical perspective

The recognition that things wear out with use, and that the more we use them the faster they wear out, must be very old. The identification that this principle might also pertain to the phenomena of human ageing and death, however, appears to have first been made by Aristotle, who suggested that among other things our deaths are hastened by engagement in sexual activity: *"salacious animals and those abounding in seed age quickly"*. Aristotle also made a prescient comparison of life and fire with respect to age: *"A lesser flame is consumed by a greater one, for the nutriment, to wit the smoke, which the former takes a long period to expend is used up by the big flame quickly*", and he observed that larger animals live longer than smaller ones (Aristotle, 350 BC), but his primary thesis was that ageing and death are linked to the process of dehydration. It was not, therefore, until the late 1800s that the general idea of ageing reflecting the body 'wearing out' gained widespread popularity. It is around this time that many popular idioms that capture the idea originate – such as "*burning the candle at both ends*" (reviewed in Speakman et al., 2002). It is probably not a coincidence that this was the time of the industrial revolution, and the origins of modern capitalism. When attempting to get complex industrial machinery to deliver greater productivity, the fact that things break down the harder you work them would have been widespread and obvious. The German biologist Auguste Weismann, who originated the idea of the germline, was among the first biologists to promote the idea that ageing and death of the soma is an analogous process to 'wear and tear'. Humans and animals, however, engage in a wide variety of activities, although which activities, and by how much they contributed to the 'wearing out' process, was unclear. A degree of clarity was brought to the field in 1908, when, recapitulating the flame idea of Aristotle, it was suggested that the linkage between what we do now, and why we age and die, lies in our energy metabolism (Rubner, 1908).

Rubner (1908) compared the energy metabolism and lifespans of five domestic animals (guinea pig, cat, dog, cow and horse) and man. He noted that the rate of metabolism of these animals increased as a function of body size, and that the larger animals also lived longer. When he multiplied the mass-specific rate of energy expenditure by the maximum lifespan, the result was relatively independent of body size (if data for humans was excluded from the comparison). The range of variation in expenditure per gram per lifespan was only a factor of 1.5 compared with the 50 000-fold difference in body mass between the smallest and largest species. Even including the data for humans the range was only fivefold. In other words, a gram of body tissue expends about the same amount of energy, before the animal dies, whether the tissue is in a guinea pig, cat, dog, cow or horse.

If the total energy expenditure per lifespan is fixed, it follows logically that using energy up faster will hasten death. This has become known as the 'rate of living' (ROL) theory. In his book '*The Biology of Death*', Pearl (1922) concluded that life duration is a function of only two variables – the genetic constitution and the rate of energy expenditure. The idea was perhaps most eloquently summarised by Murray (1926) in his statement *'If aliveness is measured by the velocity of chemical activity (heat production) an organism may in this sense be said to dig its own grave. The more abundant its manifestations of life, the greater will be its rate of senescence'*. This idea had been strongly supported two years earlier by observations that once occupational accidents were excluded from the statistics, the rates at which males died after the age of 45 were directly related to the levels of energy expenditure in their occupations (Pearl, 1924).

By a rigorous statistical analysis of mortality rates in *Drosophila* and cantaloupe seeds, in the absence of any external sustenance, Pearl (1928) suggested that animals are endowed with an 'inherent vitality' that is depleted in relation to the rate of growth. He suggested that this 'inherent vitality' was an inherited factor related to 'organisation'. There are, however, some clear problems with the 'inherent vitality' idea as a factor governing lifespan – not least of which being that the idea was developed from studies of animals that were starving to death. In this circumstance it is hardly surprising that the duration of life was inversely linked to the rate of energy expenditure, since the animals have a roughly fixed energy storage at emergence from the pupae (and the same is true of germinating seeds), which will be exhausted in relation to its rate of use. The wider relevance of 'inherent vitality' to 'total vitality', when animals can derive external sustenance, is less clear.

A rather different idea was proposed in the 1950s that resonated with the ROL theory, and builds on much earlier suggestions by, for example, Metchnikoff (1908), that ageing and death are consequences of toxic by-products of metabolism. This idea is the free-radical damage theory of ageing (Gerschmann et al., 1954; Harman, 1956). Free radicals and oxidants (collectively called radical oxygen species: ROS) are highly reactive agents that react readily with macromoleules in the body causing damage. Some ROS originate from exogenous sources – typical examples include gamma and UV radiation. However, the largest source of free radicals is the process of oxidative phosphorylation. Estimates of the rate at which oxygen radicals are generated during oxidative phosphorylation are frequently quoted as being up to 3% of the inspired oxygen (Beckman and Ames, 1998; Castiella et al., 2001; Golden and Melov, 2001; Acuna-Castroviejo et al., 2001). More recently, however, these estimates have been questioned and it is likely that the actual productions are much lower – of the order of 0.1% (St Pierre et al., 2002). Whatever these estimates finally turn out to be, the implication of expressing the value as a percentage of the total oxygen consumption is that as oxygen consumption increases (per gram of tissue) then radical oxygen species generation will do so as well. The idea behind the free-radical damage theory is that macromolecular components of the cell are under perpetual attack from ROS. Animals have a battery of protective mechanisms that aim to protect them from this

damage, as well as a number of repair mechanisms that aim to ameliorate its effects. However, despite these defence and repair processes some damage always evades these systems and the consequence is a progressive lifetime accumulation of oxidation (Sohal and Weindruch, 1996; Beckman and Ames, 1998) that leads to advancing physiological attrition and ultimately failure (death). Oxidative phosphorylation is also the molecular mechanism that underpins the generation of ATP, which powers energy metabolism. The free-radical theory therefore provides a mechanism by which the ROL theory might work. In fact measured rates of free-radical production by mitochondria correlate to the resting rate of metabolism, and in turn these are also related to longevity (Ku and Sohal, 1993).

The free-radical theory of ageing has gone from strength to strength and it is probably true that most modern gerontologists believe that free-radical damage is an important aspect of the ageing process (e.g. Huang and Manton, 2004; Fukagawa, 1999; Finkel and Holbrook, 2000; Golden et al., 2002; Dufour and Larsson, 2004). Surprisingly, despite their evident synergies, while the free-radical theory has blossomed, the rate of living theory by contrast has fallen into general disrepute. The main reason why the ROL theory has diverged from the free-radical theory reflects two vital pieces of information. The first relates to the comparison of lifespans and rates of energy metabolism when the database of mammal species is expanded (Austad and Fischer, 1991; Austad, 1997), beyond the species included in the original comparison by Rubner (1908). To illustrate this point I have plotted in Fig. 1 accumulated data on lifespans and RMR for both mammals and birds. (RMR is defined as the rate of metabolism for an animal at rest within the themoneutral zone). When this enlarged database is examined the generalities remain – bigger mammals expend more energy, but at a declining rate with increasing body mass (Fig. 1A), and they live longer (Fig. 1B) – but the specificities, that animals expend the same amounts of energy per gram of tissue per lifespan, are seriously challenged. The trend is still broadly independent of body mass (r^2=0.026, b=–0.06, although this shallow gradient is significantly different from 0 because of the large sample size; P=0.03), but within the mammals there is a 17-fold range in the lifetime expenditures of energy (Fig. 1C). Probably the most persuasive evidence against the ROL theory, however, comes from the inter-class comparison of birds and mammals. Within birds the patterns are very similar to the mammals. Bigger birds expend more energy but at a declining rate with size (Fig. 1D), and they live longer (Fig. 1E). In combination, these trends mean that birds also have rates of energy metabolism per gram per lifespan that are highly variable and relatively independent of body size (Fig. 1F; r^2=0.126, b=–0.109, although again the gradient of this relationship is significantly different from 0, P=0.001). However, when comparing birds with mammals (Fig. 2) some striking things emerge. On average birds of any particular mass have rates of metabolism that are higher than equivalent-sized mammals, but at any particular mass they combine these higher rates of metabolism with longer lives. In consequence, lifetime

