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Living Fast, Dying When? The Link between Aging and Energetics[1,2]

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ABSTRACT The idea that aging should be linked to energy expenditure has a long history that can be traced to the late 1800s and the industrial revolution. Machines that are run fast wear out more quickly, so the notion was born that humans and animals might experience similar fates: the faster they live (expressed as greater energy expenditure), the sooner they die. Evidence supporting the "rate-of-living" theory was gleaned from the scaling of resting metabolism and life span as functions of body mass. The product of these factors yields a mass-invariant term, equivalent to the "amount of living." There are at least four problems with this evidence, which are summarized and reviewed in this communication: *1*) life span is a poor measure of aging, *2*) resting metabolism is a poor measure of energy expenditure, *3*) the effects are confounded by body mass and *4*) the comparisons made are not phylogenetically independent. We demonstrate that there is a poor association between resting metabolic rate (RMR) and daily energy expenditure (DEE) measured using the doubly labeled water (DLW) method at the level of species. Nevertheless, the scaling relation between DEE and body mass still has the same scaling exponent as the RMR and body mass relationship. Thus, if we use DEE rather than RMR in the analysis, the rate-of-living ideas are still supported. Data for 13 species of small mammal were obtained, where energy demands by DLW and longevity were reliably known. In these species, there was a strong negative relationship between residual longevity and residual DEE, both with the effects of body mass removed ($r^2 = 0.763$, $F = 32.1$, $P < 0.001$). Hence, the association of energy demands and life span is not attributed to the confounding effects of body size. We subjected these latter data to an analysis that extracts phylogenetically independent contrasts, and the relationship remained significant ($r^2 = 0.815$, $F = 39.74$, $P < 0.001$). Small mammals that live fast really do die young. However, there are very large differences between species in the amounts of living that each enjoy and these disparities are even greater when other taxa are included in the comparisons. Such differences are incompatible with the "rate-of-living" theory. However, the link between energetics and aging across species is reconcilable within the framework of the "free-radical damage hypothesis" and the "disposable soma hypothesis." Within species one might anticipate the rate-of-living model would be more appropriate. We reviewed data generated from three different sources to evaluate whether this were so, studies in which metabolic rate is experimentally increased and impacts on life span followed, studies of caloric restriction and studies where links between natural variation in metabolism and life span are sought. This review reveals that there might be contrasting effects of resting and nonresting energy expenditure on aging, with increases in the former being protective and increases in the latter being harmful. J. Nutr. 132: 1583S–1597S, 2002.

KEY WORDS: • *metabolic rate* • *body mass* • *aging* • *survival* • *energetics* • *doubly labeled water* • *phylogenetics*

The idea that aging should be linked to energy expenditure is intuitively attractive. This can be readily appreciated by the number of idioms that express the generalized concept that increases in our energy expenditure are likely to result in decreases in our life span. Sayings such as "*to burn the candle at both ends*," which was originally used in English in a poem by the American poet Edna St. Vincent Millay in 1920, but appears to have an earlier French origin dating to around the 1860s, and "*the candle that burns twice as bright burns half as long*," which was probably first used by Michael Faraday in his lectures on candles at the beginning of the last century, encapsulate our strong impression that the association between the rate of expenditure and life span should be negative. It is probably not coincidence that these quotations coincide with the industrial revolution and the beginnings of the industrialization of the Western world. Our general experience of mechanical objects is that the more vigorously we use them, the more rapidly they will malfunction. Because there is a tradition of interpreting the functioning of biological systems

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[4] Abbreviations used: BMR, basal metabolic rate; DEE, daily energy expenditure; DLW, doubly labeled water; M, body mass; PDAP, phenotypic diversity analysis program; PIC, phylogenetically independent contrasts; Q, ubiquinone; QH, ubisemiquinone; RMR, resting metabolic rate; ROS, reactive oxygen species.

by analogy to the functioning of our man-made mechanical systems (c.f., the various interpretations of the functioning of the brain by analogy to a functioning factory and lately by analogy to computers), it is not surprising that this generalized phenomenon pertaining to all mechanical objects should be broadened and assumed to apply to living things as well. Scientists in the late 1800s, such as Rubner, the pioneering German researcher of animal metabolism, had already started to formulate the hypothesis that the link between energetics and aging was probably the basis of a fundamental biological law. It was not until the early 1900s, however, that the ideas became crystallized and known as the "rate-of-living" theory, which is generally attributed to Pearl, who summarized the consensus on this issue, generated over the previous decades (1). The rate-of-living theory, in essence, postulates that the amount of life that any organism has available to it is fixed. Animals may choose to utilize that life extravagantly, resulting in a shortened life span, or frugally, resulting in a prolonged life span. It is not difficult to imagine that such a concept found widespread favor in the puritanical Victorian era of the late 1800s.

Although measurements of energy demands of animals had been made from the late 1700s (primarily by Lavoiser and Seguin in France; although earlier work by Mayrow and Boyle in the 1660s substantially predate the French work, it was erroneously interpreted under the Phlogiston model), it was not until the 1930s and 1940s that widespread measurements of a range of different animal species started to accumulate. The first attempts to summarize this growing body of data, by Kleiber (2) and Brody (3), clearly established that the energy metabolism per gram of tissue for animals that were in a fasted, resting state was considerably lower in larger species than in smaller ones. The so-called "mouse-to-elephant" curve exemplified this effect. Dispute over the exact scaling exponent that best fits these data occurred over several decades, although it is now widely accepted that the exponent is in the region of -0.25 to -0.28. Substantial increases in the numbers of animals for which we currently have data on basal metabolic rate have not altered this general principle (4–6). This observation was important because it was not lost on researchers that life span shows the opposite trend: larger animals lived longer lives and the scaling exponent for this relationship was almost exactly the same, but with an opposite sign (7–9). The direct consequence of this similarity in the dimensions of the exponents with opposite sign is that the product of these two equations results in a mass-invariant parameter [the "life history invariant" of Charnov (10)]. So, the "amount of living" an organism does, which is reflected in the product of rate of metabolism (per gram) multiplied by maximum life span, is constant.

Not only this, but it was also observed that the scaling exponents for various physiological parameters connected with oxygen (O_2) delivery to the sites of metabolism not surprisingly scale in similar ways. For example, because the size of the heart is an almost fixed percentage of total body mass, the rate at which it beats has to keep pace with the total summated tissue level demands for oxygen. Resting heart rate scales as body mass $(M)^{-0.29}$ and in consequence, because oxygen consumption scales as $M^{0.29}$, the product of these two parameters is also mass invariant, meaning the number of heart beats in an animal's life is also roughly independent of body size. As noted by Calder (11), "*. . .it would consequently appear that a number of physiological parameters are scaled such that the number of them in a lifetime is relatively constant.*" Perhaps it was inevitable that such a correlation would sooner or later be misinterpreted as a causal relationship, and the concept emerged that we have a fixed number of heart beats, that we can use up rapidly or slowly, and once we reach that number we die (the number is actually 955,787,040). Notwithstanding the clearly dubious nature of this latter reasoning, the fact that lifetime expenditure of energy per gram of tissue is invariant across different mammalian species provides strong support for the rate-of-living ideas.

There are at least four separate problems with the scientific case that is espoused in the rate-of-living theory and the empirical evidence adduced to support it. In the following section of this review we elaborate on these four potential problems and evaluate the seriousness of the difficulties they pose for the idea, including some novel analyses that overcome the problems, thus allowing a more rigorous evaluation of the rate-of-living theory.

PROBLEMS WITH THE RATE-OF-LIVING HYPOTHESIS

Maximum life span is not a valid measure of aging

Aging is defined as the increase in the rate of mortality as a function of age (12). When animals age more rapidly, the gradient of the relationship between mortality and age is steeper than when they age more slowly. These changes reflect a complex combination of alterations in underlying physiology at the whole organism, cellular and molecular levels. Despite its underlying complexity, it is true that, all other things being equal, if two populations differ in the aging rate, then the average age at death in the population with the slower rate of aging will be greater than that of the species with the faster rate of aging. Maximum life span is the age of the longest-lived organism of that particular population or species. Because this is a single event of a lone individual there is actually no necessity that this event will be related to the mean life span of the population in question. Although in a normal distribution the probability of a single event far to the right of a distribution is improbable, it does remain statistically possible that the highest single event from a given distribution could occur at a higher value than the highest single event from a second distribution that had a higher average. This problem arises only if maximum life span is defined from the single longest-lived member of a population. If maximum life span is defined as the life span observed in the top 5 or 10% of the population, then this value will sit a fixed number of standard deviations above the mean life span (assuming normality in the distribution). By this definition, maximum life span would give a good estimate of the rate of aging.

