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On the complex relationship between energy expenditure and longevity: Reconciling the contradictory empirical results with a simple theoretical model

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ABSTRACT

The relationship between energy expenditure and longevity has been a central theme in aging studies. Empirical studies have yielded controversial results, which cannot be reconciled by existing theories. In this paper, we present a simple theoretical model based on first principles of energy conservation and allometric scaling laws. The model takes into considerations the energy tradeoffs between life history traits and the efficiency of the energy utilization, and offers quantitative and qualitative explanations for a set of seemingly contradictory empirical results. We show that oxidative metabolism can affect cellular damage and longevity in different ways in animals with different life histories and under different experimental conditions. Qualitative data and the linearity between energy expenditure, cellular damage, and lifespan assumed in previous studies are not sufficient to understand the complexity of the relationships. Our model provides a theoretical framework for quantitative analyses and predictions. The model is supported by a variety of empirical studies, including studies on the cellular damage profile during ontogeny; the intra- and inter-specific correlations between body mass, metabolic rate, and lifespan; and the effects on lifespan of (1) diet restriction and genetic modification of growth hormone, (2) the cold and exercise stresses, and (3) manipulations of antioxidant.

1. Introduction

The relationship between energy metabolism and longevity has been a central theme in aging studies since the late 1800s (Speakman et al., 2002). Despite more than a century of theoretical and empirical efforts, the understanding of this relationship is still controversial, and existing theories fail to reconcile the seemingly contradictory empirical evidence (Speakman et al., 2004). The oldest theory in the field—the rate of living theory (RLT) (Rubner, 1908) suggests that the rate of mass-specific energy expenditure (metabolic rate) is negatively correlated with longevity. This theory is supported by two lines of empirical evidence. The first comes from the interspecific scaling laws of metabolic rate and lifespan in wild animals (Speakman, 2005). The negative correlation between mass-specific metabolism and lifespan holds even after the confounding effect of body mass is removed (Speakman et al., 2002). The second line of evidence is that experimentally lowering body temperature, which decreases metabolic rate, extends lifespan of both ectotherms (Klass, 1977; Loeb and Northrop, 1917; McArthur and Sohal, 1982; Miquel et al., 1976; Partridge et al., 2005; Rose, 1994; Van Voorhies and Ward, 1999) and endotherms (Conti et al., 2006; Sohal et al., 2000).

Nonetheless, the RLT faces four types of challenges. First, the predicted correlation between energy expenditure and lifespan does not hold when comparisons are made across taxons. A typical example is that birds have higher metabolic rate than mammals with the same body mass, yet live much longer. Second, RLT also fails to explain why within a species, such as domestic dogs, the larger breeds with lower mass-specific metabolic rates, usually have shorter lifespans (Speakman et al., 2003). Third, a few lifespan extending interventions, such as diet restriction (DR) and genetic modification (GM) of growth hormone, generally do not alter, or only slightly reduce, mass-specific metabolic rate (Brown-Borg, 2003; Hou, 2013; McCarter et al., 1985; Merry, 2002; Westbrook et al., 2009). Moreover, a few studies even showed that when metabolic rate is altered by DR, it is positively correlated with lifespan (Cooper et al., 2004; Houthoofd et al., 2002; Liao et al., 2011; Lin et al., 2002; Roark and Bjorndal, 2009). The fourth challenge comes from experimental manipulations that increase metabolic rate, but do not shorten lifespan. For example, long-term cold expo-

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sure largely increases energy expenditures in mice (Vaanholt et al., 2009), rats (Holloszy and Smith, 1986), and voles (Selman et al., 2008), but has no effects on lifespan. Moreover, voluntary exercises increased food intake in female rats while increasing lifespan (Holloszy, 1993).

The oxidative stress theory of aging (OST), another theory that links energy metabolism and longevity, suggests that the deleterious productions of oxidative metabolism (e.g., reactive oxygen species, ROS) cause various forms of molecular and cellular damage, and the accumulation of the damage is associated with the process of aging (Balaban et al., 2005; Barja, 2004; Hulbert et al., 2007; Sohal et al., 2002). Widely considered by many researchers as a modern version of the RLT at the molecular and cellular level, this theory shares all the supports and challenges of the RLT, as well as a few of its own. New sources of supports include the evidence that (1) external oxidative insults shorten lifespan, (2) the level of oxidative damage to macro-molecules increases with age, and (3) genetic interventions and diet restriction, while extending lifespan, reduce the oxidative damage (Bokov et al., 2004; Muller et al., 2007). New challenges to OST mainly come from the studies, in which adding antioxidants to diet (Bjelakovic et al., 2007; Ristow et al., 2009) or genetically altering the expression of antioxidant enzymes (Pérez et al., 2009; Van Raamsdonk and Hekimi, 2012), which were assumed to change the oxidative damage, failed to affect longevity. In some cases these interventions even yielded results that opposed the theory's predictions (Bjelakovic et al., 2007; Ristow et al., 2009).