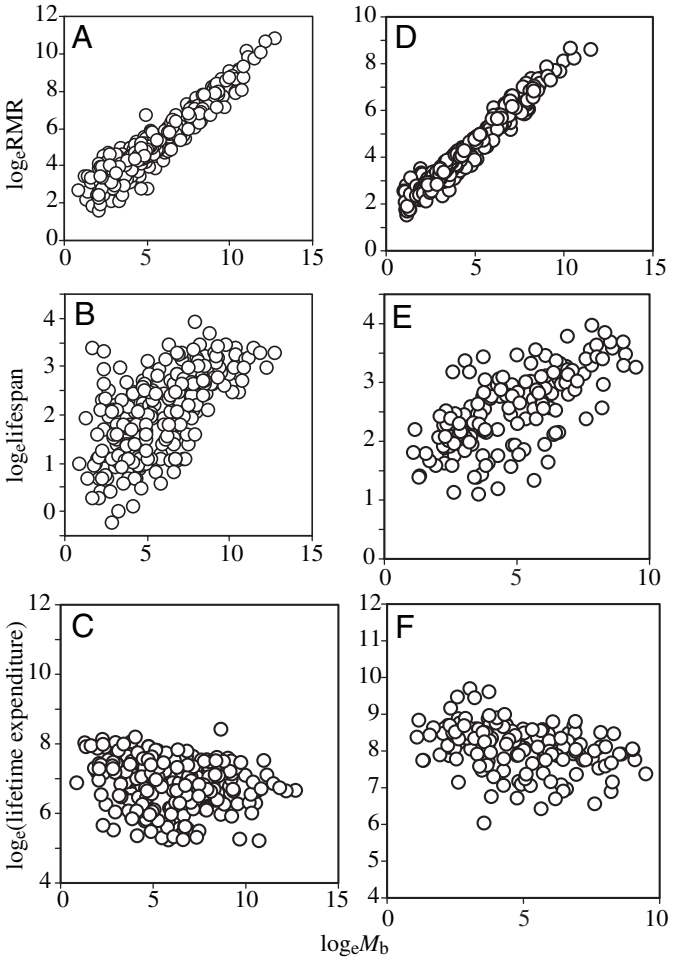

Fig. 1. Resting (basal) metabolic rates (RMR; $\log_e$RMR in kJ day^{-1}) of 639 mammals (A) and 381 birds (D) as a function of body mass (M_b, in g). [Least-squares fit equations: mammals, $\log_e$BMR=0.781+0.677$\log_e M_b$, F=6605.89, P<0.001, r^2=0.915, RMA gradient=0.708; birds, $\log_e$BMR=1.224+0.671$\log_e M_b$, F=8574.1, P<0.001, r^2=0.958, RMA gradient=0.685.] Lifespans (years) of 249 mammals (B) and 164 birds (E) as a function of body mass. [Least-squares fit equations: mammals, $\log_e$lifespan=0.85+0.209$\log_e M_b$, F=157.66, P<0.001, r^2=0.390, RMA gradient=0.334; birds, $\log_e$lifespan=1.514+0.216$\log_e M_b$, F=134.12, P<0.001, r^2=0.458, RMA gradient=0.319] and lifetime expenditure of basal energy per gram of body tissue (kJ g^{-1} life^{-1}) plotted against M_b (g) for 240 mammals – excluding 9 bat species (C) and 141 birds (F) [least-squares fit equations: mammals, $\log_e$(lifetime expenditure per gram)=7.268–0.0734$\log_e M_b$, F=17.97, P<0.001, r^2=0.07, RMA gradient=–0.278; birds, $\log_e$(lifetime expenditure per gram)= 8.635–0.109$\log_e M_b$, F=23.28, P<0.001, r^2=0.126, RMA gradient= –0.307].

expenditures of energy per gram of bird tissue are on average substantially greater than the equivalent values in mammals (Fig. 2), as observed by Holmes and Austad (1995a,b) Ogburn et al., (1998, 2001) and Holmes et al. (2001). On this basis it is argued that the 'rate of living' theory cannot be correct.

The arguments that have been used to undermine the ROL theory and the data that are used to test the theory are, however,

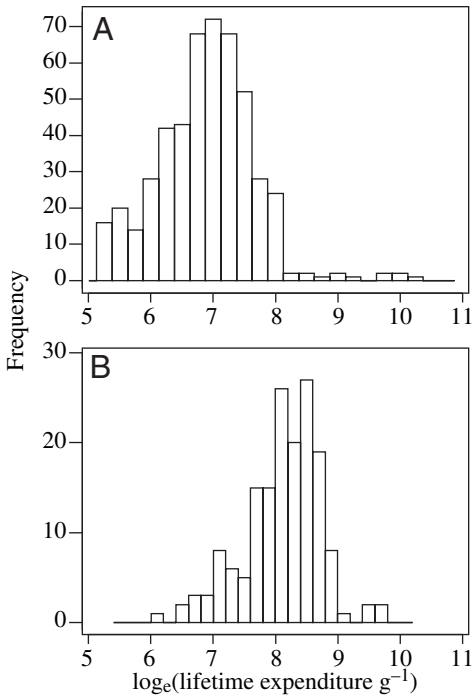

Fig. 2. Histograms of $\log_e$[lifetime expenditures of energy (kJ) per gram of tissue] for (A) mammals (N=249) and (B) birds (N=164).

fraught with problems. In the second part of this review I will highlight these issues and in the third part of the paper I will perform some novel analyses that attempt to overcome some of these difficulties and thus revaluate the validity of the original idea.

Some problems

Maximum longevity is not a good measure of ageing

Ageing refers to the age-specific increase in the probability of death. In animals that age more rapidly the age-specific increase has a greater slope. It is well established that maximum lifespan is a poor reflection of the rate of ageing because it is influenced by many additional factors (Carey, 2003) and reasons for shortening life may not reflect ageing at all (Miller, 2004). A classic example is the difference in maximum lifespans of human males and females. On average females live longer than males, and the maximum lifespan reported for a woman is about 8 years longer than the equivalent value for a man. Yet the rates of ageing for males and females are the same – the increase in the probability of mortality just starts to increase later in females than males. The second difficulty particularly concerns studies of non-domesticated species. In the wild, there are many reasons why an animal might die before it gets a chance to experience senescence – disease, starvation and predation being obvious examples. In most wild populations these factors are of considerably more importance than senescence and ageing phenomena. For example, the life expectancy of a bank vole *Clethrionomys glareolus* in the wild is about 2.6 months, and the maximum lifespan is about 18 months (Bobeck, 1969). Yet in captivity, where there is no risk of predation or starvation, these voles can live up to 40 months (Godfrey, 1958).