The second caveat in the utility of maximum life span, even if it is defined as the top 5 or 10% of life spans, is the phrase "all other things being equal." Generally, this condition is unlikely to be met, and there are several situations where aging may not differ between two populations (or species) but life span does. The first of these situations is where selective breeding of a population has resulted in fixation of a mutant gene (or genes), which has an early mortality phenotype. SCID mice, for example, are born with compromised immune systems, which means they generally die of infections very young (under 1 y), unless they are barrier-reared in isolation units to protect them from pathogens. Life span in this mouse is very short, on average; however, this has nothing to do with an aging effect, given that SCID mice generally all die before they start to age. This situation, however, is probably unique to animals that have been selectively bred and maintained in captivity (13). Although studies of domesticated breeds/strains may therefore be compromised, studies of outbred populations

are far less likely to be affected. However, there are other situations to which outbred populations may be susceptible, and these are where selection has favored a delay in the onset of aging. In two populations in which the aging rate (defined as the gradient of the increase in mortality with age) is constant, life spans will be much longer in the population where the onset of the age-related increase in mortality is delayed. Perhaps the most obvious example of this effect is the difference in life span between male and female humans. This difference does not reflect a difference in the rate of aging, but rather an early onset in the increase in age-related mortality in males. Because life span is a composite measure of both the rate of aging and delay in increased mortality onset, it is not itself a pure measure of aging rate.

Nevertheless, despite this, both aging rate and time of aging onset are important and interesting phenomena. A composite measure, which incorporates both these traits into a single figure, may not therefore satisfy purists wishing to study only onset or aging rate, but it does represent a trait of valid interest. If elephants have evolved to delay their aging onset for 50 y, which results in their longer life span than that of mice, this is surely no less important than if the life span difference came about because of a difference in the rate of aging. Because the argument supporting the rate-of-living theory deals with data derived from outbred species, where single-gene effects on pathology are unlikely, we can probably safely ignore the problem that maximum life span and aging rate are not synonymous.

Resting metabolism is not a valid measure of total energy metabolism

Measurements of resting metabolic rate (RMR) are made on animals when they are fasted and at rest because food ingestion, thermoregulation and physical activity all elevate the rate of metabolism. Organisms can survive at basal metabolic rate (BMR ≅ RMR) for a short time, but they cannot function at this level for any protracted period. There is a clear mismatch therefore between the conditions necessary to generate the RMR measure and the conditions necessary for an animal to live to an old age. If resting metabolism is only a minor contributor to total daily energy expenditure (DEE), it seems unlikely that any association between RMR and aging/life span could be causal. This also exposes the fallacy of the notions that there are "invariants" that control our life span, because these invariants are generated by reference to resting processes. It is improbable in the extreme that our life duration is determined by an "amount of living" it does when at rest, but the amount of living over and above that is irrelevant. Similarly, it is difficult to imagine a metabolic process that apportions us 955 million heart beats to spend on resting activities, but is unaffected by the numbers of heart beats we spend on everything else, particularly when these other heart beats may be substantially the majority.

Estimates of resting energy expenditure are generally made by respirometry (indirect calorimetry). During such measurements, subjects are confined into chambers, or alternatively have hoods placed over their heads, through which there is a continuous flow of gas. The gas flowing into the hood or chamber has a composition of atmospheric air (20.95% O_2 and 0.03% CO_2) but the gas coming out of the hood is altered by the respiratory activity of the subject. The O_2 content of the outflow gases is reduced and the CO_2 content elevated. These differences in gas concentrations can be translated into actual O_2 consumptions and CO_2 productions if the flow rate through the system is accurately known. It is possible to confine subjects continuously in chambers for 24 h to measure their daily energy demands. However, the resulting value is not a good reflection of actual daily energy requirements because of the restriction under which the subject is imposed.

An alternative method of estimating daily energy demands is to construct a time and energy budget over an entire 24-h period. Subjects are continuously observed and their time spent in various behaviors is logged, after which these times are multiplied by the energy costs of the behaviors in question. The main problem with this time and energy budget method is that, although hood respirometry performs adequately as a method for measuring the energy demands of subjects when they are at rest, there are a large number of activities in which animals and humans engage during their daily lives that are not amenable to study by this approach. Quantifying daily energy requirements by a time and energy budget approach is therefore extremely difficult because, though it may be possible to define the time spent doing various things, defining exactly how much these activities cost is not straightforward. Moreover, as can be readily appreciated by consideration of resting metabolism, no single value for the energy cost of this behavior is routinely applicable. An animal at rest shortly after a meal, for example, may have a considerably elevated metabolic rate compared to that of one that has been fasting.

Fortunately, there is an alternative approach, which is to measure daily energy demands directly using elimination rates of stable isotopes. Two main methods have been used, the labeled bicarbonate method (14) and the doubly labeled water (DLW^4) technique (15–17). This latter method has been considerably more popular and to date (2001) has been applied to estimate daily energy requirements for at least 80 different species of mammal (18,19) and over 150 species of bird (18), as well as many species of reptile [e.g., Christian et al. (20)]. In principle the method works on the assumption that two things dominate the flux of hydrogen and oxygen in body water: water and the continuous inflow of respiratory O_2 and outflow of CO_2. Because labeled atoms of hydrogen are eliminated only in the outflow of water from the body, but labeled atoms of O_2 are eliminated by both the outflow of water and CO_2, the difference in the elimination rates provides a direct estimate of the CO_2 production. The real advantage of this method is that the exponential elimination rates are linear when logarithmically transformed. To estimate the gradient of a straight line of logged isotope enrichment against time it is necessary only to have estimates of the enrichments at two time points (21,22). Consequently, shortly after the isotopes have been administered to an animal, a blood sample can be collected and the animal released to behave as it would normally in its natural environment, integrating the myriad of factors that determine its overall metabolic rate. It can then be recaptured some time later (1–30 d, depending on body mass) and a second blood sample removed. The difference in the gradients of the straight-line relationships derived from the start and end points define the CO_2 production of the animal over the time between these two times.

In practice, although the method sounds routine to apply, there are numerous simplifying assumptions that have to be made (16,17,21,23) and violation of these assumptions can exert a negative impact on the accuracy and precision of the method. For example, it is assumed that the background level of isotopes remains constant throughout the measurement period. This and the other issues have been treated exhaustively in several publications (16,17,21) and the conclusions of these assessments of the technique is that, on average, across a group of subjects, the method provides a useful estimate of daily energy demands, with an average discrepancy to simul-

taneous respirometry in validation studies of around 3% [see review in Speakman (17)]. For individuals, it provides a value of less utility because the precision of the estimate is lower, although because this is the only reliable method for estimating daily energy demands, such individual estimates are generally preferable to having nothing at all.

The key question in the context of the current discussion about links between energetics and aging is whether resting metabolism provides a reasonable proxy for daily rates of energy expenditure. This would be the case, for example, if RMR contributed a substantial amount to the total expenditure. Studies that have documented both resting metabolism and daily energy expenditure are available for 73 species of small mammal (weighing <4 kg), allowing evaluation of this suggestion [reviewed in Speakman (19)]. These data indicate that on average in small mammals RMR contributes 35% of the total DEE as estimated by DLW [$n = 73$ (19)]. This is a large proportion of the total, but it is not a majority. Hence, in terms of evaluating the utility of RMR as a proxy for DEE this calculation clearly indicates that it is deficient. However, this need not be the only criterion for evaluating the usefulness of RMR as a measure of total energy metabolism. For example, RMR might also be useful, despite having a low absolute contribution to DEE, if there was a fixed ratio between the two. RMR might contribute only 35% of the total, but if it was always 35%, then a relationship between RMR and life span might validly reflect a relationship between DEE and life span.

In fact, ratios between RMR and DEE vary from 1.4 to 8.0 with a maximum frequency at 2.6× RMR (**Fig. 1**). RMR clearly does not have a fixed ratio to DEE, making its use as a proxy for DEE tenuous. An example may clarify this point. The Kangaroo rat (*Dipodomys merriami*) weighing 28.7 g has an RMR of 15.8 kJ d^{-1}. This is almost identical to the RMR of the small marsupial mouse (*Antechinus*) of almost equal body mass (25.7 g), with an RMR of 15.6 kJ d^{-1}, yet the directly measured DEE of the Kangaroo rat at 34.3 kJ d^{-1} (24) in winter is only half the DEE of the marsupial mouse at 72.0 kJ d^{-1} (25). In spite of this wide range of ratios between RMR and DEE, across a wide range of species there was still a strong negative relationship between DEE (per gram body mass) and body mass (**Fig. 2**), which has approximately the same scaling exponent as that found between RMR (per gram body mass) and body mass. This relationship occurs because the range of DEE covers a 50-fold range of variation, but the variability in translating RMR to DEE only differs in the range two- to eightfold. As the ratio of RMR to DEE does not differ systematically with body mass (19), the overall nature of the relation between RMR and body mass is mirrored in the relation

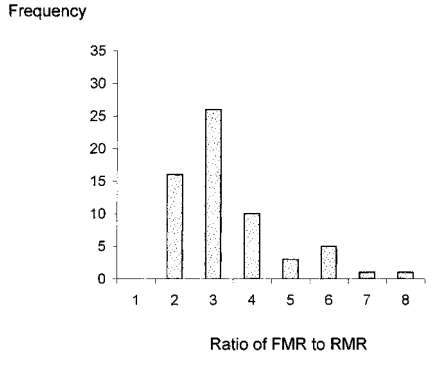

FIGURE 1 Histogram showing the distribution of ratios of resting metabolic rate to daily energy expenditure (DEE/RMR) measured across 73 species of small mammal [reviewed in Speakman (19)].