The controversial correlation between energy, metabolic rate, and longevity has been considered a long-standing question (Balaban et al., 2005; Brys et al., 2007; Hughes and Reynolds, 2005; McCarter et al., 1985; Pérez et al., 2009; Speakman et al., 2004; Stuart and Brown, 2006). Here we suggest that in considering this question the detailed energy tradeoffs between life history traits and the efficiency of energy utilization have been largely ignored, which, we hypothesize, are the keys to understanding the complex nature of the energy longevity correlation. In this paper we present a simple quantitative model driven by this hypothesis, and use the model to reconcile a series of seemingly contradictory empirical results on the relationship between energy metabolism and longevity.

2. Methods

2.1. The conceptual framework of the theory

We illustrate the framework of the theory in Fig. 1. The oxidative damage producing process starts from the overall energy expenditure (measured as oxygen consumption rate). Under many circumstances, energy expenditure is proportional to the production rate of ROS, which is in turn proportional to the net oxidative damage. Assuming that the net oxidative damage is the cause of aging and the determinant of lifespan, in these cases there is a direct and simple link between lifespan and metabolic rate. However, as shown in Fig. 1, two factors, namely antioxidant scavenging and damage repair mechanisms, can alter the damage level (the output of the process) while keeping the energy expenditure rate (the input) roughly unchanged (Bokov et al., 2004; Sohal et al., 2002). Scavenging ROS is carried out by a series of anti-oxidative enzymes, such as superoxide dismutase (SOD), peroxidredoxin (Prx), and glutathione peroxidase (GP), and non-enzymatic antioxidants such as vitamins, (Balaban et al., 2005). Non-scavenged ROS causes damage to lipid, DNA, and protein. Organisms have evolved highly efficient mechanisms to repair the damage, such as removal of peroxidized acyl chains from phospholipids (Hulbert et al., 2007), DNA double strand break or base excision repair (Madhusudan and Middleton, 2005), and methionine sulfoxide repair (Stadtman,

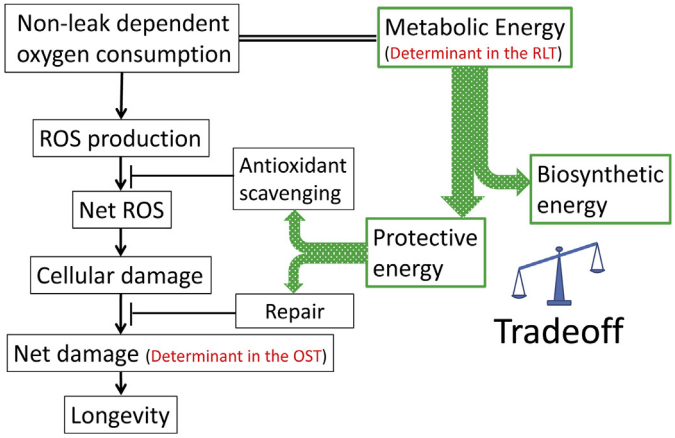

Fig. 1. Schematic illustration of the oxidative damage producing process. The discussion of this process in this paper starts with the non-leak dependent oxygen consumption, which is equivalent to the metabolic energy available to the animals, and is the determinant of lifespan in the rate of living theory (RLT). The metabolic energy is partitioned between the energy for protection and the energy for biosynthesis. The energy for protection and the efficiency of utilization of it determine the overall protective effects of radical scavenging and cellular damage repair. The net damage, which may or may not be proportional to oxygen consumption, is the determinant of lifespan in the oxidative stress theory (OST).

2006). Enhancing or weakening these two factors can result in a nonlinear correlation between net cellular damage level and oxygen consumption, and therefore a complex relationship between energy expenditure and longevity. The nonlinearity between damage and oxygen consumption may also be partially attributed to the incomplete mitochondrial coupling due to proton leak and electron leak, which causes a fraction of consumed oxygen not to produce ROS (Barja, 2013; Brand, 2000). But, as we discuss in Appendix A, if these two factors are kept fixed, incomplete coupling alone cannot fully explain the disproportionality between oxygen consumption and net damage level (Appendix A). Thus, in this paper our discussion starts from the non-leak dependent oxygen consumption as shown in Fig. 1.