For many animals we have estimates of their longevity from records kept by zoological collections. It would therefore appear simple just to use these as the estimated maximum lifespans that animals could achieve when not constrained by predation or starvation. However, while keeping animals in captivity overcomes the problems of most extrinsic mortality sources, there is a problem that for many animals we do not know their long-term nutritional requirements (particularly with respect to micronutrients) with any precision. Animals in captivity may therefore regularly fail to achieve their maximum lifespans because they are chronically malnourished.

Another problem with using maximum lifespan is that this refers to a single event in a single individual animal. Because this is a stochastic event the value for a single individual may be unrepresentative of the general trend (Carey, 2003). Thus two species may age at different rates, and have different average lifespans, but the longest lived animal could come from either species. The likelihood of the longest lived individual coming from the species that has the faster rate of ageing and lower mean lifespan diminishes as the total sample of animals from which the longest individual is recorded increases. This raises another related point, that the maximum is itself dependent on the sample size of individuals included in the sample (Carey, 2003). This is because as one continues to sample individuals from a population, the maximum in that sample can only get larger, and the probability of encountering an exceptional individual increases. Humans again provide a fine example of this. To date (2004) the best authenticated human longevity record is 122 years and 164 days (for Jeanne Calment), although this is widely disputed by claims of greater antiquity, but lower veracity. This record, however, has only been reached in a sample probably in excess of a billion accurate records of birth and death dates. In contrast, in a single sample of 100 subjects drawn from the human population in western society one would only have a 50:50 chance of getting one individual living to more than 100. For most animals, the maximum lifespans are estimated on samples that are probably considerably lower than 100 individuals. However, we can only state this as ‘probably’ because while we tend to have records of the ages of the longest lived individuals, because of a human tendency to record exceptional events, there are few records kept for individual animals that die sooner, because these are mundane events. Thus the total sample from which the maximum is gleaned is unknown, which is unfortunate because it means we cannot remove this effect by including it as a cofactor in any analyses. Finally the reliability of single exceptional events is also potentially questionable because there is seldom any independent verification of these figures.

RMR is not a good measure of energy metabolism

The arguments regarding lifetime expenditure of energy per gram of tissue developed by Rubner (1908) and expanded

much later by, for example, Lindstedt and Calder (1976) and Calder (1984), are based on estimates of resting (or basal) energy metabolism. This, by definition, involves the measurement of subjects at rest, under thermoneutral temperatures (hence no thermogenic stress), in a post-absorptive state (hence not digesting food) and inactive. The rationale for using RMR in this context is simply that it is an available equivalent measure that has been commonly determined in a large number of species for comparative purposes. Yet the justification for why there might be a direct link between the level of RMR and ageing phenomena is unclear. Animals and humans expend enormous amounts of energy on things other than resting metabolic rate. Indeed, this is why the terms of its definition need to be so prescribed for comparative purposes because metabolism is responsive to many different factors. Measurements of total energy demands using the doubly-labelled water method (see below for a full description) suggest that on average in small mammals the contribution of basal or resting metabolism to the total expenditure of energy is only about 40% (reviewed in Speakman, 2000).

The underlying machinery that fuels basal metabolic rate is, however, identical to the machinery that fuels all the other sources of energy utilisation – namely the process of oxidative phosphorylation that occurs in mitochondria to generate ATP, which is subsequently hydrolysed to ADP and phosphate to release energy for useful work. Virtually everything animals do in terms of energy utilisation is fuelled by this common biochemical mechanism. The only exception is when animals use exogenous sources of heat to supply their thermoregulatory needs. This process of electron transport during oxidative phosphorylation is the primary source of oxygen radical species. It would be remarkable indeed if the rate of oxygen free-radical production was linked in some manner to the ultimate fate for the utilisation of the ATP that acts as the primary energy storage molecule, the ultimate fate of which is not determined at its point of formation.

Testing for constancy in the lifetime expenditure of energy per gram of tissue or comparisons of birds to mammals is not the best way to test the rate of living theory

The rate of living (ROL) theory predicts that animals with greater rates of metabolism should die faster. In the original development of the idea, Rubner (1908) indicated that the product of lifespan and metabolic rate per gram of tissue was constant. Following his example, this has become the standard method for evaluating the theory – and refutations have partially hinged on the demonstration that this trait is not constant. The other main refutation is the comparison between birds and mammals (Fig. 2), which indicates that birds combine longer lives with higher metabolism and these data consequently expand even further the lack of constancy in the lifetime expenditure of energy per gram.

A major problem with the 'lack of constancy' test, however, is how to evaluate it statistically. In other words how little variation would suffice to meet the criterion of constancy. In fact, looking at the original data from Rubner (1908) it is clear that these data are also not 'constant', but vary from 141 000 kcal g^{-1} to 265 500 kcal g^{-1} (excluding man), extending to 723 000 kcal g^{-1} if man is also included (a fivefold range). However, this range is tiny in comparison to the 50 000-fold range in the body masses of the animals that generate it. In this context even though the variation in lifetime expenditure of energy per gram of tissue in the expanded data set shown in Fig. 1 covers a 17-fold range within each of the classes, this is still orders of magnitude lower than the range of body masses and metabolic rates over which it is observed. This statistical difficulty aside, by 1928 the notion that the product of lifespan and expenditure is a constant appears to have already been discarded (Pearl, 1928) in favour of a much looser inverse association. Hence, Pearl (1928) concludes his 'rate of living' book with the statement "*All of the evidence presented in this book converges to the conclusion that,* **in general**, *the duration of life varies inversely as the rate of energy expenditure during its continuance*" (original emphasis preserved). Direct proportionality in the inverse relation is never mentioned in the entire 151 pages. Refuting the theory on the basis that such proportionality is absent is, therefore, a straw-man.

Instead of multiplying these traits together to infer that lifetime expenditure of energy per gram of tissue is constant, a better test of the ROL idea would be to ask whether there is an independent association of metabolism and lifespan once the shared variation due to body mass is removed from both traits. In other words if an animal lies above the fitted line relating mass to energy expenditure, does it fall below the corresponding line relating body mass to lifespan? This is a hypothesis that can be statistically tested. This relationship between the residuals is shown in Fig. 3A,B for mammals and birds, respectively. In both groups there was no significant association between the residual lifespan and residual resting metabolic rate (mammals: F=0.09, P=0.775, r^2=0.001, N=239; birds: F=2.29, P=0.132, r^2=0.013, N=164).

The extent of variation in the lifetime expenditure of energy per gram within the mammals and birds is only one part of the argument against the ROL theory, another major argument being the difference between birds and mammals illustrated in Fig. 2. This issue will be discussed below in the context of the reanalysis of data on lifetime expenditure of energy using resting rather than total daily energy demands.