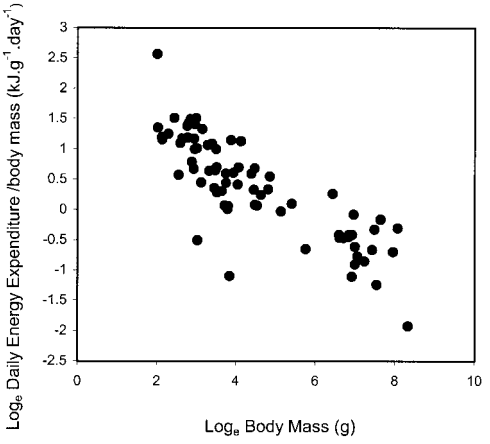

FIGURE 2 Relationship between mass specific daily energy expenditure measured by the doubly labeled water method and body mass [data derived from original data reviewed in Speakman (19)].

between DEE and body mass. As an overall trend, therefore, describing the nature of energetics scaling at the interspecific level RMR does capture the essence of the relationship between total daily energy demands (by DLW) and body mass. It is important to remember, however, that at the level of individual species RMR does not function quite so advantageously.

Because the relationship between DEE and body mass has the obverse exponent of the relationship between longevity and body mass, it remains true that despite the inadequacies of using RMR at the individual species level, the product of the two relationships (total energy expenditure per gram per life span) is mass invariant. On average a gram of tissue in a small animal expends the same amount of energy over the course of an entire life as does a gram of tissue from a large animal.

The empirical data are compromised by the confounding effect of body size

Using the scaling exponents in relation to body mass to deduce similarities in the scaling effects of different traits cannot be used to infer causality between those traits. This is because there is a confounding effect of body mass itself, with almost all physiological traits varying in relation to body mass. Hence, given that longevity also varies in relation to body mass, by comparing the respective scaling exponents it is possible to draw inferences that almost any trait that varies with an exponent around −0.25 to −0.3 is an important "life history invariant" controlling longevity [e.g., food-intake scales with an exponent around −0.29 (26)]. Hence, lifetime food intake per kg scales as $10.7\ M^{-0.29} \times 237\ M^{0.29} = 2537\ M^{0.00}$, which is a "mass-invariant" trait. Using the scaling argument to infer causality, one might reason that life span is controlled by the amount of food (energy) that is eaten. In other words, animals have a fixed amount of food to eat in their lives, which is actually equal to 2537 MJ per kg of body mass, and once they have eaten that amount, they die. [Using an average energy content for food of about 4 MJ/kg (wet weight) and an average body mass of an adult human of 80 kg, this calculation leads to the prediction that the amount of food that determines our life span is 50,740 kg or about 50 metric tons.] The idea that a fixed amount of total food intake "determines" our life span by direct causality is extremely unlikely. Yet, exactly the same arguments are brought into

play when we start to talk about lifetime energy expenditure determining life span.

One possibility therefore is that the association comes about because of causal links between both traits and body mass, although the traits themselves are completely independent. For example, we know that larger animals must have lower metabolic intensities than those of small animals because of the physics of heat dissipation at the animal surface. For a mouse weighing 30 g with a surface area of 30 cm^2, each gram of metabolizing tissue has 1 cm^2 of surface at which to dissipate the heat that is a by-product of its metabolism. For an elephant weighing 3000 kg, however, the amount of surface available per gram of tissue to dissipate heat is only 1/90th of that available to the mouse. Consequently, if the elephant had the same metabolic intensity as that of the mouse, each square centimeter of the elephant's surface would have to dissipate heat at 90× the rate achieved by the mouse surface. This can occur only by reducing surface insulation and increasing the skin/body temperature. There are limits to which surface insulation can be reduced and elephants have lost all their external insulation (fur). However, once all the external insulation has been eliminated, no further reduction is possible. The only other mechanism available is to increase surface temperature. To achieve the same rate of heat loss as that of a mouse the elephant's surface would need to heat up to greater than the boiling point of water. These physical constraints establish the fact that metabolic intensity (energy expenditure per gram of tissue) in the elephant cannot be as high as that in the mouse.

The converse arguments are less sustainable. There are no apparent physical constraints that prevent the mouse (at thermoneutrality) from having as low a resting metabolic rate as that of the elephant. The physical constraints in this system *permit* mice and other small animals to have higher rates of metabolism than those of large animals, but the constraints *require* large animals to have lower metabolic rates than those of smaller ones. Below thermoneutrality the high energy demands of small animals become obligated by elevated heat loss at the body surface. There are limits not only on the minimum level of external insulation, but also practical limits on the maximum. Fur thickness can increase to only a certain point, at which it starts to impede mobility. At this point low ambient temperatures can be tolerated only by greatly increasing the metabolic rate to balance heat loss. Consequently there are no small animals with metabolic rates that are as low as those observed in large animals.

The association between longevity and body mass may come about for completely different reasons. Predation is primarily a size-related event. Animals generally predate other animals that are only at least an order of magnitude smaller than themselves. This asymmetry in sizes is probably driven by the selective advantage to the predator of selecting smaller prey because they are less likely to fight back and injure the predator during a predation event. Inevitably then, smaller animals face greater levels of predation risk because there are lots of animals bigger than they are, whereas very big animals, such as elephants, are effectively immune from predation once they reach adult size. Predation plays a large part in defining the extrinsic rates of mortality, and the "disposable soma" hypothesis of aging (27–29) posits that it is these expected levels of the extrinsic rates of mortality that drive the patterns of protection and repair mechanisms that may ultimately define life span. Hence, if an animal lives in an environment where there is a predation pressure such that its expectation of living beyond 18 mo is zero, evolution cannot favor the development of a costly repair and protection mechanism that would keep the animal alive for 18 y, given that the animal would derive no benefits to offset the costs of such a system. Consequently, the global pattern of predation pressure in relation to size may define the expectations of extrinsic mortality for different sized animals, which drive the protection and repair mechanisms that ultimately define the life span.

The key point is that physical constraints in relation to body size may define the relationship between body mass and energetics. Ecological constraints in relation to size may define the association between body mass and life span. However, there may be no association between energetics and life span beyond that artifactually generated by the correlation of both with body size. The classic way to address this problem is to remove the effect of body size statistically, by calculating residual values to the established regression slope, and then seeking associations between the residual values of metabolism and life span. In other words, if an individual datum sits above the relationship between daily energy demands and body mass then, if the association between energy demands and life span is causal, we would also expect the same species point to sit below the regression line in the relation between longevity and body size. Across many such comparisons there should be a statistical association (negative) between residual metabolism and residual life span.

A problem, however, is the small number of species for which we have good data on both the maximum life span and information on their daily energy demands, the major gap being good data for maximum life span. This is because data on longevity in mammals generally derive from two separate sources: records from the wild, where mark and recapture studies made over protracted periods generate estimates of mortality profiles with age, along with maximum ages of a cohort of animals; and second, records from zoos of exceptional longevity in captivity [e.g., Mallinson and Barker (30)]. Neither of these records is ideal because in the former maximum life spans may be curtailed by natural mortality as a result, for example, of predation risk. Data for the white-footed mouse (*Peromyscus leucopus*) exemplify this problem. In the wild, records of 1800 individuals captured over 7 y revealed that only 2.2% of animals lived for longer than 345 d (31). Yet many animals were still alive after 5.5 y in captivity and the estimated maximum longevity was around 7 y (31,32). Similarly in the wild the average life expectancy of the bank vole (*Clethrionomys glareolus*) varies between 2.5 and 3.5 mo, yet in captivity animals have been recorded living for 40 mo (33). Records for single individuals in zoos may be unrepresentative of average maximum longevity (i.e., of, say, the longest-lived 10% of the population). Moreover, mortality in zoos may stem from inadequate long-term nutrition of animals [see, for example, data on nutritional problems and diseases in captive held sloths (*Bradypus* sp.) (34)]. Rychnovsky (35) found that European common voles *Microtus arvalis* kept in captivity had maximum life spans that depended on their nutritional status, and that by optimizing nutrition life span could be doubled (from 15 to 30 mo). In addition, the species represented in zoo collections seldom include common species, which form the focus of most field studies of energy metabolism.