Some solutions and some additional difficulties

To overcome the problem that measurements of RMR do not adequately capture the total daily rates of energy expenditure and to explore the links between daily energy demands and lifespan, I compiled data on the daily energy demands of mammals and birds measured using the doubly-labelled water (DLW) method. The DLW method is an isotope-based method for the measurement of whole body CO_2 production and hence energy expenditure (Nagy, 1994, 2005; Speakman, 1997). The main advantage of the technique, over traditional methods of

quantifying energy demands, is that it can be employed without the need to restrict subjects inside a calorimetry chamber (either direct, to measure its heat flow, or indirect, to measure its gas exchange). As such, the method has become the gold standard technique for measurement of free-living energy demands. The method depends on the observation that oxygen isotopes in body water are in complete and rapid exchange equilibrium with the oxygen in dissolved CO_2. The main consequence of this exchange is that an isotope of oxygen introduced into body water will be eliminated by both the flux of water through the body and the uptake of unlabelled inspired oxygen combined with the elimination of labelled expired CO_2. Since a simultaneously introduced isotopic label of hydrogen would only be washed out by the flux of water, a measure of CO_2 production and hence energy expenditure is made possible by the differential elimination of the two labels. The real power of the method, however, is that the elimination rates of the isotopes can be reconstructed by taking one sample shortly after the isotopes are eliminated and a second sample some time later. Between these samples the subject can engage in its normal daily activities unencumbered by the traditional apparatus that is used routinely to measure energy metabolism.

This simple description of the method belies some subtleties in its use. One important aspect that has emerged over the past decade is that the calculation method that provides the best fit of experimental DLW data to simultaneous validation data using conventional methods differs for animals in different size ranges. For animals weighing less than about 5 kg the best method involves multiplying the isotope turnover constants by a single estimate of the pool size. For larger animals and humans, however, the better equation includes both hydrogen and oxygen pool sizes applied to their own turnovers (reviewed extensively in Speakman, 1997). The calculation techniques differ by between 3 and 20%, depending on the actual isotope divergences. Unfortunately this fact has gone largely unrecognised, or studies were performed before the problem was recognised, and almost all the applications of the method to animals weighing greater than 5 kg have utilised the wrong calculation. This leads to a systematic bias in measurements of larger animals that makes estimates of their energy demands, and thus derivation of scaling exponents, subject to substantial error. Yet, the necessary baseline data to recalculate the original estimates using the more appropriate equations are generally not available in the original papers.

To overcome the above calculation problem I restricted the data collection to include only animals weighing less than 4 kg. This database included 73 species of small mammal and 90 species of bird. I then searched the literature for estimates of the maximal lifespans of these animals, utilising as a key reference and starting point the compiled database from Carey and Judge (2000). This generated 249 estimates of maximal lifespan for small mammals and 163 estimates for small birds. In combination, there were estimates of both maximal lifespan and daily energy demands for 48 of the 73 mammals and 44 of the 90 birds where estimates of daily energy expenditures were also available.

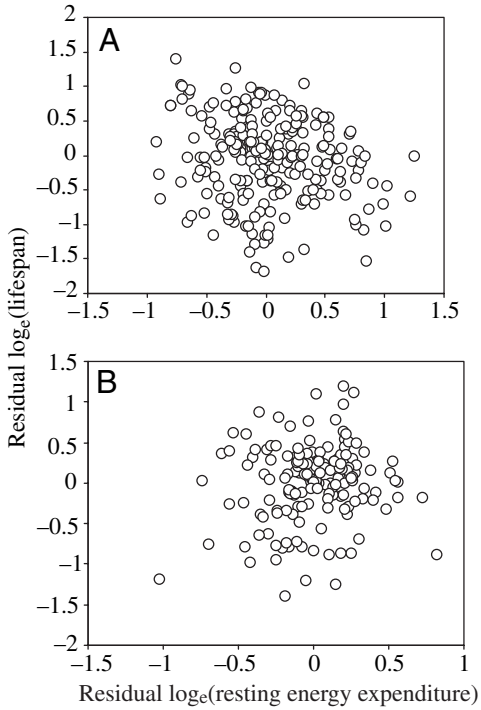

Fig. 3. Residual $\log_e$(lifespan in years) plotted against residual $\log_e$(basal metabolism in kJ day^{-1}) for (A) mammals (N=249) and (B) birds (N=164). See text for statistics.

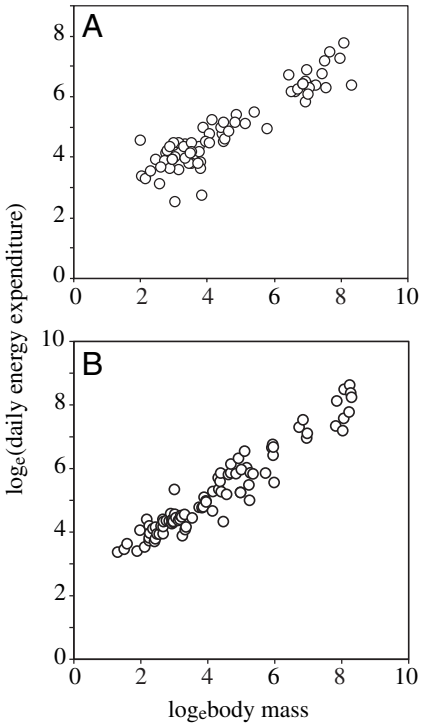

Fig. 4. $\log_e$(daily energy expenditure in kJ day^{-1}) measured using the doubly-labelled water (DLW) technique for free-living animals as a function of $\log_e M_b$ (in g) for (A) 73 small mammals and (B) 90 birds. See text for statistics.

The patterns of variation in lifespan as a function of body size in these data conformed to the previously observed patterns. The daily energy metabolism was positively related to body mass in both groups (Fig. 4A,B). In mammals, the least-squares fit regression was $\log_e$(daily energy expenditure in kJ day^{-1})=2.05+0.621×$\log_e M_b$ (in g) [F=420.5, P<0.001, r^2=0.856, N=73, reduced major axis (RMA) gradient=0.671]. In birds the regression was $\log_e$(daily expenditure of energy in kJ day^{-1})=2.31+0.692×$\log_e M_b$ (in g) (F=1087.25, P<0.001, r^2=0.925, N=90, RMA gradient=0.719). In the combined data set the difference in slopes was only marginally significant (GLM: F=3.95, P=0.048), but the difference in intercepts was highly significant (GLM: F=74.68, P<0.001). Independent of body size, birds expend on average more energy per day than the mammals. Larger mammals and larger birds both lived longer than their smaller equivalents (Fig. 1A,D), and the birds on average lived longer than equivalent sized mammals. The gradients of the scaling relationships were both shallow. In mammals the least-squares fit regression was $\log_e$(lifespan) (y)=0.851+0.209 × $\log_e M_b$ (g), F=157.7, P<0.001, r^2=0.390, RMA gradient=0.334, N=249. In birds the relationship was $\log_e$(lifespan)=1.514+0.216×$\log_e M_b$, F=134.12, P<0.001, r^2=0.458, RMA gradient=0.319, N=163. In the combined data set (N=412) the difference in slopes was again marginally significant [general linear model (GLM): F=4.77, P=0.03], and the difference in intercepts was much larger (GLM: F=62.07, P<0.001). Neither of these results differs from the patterns that were established when comparisons were made using resting metabolic rate (RMR).