The ideal situation in which to generate data on longevity is where a large number of animals are maintained in captivity with the specific purpose of giving them ideal nutritional support and recording how long they live. This has been seldom performed for any species. Even for those small mammal species that are widely used by the pet trade, such as gerbils (*Meriones* sp.) and chipmunks (*Tamias* sp.), verifiable records of dates of birth and death are seldom recorded or available. Taking the species for which cohort observations

TABLE 1

Small eutherian mammals for which we have reliable information on their body mass (M), daily rates of metabolism (DEE) measured in the field using doubly labeled water and their maximum life spans (MLS) in captivity, or for bats in the field, and the residual values of DEE and MLS (resDEE and resMLS, respectively), which are the deviations from the allometric expectations in relation to M[1]

Species	M (*g*)	MLS (*m*)	DEE (*kJ d*$^{-1}$)	resMLS	resDEE
Insectivora					
Sorex arenaeus	7.5	15	97.8	−0.32	+1.35
Chiroptera					
Plecotus auritus	8.5	260*	13.5†	+2.43	−0.72
Pipistrellus pygmaeus	7.6	130*	14.7†	+1.77	−0.56
Myotis lucifugus	8.45	335*	13.8†	+2.68	−0.69
Eptesicus fuscus	19.3	292*	24.0†	+2.28	−0.61
Rodentia					
Microtus arvalis	20	33	89.9	+0.11	+0.62
M. agrestis	26.8	30	78.3	−0.06	+0.28
Clethrionomys glareolus	23.4	40	87.9	+0.15	+0.49
Peromyscus leucopus	19.4	84	53.0	+1.06	+0.09
P. maniculatus	19.5	66	64.3	+0.81	+0.26
Mus musculus	15.0	26	46.3	+0.28	+0.19
Marmota flaviventris	3120	130	1217†	+0.01	−0.07

[1] Adapted from Speakman (19) and Sacher (7). Data for DEE are derived from Speakman (19). Longevity data are from Refs. 32, 33, 35–38, and personal observations of cohorts of animals in captivity (*S. arenaeus, M. agrestis, Clethrionomys glareolus and Mus musculus*).

* Denotes that, where the maximum life span was reported for a single individual in the field, the estimate is assumed to be 10% lower than this limit.

† Denotes that, for individuals that hibernate, the summer DEE is reduced by 50% to get the average DEE over a complete annual cycle.

have been made in captivity, and combining them with field data on longevity of four bat species and one large rodent, there were a total of 12 species for which good data are available on aspects of daily energy metabolism as well as estimates of maximum longevity that we considered reliable (**Table 1**).

One question in connection with the estimates of daily energy demands is how representative these estimates are for the metabolism of the animals throughout their life spans. The answer to this important problem is that we just do not know. Factors influencing intraspecific variation in DEE have seldom been studied and extending the few studies to these species is not possible. However, for five of the species included in Table 1 we know there is a definite problem because they are hibernating animals. This is a problem because estimates of daily energy expenditures of these animals have all been made during the summer months, when the animals are endothermic and regulating their body temperatures at the euthermic mammalian level of around 37°C. Assuming that the average annualized rate of metabolism is equivalent to the summer rate is clearly erroneous. We have made some assumptions to convert these values to average daily energy expenditures over a complete annual cycle. Although hibernal animals periodically arouse in winter, these arousals are brief in duration. During continuous torpor the metabolism is barely detectable. We have thus made the assumptions that these animals hibernate (are in torpor) for half the year and that their energy metabolism during this time is effectively zero. Annual rates of energy expenditure are therefore assumed to be half the rates measured during the summer months. This assumption pertains to the bats and the marmot.

We calculated the residual metabolism and also the residual life spans of these 12 species by taking the expectations generated from Speakman (19) for the DEE and from Sacher (7) for the life spans, using the average body masses at which measurements of energy demands had been made in the field. These residuals were then examined to see whether there was an association between residual life span and residual total daily energy expenditure using regression analysis. There was a strong negative relationship between the two (**Fig. 3**), with variations in residual DEE explaining 76.3% of the variation in residual life span ($F = 32.15$, $P < 0.001$). These data therefore indicate that the negative association between energy expenditure and life span is not an artifact of the covariation of both traits with body mass.

Comparisons that include species as individual data are statistically invalid

It has been recognized for almost 20 y now by comparative biologists that making comparisons between species is not a statistically valid procedure (39,40). Most statistics are founded on the assumption that individual data points in-

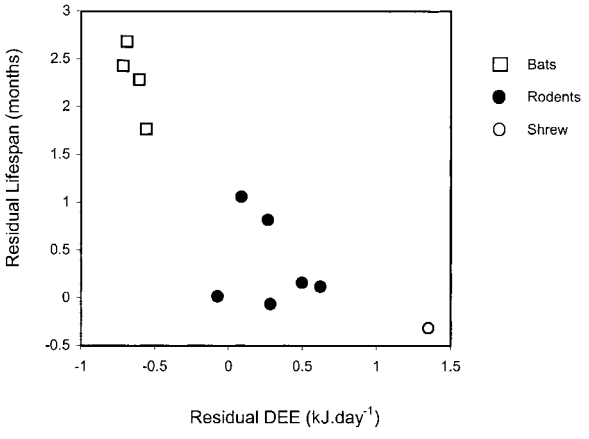

FIGURE 3 Residual life spans of 12 small mammals plotted against residual daily energy expenditure measured by doubly labeled water. Data extracted from Table 1.

cluded in the analyses are independent of each other. However, different species are not independent because they are not independently created entities, because they are generated by the process of evolution. Any two mammalian species, therefore, share some of their evolutionary history, simply by virtue of the fact they are both mammals. If we take two species that diverged and became reproductively isolated 50,000 y ago, these two species share a great amount of their evolutionary history in common, compared, for example, with two species that diverged 50 million years ago. If we include both species that diverged only recently in our sample used to construct the relationships between longevity and body mass, and between daily energy expenditure and body mass, then we artificially inflate the degrees of freedom in our comparison, and potentially bias the slope of the relationship between the traits. Indeed, the very existence of a relationship at all may be thrown into doubt by the "phylogenetic lack of independence" of the component species (40–44).

Solutions to this problem have been worked out (41–44), but they depend on knowledge of the historical phylogenetic relationships between the species involved in the analysis. In some cases these relationships are still a matter of dispute and hence any phylogenetically "correct" analysis can only generate a result that is "correct," subject to the possibility that the phylogeny upon which it is based may be erroneous. This is not a trivial problem because rearrangements of phylogenetic interrelationships in all taxa are ongoing and are often major in their nature and ramifications. Perhaps the accumulation of molecular evidence in connection with disputes over phylogenetic relationships will ultimately lead to resolution of the problems, which primarily stem from interpretations of whether aspects of morphology are shared (hence indicative of shared evolutionary history) or derived (and hence ascribed to convergent evolution of unrelated species). However, at present there are still many regions where our understanding of phylogenetic relationships are unstable and unresolved. Although those responsible for generating algorithms that "solve" the phylogenetic independence problem have minimized the importance of having a "correct" phylogeny (41–44), the implications of not having a correct phylogeny may not always be trivial. Symonds (45), for example, noted that interpretation of the role of energy metabolism in the life histories of insectivores was dependent on the phylogeny that was employed to make the phylogenetic correction.

Examination of the data on longevity and daily energy demands (Fig. 3; Table 1) makes clear the possible effects of phylogeny on the independence of the data. Although there was a significant relationship between residual energy metabolism and residual longevity (Fig. 3), the data comprising this relationship fall into three groups, with four data displaying low metabolism and long life spans, a central group of eight species exhibiting intermediate levels of both traits and a single datum displaying high metabolism and a short residual life span. Further examination (Table 1) reveals that these three groups are phylogenetically distinct. The four data with low metabolism and long residual life spans all refer to bat species, the central group is composed exclusively of rodents and the single datum with high metabolism and a short life span is an insectivoran. If low metabolic rates and long life spans evolved very early in the chiropteran lineage, then all four of the bat species may have the same traits by virtue of the fact they are evolved from an ancestral bat that had this trait distribution, and not because they are independent adaptive combinations of the two traits. Similar arguments might be made for the rodent group and the insectivoran. Treating these as independent data is clearly questionable, and the actual degrees of freedom in this analysis may be closer to 2 (3 orders − 1) instead of 11 (12 species − 1). To statistically remove the effects of phylogeny, it is necessary to reconstruct the phylogenetic interrelationships for the species involved in the analysis. Fortunately, this reconstruction does not include any species where major disputes are currently being fought about their phylogenetic locations, although the lack of dispute today is no guarantee for the future. The phylogeny we used is illustrated in **Figure 4**.

We used the phylogenetic diversity analysis program (PDAP) package (41) to transform the raw data for residual life span and residual daily energy demands into phylogenetically independent contrasts (PIC) using the Felsenstein (39) method. We made three different assumptions about branch lengths in the phylogeny: *1*) branch lengths equal to those illustrated in Figure 4, *2*) branch lengths set by Pagel's arbitrary method (46) and *3*) branch lengths set by Grafen's arbitrary method (47). Independent of these assumptions, the diagnostic plots in PDAP (41–44) indicated the branch length assumptions were adequate to standardize the contrasts, and thereafter analyses were performed on the tree using only the branch length assumptions illustrated in Figure 4. The PIC of residual life span was significantly negatively associated with the PIC of residual daily energy expenditure (**Fig. 5**). The least-squares-fit regression equation explained 81.5% of the variation in PIC residual life span ($F = 39.74$, $df = 11$, $P < 0.001$). These data indicate that the negative relationship between residual life span and residual daily energy expenditure is not a consequence of elevated degrees of freedom in the analysis because of failure to take into account the relatedness of the species included in the original analysis.

Summary of problems

The analyses we have performed show that RMR is not a good proxy for daily energy demands when employed at the level of individual species. This is because the ratio of RMR to DEE can vary between 1.6 and 8.0. Unfortunately, there are fewer species for which we have data on DEE directly measured by the doubly labeled water method than on RMR. Nevertheless, the negative relationship between RMR (per gram body mass) and body mass is paralleled by a similar

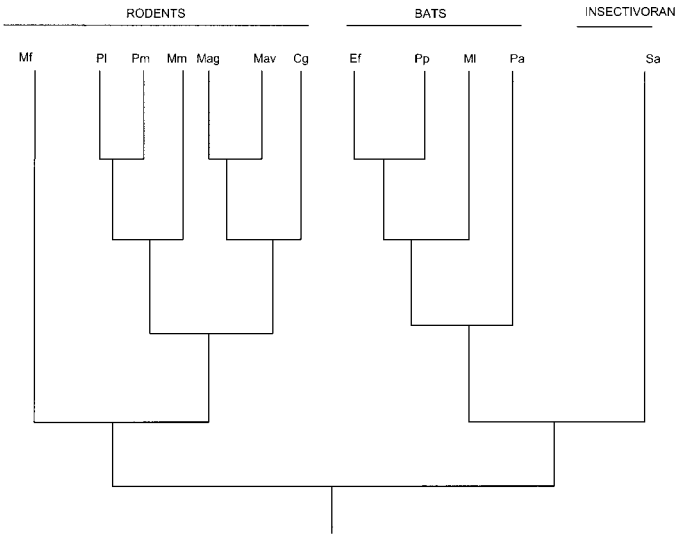

FIGURE 4 Phylogeny used to remove the effects of phylogenetic lack of independence in the plot in Figure 3.

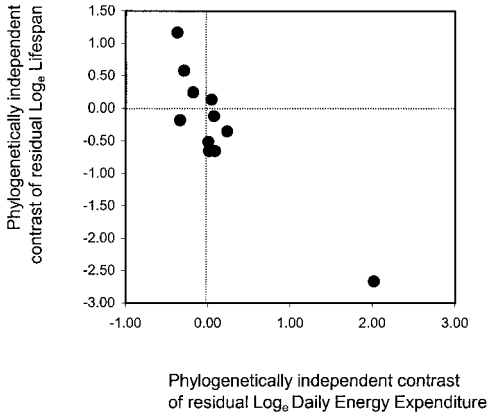

FIGURE 5 Phylogenetically independent contrast (PIC) of residual life span plotted against PIC of residual daily energy expenditure for 12 species of small mammal.

relation in measures of DEE (per gram body mass) and body mass. Consequently, there is an apparent association between DEE and longevity because the gradients of the scaling exponents to body mass are similar in magnitude but opposite in sign. This association could be a consequence only of the confounding effect of body mass. To remove the effect of body mass we scoured the literature for records of small mammals for which we have good data on their daily energy demands and also reliable estimates of their longevity. There were only 13 species that met our criteria for inclusion in this analysis, but across these species there was a strong negative association between residual DEE and residual life span. This association, however, is marred by the problem of a lack of statistical independence of the component species included in the analysis. When we removed the effect of phylogeny by calculating the PIC, the association was confirmed. Despite the four major problems that attend the original data that had been adduced to support the idea of the rate-of-living hypothesis, reanalysis of the data eliminating these problems reveals that there is still a statistical association between high rates of daily energy metabolism and shortened life spans. Small mammals that live fast really do die young.

More problems with the rate-of-living hypothesis

Although our new analysis of data concerning the issue of whether life span is linked to energetics provides some support for the idea that increased metabolic energy expenditure is associated with reduced life span, these data also contain information that indicates the reason underlying this association cannot be attributed to the simple rate-of-living ideas. This is because the theory predicts not only that the relationship between metabolic intensity and life span should be negative, but that the total amount of energy expended by each gram of tissue should be roughly constant and independent of the animal from which that tissue is considered. We calculated the lifetime expenditure of energy per gram of tissue for each of the 13 small mammals for which we had data (**Table 2**) and found that there was, in fact, a 10-fold variation in this trait: from 1480 kJ g^{-1} $life^{-1}$ in the yellow-bellied marmot (*Marmota flaviventris*) to over 16,000 kJ g^{-1} $life^{-1}$ for the little brown bat (*Myotis lucifugus*). Even across this relatively small size range and restricted taxonomic grouping, the rate-of-living theory does not appear to hold as a valid explanation for the differences in life span of these species, or the negative association between their daily energy demands and how long they live.

This comparison is made even worse if we start to consider a broader taxonomic framework. In particular, the birds (Aves) have combined elevated energy demands above those of small mammals (48) with an increase in their longevity (49–51). On average, birds appear to combine living fast with dying old (52). In contrast, analysis of the Marsupial mammals (Marsupialia) indicates that they combine their generally lower metabolic rates (resting) than equivalent sized Eutherians with shorter average life spans. Marsupials, it has been suggested (53), have evolved to live slow and die young. However, we should bear in mind the inadequacies of RMR as a proxy for DEE. Speakman (19), for example, reviewed the energy demands of small marsupials and concluded they do not have particularly lower DEEs despite their lower RMRs. Thus they may die young, but not necessarily combined with living slowly.

Selected examples from these two groups may serve to illustrate these general points. The Northern fulmar (*Fulmarus glacialis*) is a small oceangoing seabird from the tubenose family. Long-term monitoring studies of fulmars breeding on the island of Eynhallow in the Orkney islands (61°N) off the north of Scotland (UK), which were initiated by George Dunnet in the 1950s, have indicated that these 1.1- to 1.2-kg birds live for a very long time (54,55) relative to the expectation from Sacher (7) for the life span of an equivalent sized mammal (12 y). In fact, fulmars do not routinely even start to breed until they are 8–12 y old, and exceptionally may postpone the start of their breeding lives until they are well into their 20s (55). Several birds from the original cohort ringed at Eynhallow in the 1950s were still alive in the late 1990s (P. Thompson, personal communication, 2001) and calculations from life tables indicate that maximum longevity is probably in the region of 60–70 y. Fortunately, the fulmar is a species for which we have estimates of their daily energy demands using DLW (56). This estimate averaged 1444 kJ d^{-1}. Combined with the body mass and estimated life span this leads to an estimated lifetime energy metabolism of fulmar tissue of 26,400 kJ g^{-1} $life^{-1}$, about 60% more "living" than the highest

TABLE 2

Estimated maximum "amount of living" for the small mammals detailed in Table 1, expressed as maximum lifetime expenditure of energy (maxLEE) and lifetime expenditure of energy per gram of body mass (maxLee g^{-1})

Species	maxLEE *MJ* *life*$^{-1}$	maxLEE g^{-1} *kJ* *life*$^{-1}$ g^{-1}
Insectivora		
Sorex arenaeus	43.3	7220
Chiroptera		
Plecotus auritus	105.3	12,390
Pipistrellus pygmaeus	57.3	7540
Myotis lucifugus	138.7	16,410
Eptesicus fuscus	210.2	12,080
Rodentia		
Microtus arvalis	89.1	4460
M. agrestis	74.7	2480
Clethrionomys glareolus	95.0	4060
Peromyscus leucopus	156.7	7760
P. maniculatus	111.0	5390
Mus musculus	48.7	3480
Marmota flaviventris	4746.0	1480

"amount of living" in the sample of considered mammals in Table 2.