When the daily energy expenditure and lifespans were combined to yield the lifetime expenditure of energy, however, a novel pattern emerged. In the mammals there was a strong negative relationship between lifetime expenditure of energy per gram and body mass; the least-squares fit regression was $\log_e$(lifespan expenditure of energy per gram) (in kJ g^{-1} life^{-1})=9.04–0.208×$\log_e M_b$ (in g) (F=27.82, P<0.001, N=49, r^2=0.377). In birds the relationship also had a negative trend but in this case failed to reach significance: $\log_e$(lifespan expenditure of energy per gram) (in kJ g^{-1} life^{-1})= 9.693–0.0696×$\log_e M_b$ (in g) (F=2.5, P<0.121, N=44, r^2=0.056). In the combined data set the difference in slopes was significant at P<0.05 but P>0.01 (GLM: F=5.65, P<0.020). Excluding this minor slope effect the intercept effect was highly significant (F=139.65, P<0.001). Combining the data, and excluding the marginally significant interaction, the effects of body mass and class were both highly significant, and the best-fit pooled regression equation was $\log_e$ (lifetime expenditure of energy per gram) (kJ g^{-1} life^{-1})= 8.75–0.145×$\log_e M_b$ (in g) (t=–4.85, P<0.001)+1.26×Class (t=12.22, P<0.001, where Class is a dummy variable; for mammals Class=0; for birds, Class=1) (overall regression F=92.78, P<0.001). This equation indicates that independent of body mass, a gram of tissue in a bird expends about 3.5 × the amount of energy over a lifespan as a gram of tissue in a mammal of the same body mass. In both the birds and mammals there was substantial inter-individual variation in the lifetime expenditure per gram (Fig. 5). I explored the nature of this variation in the mammals and found that residual variation in the relationship was related to the average temperature where the measurements of daily energy expenditure had been made (Fig. 6). Thus, independent of the body size effect, animals living in colder habitats tended on average to have greater lifetime expenditures of energy per gram of body tissue. In combination, body mass and ambient temperature explained 45% of the variability in the lifetime energy expenditure per gram of the mammals.

I removed the shared effects of body mass on both lifespan and daily energy expenditure and sought associations between

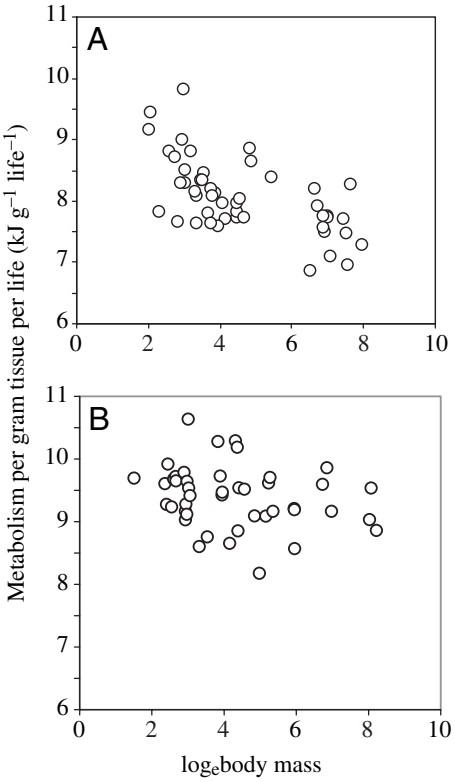

Fig. 5. Lifetime expenditure of energy per gram of body tissue plotted against $\log_e M_b$ for (A) mammals and (B) birds. See text for statistics.

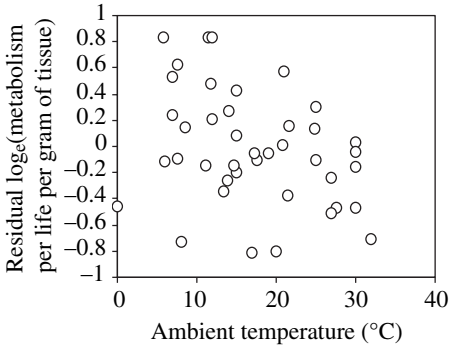

Fig. 6. Residual $\log_e$(lifetime expenditure of energy per gram of body tissue) plotted against ambient temperature at which the measurement was made for small mammals.

the residuals. In the mammals there was a significant negative association between residual lifespan and residual daily energy expenditure. Mammals that had high rates of expenditure for their body masses died sooner (F=18.47, P<0.001, r^2=0.139: Fig. 7). In birds, however, the association was not significant (F=1.85, P=0.181).

In part, therefore, replacing the estimate of RMR with daily energy expenditure confirms several of the basic arguments that have been used to undermine the rate of living theory – namely that birds expend more energy per gram of tissue over their lifespans than mammals of equivalent sizes, and within each class there is an enormous variation in the lifetime expenditure of energy. However, these analyses have revealed some evidence in support of the rate of living theory and some additional evidence against it. In support of the theory, the residual lifespans were negatively associated with the residual daily energy expenditures – at any given size mammals that expend more energy appear to expire sooner, but in birds this did not hold. However, the independence of the lifetime expenditure of energy per gram of tissue as a function of size (Fig. 1C,F) was not confirmed when RMR was replaced by FMR. The amount of energy a gram of tissue expends over a lifetime does appear to differ as a function of body size (particularly in mammals) – such that this level is about 62% lower in a mammal weighing 2000 g (lifetime expenditure per gram averaging 1737 kJ g^{-1} $life^{-1}$) compared with one weighing 20 g (4518 kJ g^{-1} $life^{-1}$). In fact this negative effect of mass was evident (and highly significant) in the original data based on RMR (Fig. 1C,F – see statistics in the legend), but the effect becomes magnified when considering daily energy expenditure rather than RMR.

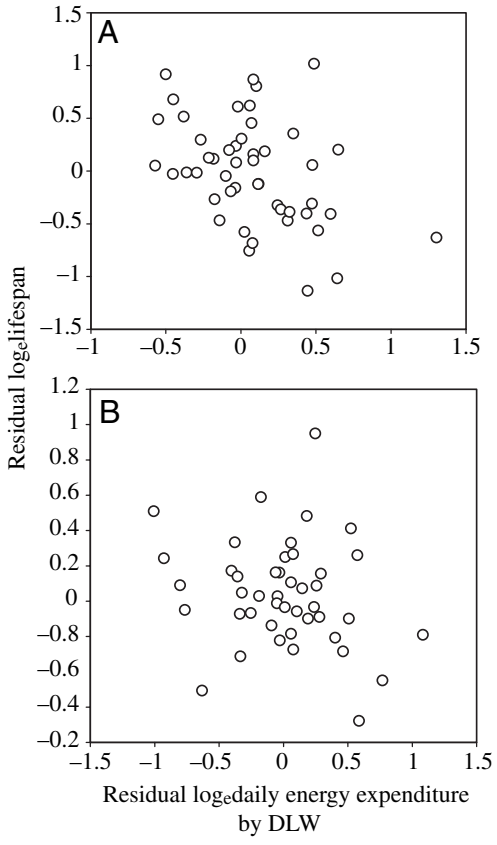

Fig. 7. Residual log$_e$lifespan (years) plotted against residual log$_e$(daily energy expenditure in kJ day^{-1}) measured using the doubly-labelled water method for (A) mammals and (B) birds. See text for statistics.