The second example is the marsupial sugar glider (*Petaurus-breviceps*), which weighs around 100–120 g. Their daily energy expenditure in the wild during the summer is around 150 kJ d^{-1} (57), although in winter the animals make extensive use of torpor. Field estimates of winter metabolism are not yet available. Assuming that the costs in winter during torpor are negligible and that torpor occurs on one-third of all days, then the average metabolic rate over an annual cycle is probably around 100 kJ d^{-1}. In captivity the maximum life span reported for a sugar glider is 8 y (2920 d). Using the same criteria used previously to estimate the longevity of the top 10% of the population, this can be reduced to around 2630 d. Accordingly, the lifetime expenditure of energy equals about 2320 kJ g^{-1} $life^{-1}$. This is at the lower end of the range established for eutherian mammals (Table 2) but is not particularly low. Hence, marsupials may have short lives and low metabolism, but not exceptionally so, consistent with the fact their DEEs are not significantly reduced below that of equivalent sized eutherian mammals in the size range below 4 kg body mass (19).

Overall, the variation in "amount of living" when considered across both the mammals and birds covers an approximate 20-fold range (=26,400/1480 kJ g^{-1} $life^{-1}$). This is well in excess of what would be anticipated if the rate-of-living theory was correct because this theory predicts the "amount of living" should be constant, and hence observed variability should reflect only the error variance in the component traits involved in its calculation: DEE, body mass and longevity. As we have already suggested, the error in group estimates of DEE is about 3–5%, group estimates of body mass might have an error of around 5% and estimates of maximum longevity are unlikely to be erroneous by more than about 20%. Even neglecting covariances, the maximum variation one might expect in the "amount of living"—were the rate-of-living theory correct—would be about 40%, only 1/50th of the actual observed range.

In the introduction we noted that the attractiveness of the rate-of-living theory was that it accords with our everyday experience of the behavior of mechanical objects. Our analysis has shown that animal species do not conform to this theory because they behave in a manner fundamentally different from that of mechanical objects. Why might this be the case? To understand why the range in "amount of living" is so vast in animals, it is necessary to consider the mechanisms by which animals wear out and ultimately die. One mechanism that enjoys widespread favor is the idea of physiological attrition, leading to aging and death, is a consequence of damage induced by oxygen free radicals generated during oxidative metabolism (58,59). This hypothesis is attractive because it forms a link between the rate-of-living theory and more evolutionary based ideas about the aging process (e.g., the disposable soma hypothesis).

During oxidative phosphorylation electrons from reduced substrates are picked up by ubiquinone (Q) on complex 1 of the mitochondrial membrane. As these electrons are passed from complex 1 down the cytochromes the released energy is used to pump protons across the inner mitochondrial membrane creating a protonmotive force. Finally, in complex 4 the electron combines with a proton and oxygen to form water. The hydrogen ions pass back across the membrane via ATP synthase, resulting in the generation of ATP from ADP and inorganic phosphate, although occasionally protons leak back through the membrane without the creation of ATP, either as a membrane leak or via a specialized protein called an uncoupling protein (UCP), which allows the proton to pass uncoupled from the generation of ATP, but resulting in release of the stored energy as heat. Occasionally, however, this process goes wrong and the oxygen reacts with a reduced form of Q, called ubisemiquinone (QH), which results in generation of a superoxide free radical (O_2^-) (60–63).

The superoxide radical is reactive, but can be detoxified in a series of reactions, which convert it into intermediary radical species (hydrogen peroxide) and ultimately to water. This detoxification is catalyzed by a number of antioxidant enzymes (superoxide dismutase, glutathione peroxidase and catalase). Each of these steps, however, produces radical species that may escape the detoxification process and cause cellular damage (59). In addition to the free-radical scavenging enzymes, the radical oxygen species can be also detoxified by reactions with antioxidants. These include dietary components such as vitamins E and C, as well as other micronutrients such as folate, and carotenoids and endogenous antioxidants such as glutathione. Despite this armory of defense mechanisms, some of the radical oxygen species slip through the net and cause oxidative damage to macromolecules such as fats, proteins and DNA. Animals, however, have one last line of defense, and that is to repair the damage that occurs, in particular to DNA, using repair enzymes (64), but also to other macromolecules such as proteins by refolding them, or tagging them for disposal and recycling of their constituent amino acids. The so-called heat-shock proteins appear to play a critical role in these repair and regeneration processes (65,66).

The importance of free-radical damage for animals can be appreciated by considering transgenic mice, which lack mitochondrial manganese-dependent superoxide dismutase (67,68). These mice survive for only 30–40 d before they succumb to accumulated oxidative damage, particularly in the brain, where a form of spongiform encepalopathy develops (67,68). This phenotype can be partially rescued by administration of catalytic exogenous antioxidants, demonstrating the importance of oxidative damage in the shortened life span and tissue damage (68). The free-radical damage hypothesis thus provides a mechanism for the rate-of-living theory, because long-term energy demands must be met by hydrolyzing ATP, which originates in the mitochondria, primarily as a result of oxidative phosphorylation. The more ATP that is required, the more oxygen that must pass through the mitochondria and the more oxygen radicals that are likely to be generated, and all things being equal, the more oxygen radicals that are generated, the greater will be the tissue damage. The critical difference that separates the behavior of animals from machines, in this context, are the phrases "likely to be" and "all things being equal" because in real animals they are not necessarily "likely to be" and generally "all things are not equal."

Different species of animals differ in many aspects of the free-radical generation, protection and repair processes. For this reason, although it is generally true that greater levels of oxygen demand might lead to greater amounts of free-radical production, some species have evolved mitochondria where the generation of radicals per gram of oxygen utilized is very much reduced (69,70). Species may differ in the levels of activity of the major scavenging enzymes, levels of endogenous and exogenous antioxidants, and may also differ in the effectiveness of their repair and degradation systems. Why these differences between species might evolve has been addressed by the disposable soma hypothesis (27–29), which places the mechanistic aspects of aging into an evolutionary framework. The transgenic mouse lacking superoxide dismutase clearly demonstrates that animals do need to protect themselves against oxidative damage. The disposable soma hypothesis

postulates that animals will evolve mechanisms to defend themselves against the levels of tissue damage that they sustain, in relation to the extrinsic expectation of mortality. For example, if an animal has a high extrinsic rate of mortality, such that 98% of the population is dead within 345 d (see above), natural selection will not favor the evolution of an antioxidant defense and repair mechanism that would allow the animal to survive for 80 y. Implicit in this argument is the idea that defense and repair mechanisms have costs associated with them as well as benefits. The costs of an antioxidant defense system to enable an animal to live for 80 y will be worth paying for only if the animal lives in an environment where it stands a chance of living that long despite extrinsic mortality rates; thus no animals have ever evolved immortality because they have all evolved in environments with nonzero levels of extrinsic mortality. Dawkins (71) paints an interesting picture of how we might behave if we were immortal, never performing behaviors like crossing roads, for example, because the risks of this behavior would be unacceptable. However, the reality is that as long as we do perform behaviors that carry such risk, immortality will never evolve.

By removing animals from the environments in which they evolved into environments with minimal extrinsic mortality risks (i.e., captivity) we observe the legacy of an evolved system that aims to sustain them from oxidative damage for the duration of their "expected lives." Evolution cannot engineer a system that protects the animal from oxidative damage for its expected life span, but then catastrophically fails, resulting in the animal's immediate death once this duration is exceeded. The legacy of this inability of evolution is that animals released from their natural environment show protection from damage over the "expected life span" but then a gradual accumulation of damage and physiological attrition occurs, such that systems start to degenerate in a stochastic manner over time. Aging does not result in a given "cause of death" because the system that fails first is largely a matter of chance. Although we have relatively few observations on which to base an estimate, it appears generally that the maximum life span sits at around three to four times the "expected life span" under the conditions in which the animals evolved. Hence, long-lived humans survive to between 90 and 120 y, but estimates of their original life span during the period when humans first evolved are around 30 y. Similarly, estimates of the expected life spans of small mammals such as mice and voles indicate maximum life expectancies in the wild of the order of 9–12 mo (30,31), and maximum longevities in captivity of 2.5–3 y (30,31). Thus, under the disposable soma hypothesis aging and death are simply by-products of selection for protection under the pressure of extrinsic mortality expectations.

Drawing together these three theoretical treatments of aging provides a useful platform on which to build our understanding of the interrelationships between energy expenditure and longevity. In general, there is a trend for those species that consume the greatest amount of oxygen also to be those that sustain the greatest damage and also die faster (Figs. 3, 5), consistent with the rate-of-living theory and the free-radical damage hypothesis. However, this relationship is not perfect and the calculated "amounts of living" of different species are very different, both of which are inconsistent with the rate-of-living theory. This imperfection can be understood in the evolutionary context provided by the disposable soma hypothesis. Animals that have low extrinsic rates of mortality (such as those that have evolved flight) will evolve low radical production per mL oxygen consumption, greater radical scavenging defenses and higher levels of repair and, thus, tend to live long lives for the amounts of oxygen they produce (e.g., birds and bats), whereas animals with high extrinsic rates of mortality show the converse (e.g., most small terrestrial mammals).