There are, however, some aspects of this analysis that require further consideration. Firstly, it is generally the case that the estimates of energy metabolism utilised in this analysis (i.e. daily energy expenditures measured on animals living in their natural environment) have not been made under the same conditions that pertain for the measurements of maximum longevity, which generally refer to animals kept in captivity. It seems likely that the energy demand levels of captive animals will not be as high as their wild counterparts because the foraging requirements are reduced. If the rate of living theory postulates that energy metabolism rates and lifespans are causally related, then it is clearly necessary to compare data for lifespans with data for energy metabolism derived under the same conditions. So while it is certain that measurements of RMR are not an appropriate measure to evaluate the rate of living theory, we may simply have replaced one inappropriate index of energy metabolism with another. This effect is probably more significant in birds than mammals because in captivity the metabolic rates of the former are probably more reduced than in small mammals because of the restriction in their flight behaviour. This may explain why significant effects were more evident in mammals than birds. Unfortunately, there are very few measures of daily energy demands in captive conditions made under the same conditions as lifespan estimates.

Moreover, there are probably systematic changes in activity (e.g. Minois et al., 2001; Carey, 2003) and RMR (e.g. Hughes et al., 1998; Van Pelt et al., 2001; Speakman et al., 2003) as a function of age, that call into question the validity of characterising the energy demand levels for a species over its entire lifespan with estimates derived from a cohort of individuals that may be unrepresentatively sampled. The interesting patterns in lifetime expenditure of energy that emerged as a function of both body mass and temperature may be artefacts of combining inappropriate measures (but it is also true that the absence of these effects in the original data based on RMR may also be an artefact).

A deeper problem exists, however, which suggests that such suitable information may never emerge from this type of analysis as a test of the rate of living theory. As pointed out by Ramsey et al. (2000), in the context of responses of animals to caloric restriction, and more generally by Speakman et al. (2002), the rate of living theory posits only that metabolic rate is related to the rate of ageing. A fair test of the theory is therefore only feasible if one modulates the rate of metabolism while holding other variables that might have an influence constant. Obviously when comparisons are made between different species this assumption that everything else is held constant is violated because different species differ enormously in their capacities for oxidative defence and repair

(Cleaver et al., 1995; Portero-Otin et al., 2001; Pamplona et al., 2002) and also in the precise stoichometry of the production of radical oxygen species during oxidative phosphorylation (Barja, 1998, 1999, 2002). The much cited comparison of birds and mammals as a refutation of the ROL theory (Holmes and Austad, 1995a; Austad, 2000; Holmes et al., 2001) is consequently not very strong evidence, because these groups differ in many other aspects, as well as in their metabolic rates and lifespans. It rather points to a lack of generality in the concept when applied to larger taxonomic groupings.

One conclusion that can be drawn from this discussion is that because differences between species may reflect adaptive differences in the stoichiometry of free-radical production in relation to oxidative phosphorylation, or differences in the capacity of oxidative defence and repair mechanisms, many of which may have a genetic basis, tests of the rate of living theory may be better performed by considering the associations between energy metabolism and ageing within species.

The association of energy metabolism and lifespan within species

Several types of study have been performed in the context of dissecting associations between energy metabolism and ageing within species. The first studies have concerned examinations of the rates of energy metabolism in genetically modified animals that show extension of lifespan. These include studies of transgenic and natural mutant *C. elegans, Drosophila* and mice, where an ageing and lifespan phenotype has been demonstrated. These generally involve single gene mutations. The second type of study includes associations between ageing and metabolism compared between different strains of a given species where the genetic differences in the strains reflect polygenic effects. The third type of study has involved experimentally manipulating groups of animals such that some of them have increased rates of energy metabolism and then exploring the impact of these changes on ageing. The fourth type of study includes examinations of variability at the inter-individual level, asking whether individual differences in rates of energy metabolism are linked to differences in lifespan. Finally, the fifth type of study includes taking environmental manipulations that extend lifespan (such as caloric restriction) and asking whether such manipulations have their effects because of a reduction in rates of energy metabolism. I will review the current situation regarding each of these types of study, except this latter area, the metabolic responses to caloric restriction, since this whole issue was the subject of a recent comprehensive review (Ramsey et al., 2000).

(i) Studies of transgenic and natural mutant animals

The demonstration that single gene mutations can have profound effects on the rates of ageing and lifespans of model organisms like *C. elegans* (Kenyon et al., 1993; Dorman et al., 1995; Ebert et al., 1996; Murakami and Johnson, 1996), *Drosophila* (Lin et al., 1998; Tatar et al., 2001; Clancy et al., 2001a; Aigaki et al., 2002, 2003) and the mouse (Migliacco et al., 1999; Bluher et al., 2003; Liang et al., 2003) has revolutionised our whole view of the phenomena of ageing and lifespan. Van Voorhies and Ward (1999) suggested that the differences in lifespans between *daf-2* mutants and wild-type *C. elegans* could be entirely accounted for by differences in their rates of metabolism. This has subsequently led to a protracted debate regarding the role of metabolic differences in lifespan extension in this species (Van Voorhies, 2001a,b, 2002a,b,c, 2003; Vanfleteren and Braeckman, 1999; Van Voorhies and Ward, 1999; Braeckman et al., 2001; Houthoofd and Vanfleteren, 2002).

Many of the mutations identified in *C. elegans* that are linked to lifespan effects include mutations in the insulin and insulin-like growth factor (IGF) signalling pathways (Hsu et al., 2003). These effects appear to be homologous in both *Drosophila* and mice (Tatar, 1999; Coschigano et al., 2000; Flurkey et al., 2001, 2002; Bartke, 2001; Hsieh et al., 2002a,b; Tatar et al., 2003; Brown-Borg, 2003). Measurements of metabolic rates of insulin/IGF signalling mutant *Drosophila chico* (Clancy et al., 2001), which has a disruption of the insulin receptor substrate, indicate no reduction of metabolism relative to that of wild-type flies (Hulbert et al., 2004a). Similarly *Drosophila* with the INDY mutation do not have decreased metabolic rates (Marden et al., 2003). Several mutant mice with disrupted growth hormone (GH), GH receptor, IGF and IGF receptor have been constructed, all of which show dwarfism combined with extended lifespan (Kopchick et al., 1999; Carter et al., 2002a,b; Bartke et al., 2003; Coschigano et al., 2003; Heiman et al., 2003). Unfortunately direct measures of energy metabolism in these mice are not available except for the GH missense mutant SMA1 (Meyer et al., 2004), but this is the only GH related mutant for which lifespan data are not available. Many other transgenic mice have been constructed with effects on lifespan (e.g. Migliaccio et al., 1999; Purdom and Chen, 2003; Liang et al., 2003; Bluher et al., 2003; Trifunovic et al., 2004), but in all these cases detailed measurements of energy metabolism are lacking.

Oklejewicz et al. (1997) measured the energy demands of *Tau* mutant Syrian hamsters *Mesocricetus auratus*. These hamsters have a disruption of their circadian timing such that they work on a free-running cycle of 20 h rather than the wild-type 22 h. The mutants have about 20% greater energy demands – but they also live about 15% longer (Oklejewicz and Daan, 2002).

(ii) Studies of different strains

Lin et al. (2002) observed that yeast under caloric restriction varied in their lifespans such that those with the longest lives also had the highest metabolic rates. Similarly Storer (1967) reviewed the available data on phenotypes of different mouse strains, and using his data a positive link of lifespan to energy expenditure can be seen. Speakman et al. (2003) measured the energy demands of different dog breeds and found a positive

association between energy metabolism (per gram) and lifespan, which also confounded the traditional pattern of larger individuals living longer. This anomaly has been established for dogs for some time (Michell, 1999; Li et al., 1996; Patronek et al., 1997; Egenvall et al., 2000). In contrast to these data, however, several studies on different strains of *Drosophila* (Promislow and Haselkorn, 2002; Novoseltsev et al., 2002; Van Voorhies et al., 2003) show no relationship between energy expenditures and lifespan.

(iii) Environmental factors

Many environmental factors impact on energy demands of animals – the most important of which are activity and temperature. Temperature is a particularly interesting effect because it has contrasting effects on energy metabolism in ectothems (colder temperatures eliciting lower metabolic rates) and endotherms (where colder temperatures elicit higher metabolic rates to sustain constant body temperatures). The effects of cold on the metabolic rates and lifespans of ectothermic flies have long been established. Loeb and Northrop (1917) made extensive studies showing that *Drosophila* at lower ambient temperatures lived longer. These observations have been replicated many times in several different insect species (Ragland and Sohal, 1975; Buchan and Sohal, 1981; Farmer and Sohal, 1987; Miquel et al., 1976). Contrasting these effects in ectotherms, however, a single study of the effects of cold exposure on longevity in rats indicated no significant influence on lifespan (Holloszy and Smith, 1986). This, perhaps, indicates that the impact of cold temperatures on lifespan in ectotherms acts *via* an effect directly on body temperature and is not mediated *via* an effect on energy demands. This interpretation is supported by observations on hamsters. Hamsters conserve energy during winter by hibernating and reducing their body temperatures. Lyman (1981) observed that if Turkish hamsters *Mesocricetus brandti* in the laboratory were prevented from hibernating then their increased energy expenditure and increased body temperatures were associated with a decrease in their lifespan.

In both ectotherms and endotherms increased activity leads to increased metabolic rate. Several manipulations have been made of animals to try to elevate their metabolism by making them more active (or increase the costs of activity) or reduce their metabolism by restricting their activity and then following effects on lifespan. Wolf and Schmid-Hempel (1989) manipulated honey bees *Apis mellifera* by forcing them to carry weights that elevated the energy costs of flight. The bees carrying the extra weights lived shorter lives than those unburdened. Deerenberg et al. (1995) forced breeding kestrels *Falco tinnunculus* to increase their work rates when feeding young, and observed that those adults forced to work harder died sooner (Daan et al., 1996). In contrast, however, some studies of exercise have produced the opposite (positive) effects on lifespan; but, as pointed out by Ramsey et al. (2000), in these latter studies measurements of impacts of the exercise treatments on total daily energy expenditure were not evaluated, and an effect on metabolism was not therefore demonstrated.

Contrasting the above studies, where manipulations were made that elevated activity, Ragland and Sohal (1975) restricted activity in house flies *Musca domestica* and found that this led to an increase in lifespan, an observation repeated by both Yan and Sohal (2000) and Toy and Sohal (1986).

(iv) Studies of individual variation within species

Individual animals within a species vary tremendously in their rates of energy expenditure. Few studies, however, have attempted to associate these differences with lifespan despite the evident advantages of this type of comparison (Austad, 1996). Speakman et al. (2000) measured the daily energy expenditures of 42 individual mice of the outbred MF1 strain at both 6 and 13 months of age, and then followed the mice until they died. There was a positive relationship between the energy expenditure and the lifespans. There was no effect of body mass on longevity in this cohort and the association between metabolic rate and lifespan was significant, whatever method was used to express the metabolism – whole body energy expenditure, expenditure per gram of tissue, or expenditure per gram of lean body mass. The relation to metabolic intensity (energy expended per gram of tissue) was, however, the strongest. This effect was not a small trivial effect. Dividing the mice into those with the top 25 percentile of metabolic rates, compared with the lowest 25 percentile, revealed that those in the top 25 percentile had 30.2% greater daily energy expenditure and lived on average 36% longer lives. In separate cohorts that were divided by the same criteria into the upper and lower 25 centiles for total daily energy metabolism, we also showed that the mice differed in their rates of resting as well as total metabolism and also that the mice in the upper 25 centile had more uncoupled mitochondria – an effect mediated by both differences in the adenine nucleotide translocase and uncoupling protein 3 in their muscle (Speakman et al., 2004). In contrast to these data, Hulbert et al. (2004a) measured metabolic rates of individual *Drosophila* but found no relation between the lifespan and metabolic rate and these observations were repeated in the blowfly *Calliphora stygia* with the same results (Hulbert et al., 2004b).

Overview of intraspecific studies

The observed patterns of association between rates of energy metabolism and the rates of ageing (or lifespans) of animals within species include all the potential patterns of association – positive, negative and not significant. Studies that include overt manipulation of animals (by exercise or temperature manipulations), particularly in invertebrates, often result in negative associations between metabolism and longevity – as predicted by the original formulations of the ROL theory. However, some quite large temperature manipulations (e.g. Holloszy and Smith, 1986) yielded no significant effects. In the few exercise studies where effects on energy demands were quantified and significant (e.g. Deerenberg et al., 1995; Daan et al., 1996) the effects on lifespan were negative. There is an

indication that the effects of temperature manipulation may act directly *via* effects on body temperature rather than on reduced metabolic rate.

The transgenic manipulation studies are confounded by the problem that transgenic manipulations often also affect body size. This raises issues about how metabolic rate should be normalised (see discussions in Ramsey et al., 2000; Speakman et al., 2002) that are not yet resolved. How this normalisation is performed dramatically alters the conclusions that are drawn about the effects of metabolism. Some researchers therefore claim that the transgenic effects act *via* metabolism; others suggest there is an independence of the effects from metabolism. In other species, particularly mice, the measurements have simply not yet been made to indicate what is going on. This problem of normalising for effects of body size also besets the whole area of whether caloric restriction acts *via* a decrease in metabolism or not (reviewed in Ramsey, 2000). Comparisons across different strains of dogs and mice are much clearer in suggesting a positive association of metabolism and longevity, and this effect was also found in the single comparison made to date of individual variability within a strain of mice (Speakman et al., 2004).