There is considerable evidence claimed to support this generalized framework. Long-lived mammals, for example, have low levels of reactive oxygen species (ROS) production per mL O_2 consumed (72). If the levels of antioxidant enzyme activity are expressed as a ratio of the level of oxygen consumption, the resultant ratio is proportional to life span (73–75). Birds also have low ROS production per mL O_2 consumed (49,76–78), consistent with their generally elongated life spans. Measures of DNA repair correlate to life span (79) and, finally, in cell cultures derived from tissues taken from live animals the resistance to experimentally induced oxidative stress varies with life span of the donor species (80,81).

We should, however, air a note of caution about this supportive evidence. This is because almost all of the above-noted comparative studies have ignored the problem of lack of independence in the use of species as the sampling unit for comparative analysis [except the study of Kapahi et al. (80)], and most have ignored the confounding effects of body mass on the salient traits. To reiterate, any trait that varies with body mass will also vary with life span. For example, take the levels of activity of the enzyme glutamate oxaloacetate transaminase. This enzyme is involved in transamination of glutamate. We have no reason to believe that this enzyme is linked to aging. Yet its scaling to body mass about $M^{0.83}$ (82) results in a strong association between this enzyme activity and life span ($\sim M^{0.54}$). Animals with high levels of glutamate oxaloacetate transaminase live longer lives, although this result is a trivial by-product of the covariance with body mass.

INTRASPECIFIC COMPARISONS

Comparisons between species have several problems associated with them: lack of phylogenetic independence, the confounding effect of body mass and the disruptive effects of ecology on variation in defense and repair. Perhaps, then, examining the relationship between energetics and longevity within species might provide a much more favorable paradigm within which to test the rate-of-living ideas. Indeed, a close reading of the theory reveals that the original idea is actually not testable, given that it postulates that if a given individual expends energy at a greater rate, it will die earlier. Because animals cannot be resurrected to live their lives over at different rates, this is unachievable. The closest we might come, however, is to examine responses across individuals within a given species where presumably the levels of defense and repair will be set at evolutionary norms and relatively invariant across individuals. There is less of a phylogenetic independence issue (particularly if the animals are an inbred strain) and differences in body mass between large and small individuals will be orders of magnitude lower than that in most comparative studies across species. Studies of the effects of metabolic rate on longevity within species have included two distinct types of study. In the first, animals are experimentally manipulated to make them expend more (or less) energy and the consequences of these manipulations on subsequent life span or survivorship are examined. The benefits of such studies are clear, in that experimental manipulations allow us to establish causality. The second type of study relies on correlations between interindividual variations in energy metabolism (without manipulation) and life spans. These latter studies have been less frequently performed.

Experimental studies

Studies in which animals are forced to work harder and hence elevate their rates of energy expenditure are common. Relatively few include explicit measures of the impacts of the manipulation on energy demands, and consequently it is possible that because of compensatory changes in other components of the daily energy budgets some of the manipulations where costs were not quantified may not have resulted in an increase in total daily energy expenditure. An example of a study in which costs were quantified are the manipulations of kestrels (*Falco tinnunclus*) feeding their offspring (83,84). In these elegant experiments kestrel nesting boxes were mounted on the side of a shed where the experimenters could open a hatch door onto the back of the nest, and remove any prey delivered to the offspring once the parents had left, supplying a small amount of food (ground beef) to the chicks to ensure they survived. Because they were hungry the chicks kept begging for food, and this stimulated the parent birds to increase the amount of time they spent in energetically expensive foraging flights (83,84). Measurements of actual energy demands by doubly labeled water revealed that the increased flying elevated demands by about 40% (83,84). The consequence of this greater work rate was that these birds were less likely to return the following spring, which generally indicates over-winter mortality had occurred. An interesting aspect of these studies is that mortality was delayed in time compared to the manipulations, indicating the effects have a physiological dimension rather than being purely ecological, that is, the birds did not die during the manipulations because they were more likely to break a wing when out foraging, or get predated themselves. These observations match exactly the expectations from the above synthesis of the rate-of-living, free-radical damage and disposable soma ideas.

Another independent example is the work of Wolf and Schmid-Hempel (85) on honey bees (*Apis mellifera*). In this experiment worker honey bees were fitted with small weights on their backs to increase the energy demands of flying. Aerodynamic calculations suggest the cost of flight should increase dramatically with the weight of load carried by a flying animal (86). Although the actual costs were not quantified, the result of the manipulation was a negative relationship between loading and life span. This accords with the theoretical expectation, although recent studies on flight costs in birds that varied in their natural body masses did not find a strong association between body mass (= wing loading) and energy expenditure (87), perhaps calling into question the nature of the manipulation effect. The direct effects of carrying artificial loads may, however, differ from the effects of natural differences in body mass.

Manipulating costs downward is more difficult. Lyman et al. (88) found that hamsters (*Phodopus sungorus*), allowed the opportunity to reduce their metabolic rates by entering hibernation, had increased life spans relative to those that did not hibernate. However, this is really an elevation rather than a reduction experiment. The natural condition for a hamster is to hibernate, the experimental condition is to prevent it, rather than allow it, and the life span is not elevated under reduction, but rather shortened under elevation. Probably the best example of a reduction in metabolism is the manipulation of house flies (*Musca domestica*) by placing them into mazes of drinking straws or cardboard, where they were unable to engage in flight behavior (89,90). The flies that were prevented from engaging in the high-cost flight activity had metabolic rates (oxygen consumptions) that were much reduced compared with that of unrestricted flies and they lived three times longer.

Although in many manipulations of this type the consequences for energy expenditure were not quantified directly, the overall trends in such studies are very clear: increasing energy expenditure leads most frequently to a decrease in survivorship, both in the wild and the laboratory (eliminating the role of predation in this effect). Although occasionally these manipulations have no effects [e.g., the rats forced to stand in cold water 8 h/d (88)], they are generally not associated with elevated survival. In contrast, reducing expenditure has the opposite effects (either elevating survival or having no effect, but not reducing survival). These experimental manipulations strongly support the model for the association between energy demands and longevity presented above. Experimental manipulations that result in living faster generally also result in dying sooner, and the converse is also true.

Caloric restriction

No discussion of the link between energetics and life span could be complete without some mention of caloric restriction studies, not least because this is the only intervention to date that appears to successfully result in increased life span (92). Because there have been several recent reviews of this area (e.g., 93–95), we will focus this discussion only on highlighting some of the issues that attend the problem of whether the effect of caloric restriction on aging is mediated via a modulation of energy expenditure. Caloric restriction has two main effects. Energy intake is reduced (by definition) and by consequence the total daily energy demands are also reduced, so that the two come into balance; however, body mass also reduces. The key question is what the extent of reduction in body mass is relative to the reduction in overall metabolism because this defines whether the rate of energy expenditure per gram of tissue (the metabolic intensity) also declines, remains constant or even increases. This is further complicated by the fact that when animals are placed under energy restriction, they do not uniformly decrease the mass of all their body tissues. Under initial restriction there are profound reductions in the mass of metabolically active components (such as the liver and gut), but under further restriction the body component that is most reduced is fat (96), and this component has a relatively low metabolic rate.

These body mass changes over time introduce a level of complexity into the analysis that is thrown into further confusion by the fact many researchers have recognized the importance of changed body mass and have attempted to "correct" for this mass change in their expressions of metabolic rate (97–100). This correction has been attempted in two dominant ways. First, by expressing the metabolic rate divided by mass raised to an exponent (normally 0.75 or 0.67). The justification for this approach is that differences in body mass between species scale with a gradient of approximately 0.75 (2,3). The argument for using this correction, however, is extremely dubious. Differences between species that generate the 0.75 scaling exponent reflect altered body size and dimensions over orders of magnitude. The reasons for the scaling exponent of 0.75 for energy expenditure have been the matter of considerable debate (101–103), but at least one of the contributing factors is the scaling effect is not in the ratio of bone to lean tissue mass, because larger species require disproportionately greater bone masses to support their bodies under gravity (102). It is clear that the processes responsible for the 0.75 exponent bear no relation to the processes that cause altered body mass when an individual animal is placed under

energy restriction (104), where bone tissue mass actually increases as a proportion of body mass as animals get lighter, because bone tissue is preferentially spared compared with fat and lean tissue components. Consequently, utilizing an exponent of 0.75 to "correct" for body mass effects may generate a spurious indication of an effect of restriction on metabolism (or lead to an erroneous rejection of an actual effect). Similar problems attend the use of the other common scaling exponent used in these studies of 0.67, which addresses interspecific surface area changes with size.