Why do we see such a diversity in response? Interestingly, the diversity in the responses of animals to changes or differences in their metabolism has been mirrored recently by suggestions that there are some theoretical shortcomings in the entire notion of how the rate of living theory and free-radical theory are linked. The fundamental notion of the association between the rate of living theory and the free-radical damage theory is that a proportion of the oxygen that interacts with the electron transport chain generates radical oxygen species. Hence a simplistic interpretation is that the more oxygen involved in such interactions (i.e. the higher the metabolic rate), the greater will be the ROS production, leading to a positive association between oxygen consumption, ROS production, free-radical damage and thus a negative link to ageing and lifespan (for a summary, see Ramsey, 2000). Our increasing understanding of the functioning of mitochondria has, however, painted a rather different picture of how such associations might arise (Brand, 2000; Speakman, 2004). In particular, the generation of free radicals in mitochondria appears to occur predominantly due to promiscuous interactions between electrons in the transport chain and oxygen at two particular sites. The first is in complex I (Barja, 1999). The second site is in complex III (Brand, 2000). In complex III, electrons are carried by ubiquinol (QH_2) to a site adjacent to the outer membrane where an electron is transferred to the small carrier molecule cytochrome *c*, for transfer to complex IV, generating ubisemiquinone (QH^-). This ubisemiquinone almost immediately loses an electron, and the resultant electron and ubiquinone (Q) tunnel to the matrix side of the membrane. Ubisemiquinone, however, may donate its electron to oxygen generating ROS on the inter-membrane side of the complex (Han et al., 2001). The likelihood it will do this is governed by how long the QH^- exists at the inter-membrane side of the inner-mitochondrial membrane (the p site). This duration, and hence the likelihood that ROS will be formed, appears to depend on the mitochondrial membrane potential (Demin et al., 1998a,b) When the potential is large, this retards the propensity of the electron to move across the membrane and enhances the longevity of QH^- at the p site. Consequently there is an exponential increase in ROS production from mitochondria as the membrane potential increases (Nicholls and Ferguson, 2002). When the membrane potential is low, mitochondria produce very few ROS (St Pierre et al., 2002), suggesting that production from complex 1 is normally minor, or is similarly dependent on membrane potential.

The membrane potential of mitochondria can, however, be influenced by activated channels (Arechaga et al., 2001) that permit protons in the intermembrane space to move across the membrane without passing through complex V and generating ATP. Some of these protein channels are called uncoupling proteins (UCPs) because they uncouple the association between protein movement and ATP production (Klaus et al., 1991; Jezek, 2002) but other compounds also dissipate the proton gradient by translocating other charges across the membrane – such as the adenine nucleotide translocase (ANT). The greater the activation of UCPs the less efficient mitochondria are because fewer proton movements are linked to ATP generation. In this situation oxygen consumption needs to increase to satisfy a given demand for ATP. However, the benefits of this inefficiency are fewer free radicals (Erlanson-Albertsson, 2002). The consequence, however, of this interpretation of mitcohondrial function is that the association between oxygen consumption and free-radical production becomes reversed under some circumstances (Brand, 2000). When there are high rates of oxygen consumption, associated with low mitochondrial efficiency due to uncoupling, the net production of ROS may be reduced. When mitochondria are well coupled, however, the association of metabolism to ROS production may be positive because of increases in ROS at both complex I and III.

Can we understand the diversity of the experimental data in the light of this theoretical understanding of mitochondrial function? The uncoupling to survive model suggests that when increases in metabolism are linked to uncoupling of mitochondria the association between metabolism and longevity should be positive. We observed this association directly in the individual mice we studied and showed an association to differences in their mitochondrial membrane potentials linked to activation of UCP-3. In the different strains of dogs, the smaller dogs lived the longest and also had the greater rates of energy metabolism. These are potentially associated to uncoupling rates because animals at smaller body sizes are more likely to be below their lower critical temperatures (McNab, 1980), thus paying a thermoregulatory cost. The primary mechanism that small mammals utilise to thermoregulate is to generate heat in brown adipose tissue (BAT) *via* a mechanism that involves induction of uncoupling protein-1 (Cannon and Nedergaard, 2004). Indeed, accepting all the caveats that attend the data on daily energy demands and maximum lifespans (Fig. 3A,B), this interpretation is also

consistent with these data. Hence the residual variation in the data for mammals was negatively associated with ambient temperature – and animals at greater ambient temperatures would be expected to be spending less energy on thermoregulation – i.e. more coupled in their metabolism, and the overall trend was for smaller animals to have greater lifetime expenditures of energy per gram of tissue. Similar to the dogs, the longer lifespans at greater energy demands is potentially because the smaller the animal, the more likely it is to be operating below lower critical temperature (T_{lc}), since there is a negative relationship between T_{lc} and body mass (McNab, 1980) and therefore have its expenditure uncoupled. The contrasting responses of animals to manipulations of their energy demands by exercise changes are, however, likely to include the opposite effects – that is, during exercise animals generally do not need to have high thermoregulatory heat output because they can substitute the heat generated from the exercise, and their mitochondria tend to become more coupled to maximise efficiency (reviewed in Speakman and Selman, 2003). Thus manipulations of animals that include forced exercise lead to reduced lifespans (above). In the context of caloric restriction we have recently observed that while almost all tissues are reduced in size in the caloric-restricted rat the only tissue that is larger is the brown adipose tissue – perhaps indicating a shift from coupled to uncoupled respiration (Selman et al., 2005).

Recent theoretical evidence shows that the link between free-radical production and energy metabolism is far from straightforward. For some modes of energy expenditure where mitochondria are well coupled the association may be positive, as originally hypothesised in the combination of the rate of living and free-radical damage theories. In other modes, however, a completely opposite association may pertain – higher metabolism may result in less ROS production. This state of affairs is extremely unfortunate because it means that in the absence of actual data on rates of free radical production and uncoupling, almost any empirical data set can be explained by some *post hoc* arguments concerning the nature of the energy expenditure changes. It is important to recognise, therefore, that while we can understand many of the existing patterns of association between energy metabolism and lifespan, there are some features of the data that certainly do not fit with this scenario. The most compelling data that do not fit are the experiments of Holloszy and Smith (1986) involving rats forced to stand in cold water, resulting in an increase in total energy demands of about 50%. Presumably, most of the increased expenditure of these animals was thermogenic and uncoupled yet they did not have extended lifespans (or for that matter shortened lifespans predicted by the simple ROL theory). In addition, we have shown that while short-tailed field voles exposed to the cold have an increase in their thermogenic capacity mediated by changes in brown adipose tissue and increased metabolic rates, these animals show upregulation of oxidative defence (Selman et al., 2000) and repair mechanisms (Selman et al., 2002) when exposed to the cold. If the uncoupled metabolism reduced oxidative stress then why did these animals upregulate their defences?

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