A second common way to correct for body mass changes in caloric restriction studies is to express the metabolic rate in relation to the changes in lean body mass. The basis of this argument is that fat tissue has a substantially lower metabolic rate than that of lean tissue. However, whereas fat tissue has a substantially lower metabolic rate than that of lean tissue in vitro, the magnitude of the effect in vivo is less apparent (105,106). Yet, expressing metabolism divided by lean body mass makes the assumption that fat tissue has *no* metabolism, which is clearly erroneous. This can lead to some spurious inferences when large changes in body composition occur. For example, if a mouse consisted of 30 g of lean tissue and 10 g of fat, and the lean tissue expends energy at 30 mW/g but the fat tissue expends energy at only 10 mW/g, the total metabolic rate would be 1 W. The expenditure per gram of lean tissue would be 33 mW/g LBM. If the animal lost the 10 g of fat, its metabolism would fall to 900 mW and its body mass would fall to 30 g, all of which would be lean tissue. The whole animal metabolic rate per gram of lean tissue would fall to 30 mW/g LBM (10% reduced compared to the initial conditions). In contrast, the metabolic rate per gram whole body mass would change from 25 to 30 mW/g, with the same weight change, an increase of 20%. If we employ the commonly used interspecific scaling exponents, then metabolism divided by mass raised to the 0.75 power results in an initial metabolic rate of 62.8 kJ $g^{-0.75}$, which changes to 70.2 kJ $g^{-0.75}$ (an increase of 11%). Using the other commonly used scaling exponent of 0.67 gives values of 84.4 kJ $g^{-0.67}$ before weight loss and 92.1 kJ $g^{-0.67}$ afterward (an increase of 9%). Yet, clearly, the metabolic rate of the component tissues has remained constant and the perceived changes, which vary between a reduction of energy demands by 10% to an increase by 20%, are only an artifact of the assumptions built into the metabolic rate calculations.

Given variations in the underlying causes of the mass change over time, and differences in the manner in which metabolic rate is "corrected" for the body mass effects, it is not surprising that the literature on the effects of caloric restriction on energy demands is replete with confounding effects on metabolism [reviewed in Ramsey et al. (95)]. Greenberg and Boozer (107) utilized a variety of different methods to correct for body mass effects during caloric restriction in rats. This study indicated that expressing metabolic rate in relation to the summed mass of the main organs (heart, brain, liver and kidneys) was the most reliable way to understand (and correct for) effects of changes in body mass on metabolism. They suggested that, given that previous studies of the effects of caloric restriction on metabolic rate had all expressed changes in metabolism in relation to body mass raised to some exponent (0.75 or 0.67) or in relation to lean body mass, the assertions derived from these prior studies are questionable. At present it is almost impossible to resolve whether caloric restriction exerts its effects by a reduction in metabolic rate or by some other mechanism.

Correlational observations

Only two studies have explored the relationships between the habitual rates of energy expenditure across individual animals and their ultimate survival. We measured the daily energy demands of 42 individually housed female mice (MF1 strain, outbred) at ages 6 and 13 mo and then sought associations between these individual variations in expenditure and longevity (105). Against the expectations of predictions based on all the foregoing evidence, we found that the relationship between daily energy demands (at ages 6 and 13 mo) and life span was positive [life span (d) = 111 + 5.8 DEE (kJ d^{-1}), F = 5.05, df = 1,40, $P = 0.03$, $r^2 = 0.112$]. The mice that lived fastest actually lived the longest! This was not an artifact of correlations of both energy demands and life span with body mass, because the association between mass and food intake was significant but weak, and the relation to longevity not significant. Indeed, using residual energy demands or metabolic intensity (kJ g^{-1} body mass) actually strengthened the relationship between energy demands and life span (**Fig. 6**) [life span (d) = −4.1 + 270.4 metabolic intensity (kJ g^{-1} d^{-1}), F = 7.68, $P = 0.008$, df = 1,40, $r^2 = 0.161$]. It is important to note that not only was the direction of the effect unexpected, but that this was not a trivial effect. The difference in the mean life span between the top and bottom 25 percentiles of the distribution of daily energy expenditures was 38%. There is only one other study that has replicated this work to date, and that study has confirmed a positive association of expenditure and longevity in hamsters (M. Okleiwicz et al., unpublished data, 2001).

There is an indication therefore of a fundamental difference between the experimental manipulation studies and the studies based on correlations. The nature of these differences is currently uncertain but we have two working hypotheses. First, the major component of energy expenditure in a captive animal is the cost of resting, given that activity and thermoregulation costs are generally minimized by the housing conditions. In a wild animal, however, the major contributor is the cost of activity (Fig. 2), particularly if expenditure has been artificially elevated by forcing the animals to work harder [e.g., Daan et al. (84)]. These data suggest that it is not only the level of energy demand that is an integral aspect of the link between energetics and aging, but also the manner of expenditure. The implication is that nonresting expenditure might be bad but resting expenditure might be protective. Unexpect-

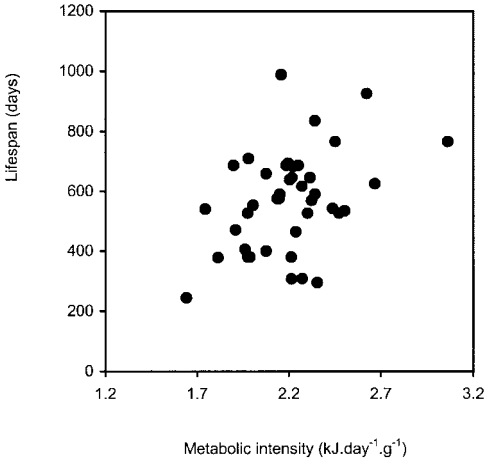

FIGURE 6 Metabolic intensities of 42 MF1 mice at age 6 mo against individual life spans.

edly, this conclusion flies in the face of almost every public health message currently being broadcast.

A salient question then is why these two modes of expenditure might exert "radically" different effects on life span. When animals exercise they use the increased energy they expend to fulfill a given role (e.g., contraction of muscles). This uses ATP and increases oxygen consumption to generate ATP by oxidative phosphorylation. There is a considerable weight of evidence that this increased oxygen consumption leads to elevated rates of oxidative stress and stress-induced damage to both protein and DNA (65,66). This effect is consistent with a negative effect of the elevations of such activity on life span. In contrast, the function of energy demands at rest remain obscure. It is widely agreed, however, that three components contribute most to the RMR: the proton leak in mitochondria (63,108–111), the costs of sustaining ion gradients by sodium potassium pumping (112,113) and protein synthesis. Within this framework there are at least two mechanisms by which elevations in RMR might be associated with decreases in oxidative damage. The first is the direct cost of sustaining all the protection and repair mechanisms, most of which are enzymes that need to be synthesized. To date nobody has attempted to correlate individual variations in the capacities of the defense and repair systems with differences in energy metabolism in the resting state. Consequently, the contribution of these effects to the substantial interindividual variation in RMR remains unknown. However, it is interesting to note that one of the assumptions underpinning the disposable soma hypothesis (27–29) is that there is a significant cost associated with the processes of protection and repair that is traded off against other processes. Perhaps what our study (105) and that of Okleiwicz et al. demonstrate is the existence of this "cost."

Another hypothesis, however, is that the elevated oxygen demands at rest do not reflect direct costs of protection and repair but rather are part of a mechanism to reduce the production of free radicals in the first place. As we noted above oxygen radicals are often formed when oxygen combines with ubisemiqunone (QH). The rates of free-radical production per mL of oxygen consumed depend on the levels of QH in the mitochondrial membrane. A key factor influencing the levels of QH is the magnitude of the protonmotive force across the mitochondrial membrane. At high levels of external protons there is lots of QH and free-radical production is high. Animals can reduce the levels of protonmotive force by increasing the extent of uncoupling in their mitochondria. To continue to generate ATP requires elevated oxygen consumption, although the net production of free-radical species is diminished. The animals uncouple respiration to increase their survival (63). This effect is diametrically opposed to the prevailing notion that increasing uncoupling should lead to an increase in free-radical production because of the elevated oxygen consumption (95). Our data are consistent with a protective effect of uncoupling respiration and, consequently, our current efforts are directed at resolving whether those MF1 mice with high-energy expenditures have more uncoupled mitochondria, or elevated levels of protection and repair processes.

In summary, the novel analyses we have performed, which for the first time link together total daily energy demands measured by DLW with life-span differences between species, accounting for the differences in body mass and ensuring phylogenetic independence of the comparisons, confirms that in small mammals a high-energy expenditure is associated with a shorter life span. However, this relationship is not because of a simple rate-of-living explanation, because across species there is a 20-fold difference in the energy expended per gram of tissue per lifetime. Within-species increases in forced expenditure generally cause reductions in life span, but natural variation between individuals in expenditure is associated with increased life span.

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