We need to emphasize that the protective mechanisms of anti-oxidative scavenging and damage repair require energy. So, the overall protective efficacy depends on the amount of energy allocated to these mechanisms and the efficiency of energy utilization for this purpose (Hou, 2013, 2014; Kirkwood, 1990; Kirkwood and Holliday, 1979). Thus, we hypothesize that there are two ways to enhance the protection.

The first way is to allocate more energy to protection. More energy for protection does not necessarily require an increase in overall energy expenditure. Some lifespan extension interventions can reshuffle the energy allocation and induce tradeoffs between protection and other life history traits. One of the most important traits that is often manipulated to tradeoff with protection is biosynthesis during growth. For example, when growth is retarded by diet restriction or genetic modification of growth hormone, the energy requirement for biosynthesis is reduced accordingly. Thus, animals can channel extra energy to protection without increasing the overall metabolic energy pool, and therefore enjoy a longer lifespan (see quantitative details in Section 3.2). We will show that the biosynthetic energy associated with growth rate also has significant effects on oxidative damage profile over ontogeny and the inter- and intro-specific relationship between lifespan and body mass.

The second way is to enhance the protective efficiency, so that one unit of the energy is associated with less molecular damage. Protective efficiency can be altered by experimental manipulations, such as down- or up-regulating genes for antioxidant enzymes

(Pérez et al., 2009), or altering the structures of molecules, such as the fatty acid composition of membranes, to change their vulnerability to oxidative insults (Hulbert et al., 2007). Again, when the efficiency is altered, the same amount of the overall energy expenditure will lead to different levels of cellular damage.

In the RLT, the overall energy expenditure is the determinant of longevity, whereas in the OST, the determinant is the net cellular damage (Fig. 1). As discussed above, because energy allocation and protective efficiency can both change in a variety of situations, these two determinants are not simply proportional to each other, and the link between longevity and energy expenditure is far more complex. Thus, we argue that the OST is not merely the modern version of the RLT at the cellular and molecular level. In Section 2.2 we introduce a simple quantitative model based on the qualitative framework described in Fig. 1. Using a few measurable physiological parameters, the model explicitly quantifies how energy tradeoffs and protective efficiency affect animal lifespan, and highlights how biosynthesis plays a role in oxidative damage accumulation and the process of aging.

2.2. The quantitative energy model

Previously we developed a theoretical model for understanding the effects of diet restriction and genetic modification of growth hormone on extending lifespan. The model also explains the complex relationship between compensatory growth and lifespan. The quantitative predictions by the model and the empirical tests of them have been published (Hou, 2013, 2014; Hou et al., 2011a). Here, we briefly summarize the model, and further extend it for the more general purpose—understanding the relationship between energy expenditure and longevity and explaining the empirical results listed in Table 1.

During growth, the total metabolic rate, B, is partitioned between the rate of energy required to maintain existing biomass, B_{maint}, the rate of energy required to synthesize new biomass, B_{syn}, and the rate of energy spent on activities, B_{act} (such as foraging) (Hou et al., 2008; West et al., 2001), i.e., $B = B_{\text{maint}} + B_{\text{sys}} + B_{\text{act}}$. The total metabolic rate is usually a constant multiple of the active metabolic rate, i.e., $B = (f-1) \times B_{\text{act}}$, where f is a dimensionless constant, usually ranging between 2 and 3 (Hou et al., 2008). The maintenance term (B_{maint}) includes the energy spent on the protective mechanisms, such as oxidant scavenging and damage repair. The synthetic term (B_{syn}) can be expressed as $B_{\text{sys}} = E_{\text{m}}\text{dm/dt}$, where dm/dt is the growth rate (change in body mass, m, per unit time, t), and E_{m} is the metabolic energy required to synthesize one unit of bio-tissue, such as the energy for assembling macromolecules from monomers (Hou et al., 2008).

We make three assumptions to estimate the oxidative damage and to link it to lifespan.

Assumption 1. The rate of damage production, H, caused by oxidative metabolism is proportional to the rate of oxygen consumption (metabolic rate, B). The relationship between oxygen consumption (metabolic rate) and ROS generation is complex when comparison is made across taxon, but within a taxon, especially within a species, they are shown to be proportional to each other after taking proton and electron leak into account (see detailed review in Appendix A and Hou, 2013). Before ROS scavenging and damage repair, we assume that the gross damage production rate is in turn proportional to the metabolic rate. Thus, we have the rate of damage accumulation (damaged mass/time), $H = \delta B$, where δ is a constant within a taxon, indicating the amount of damaged mass associated with one unit of metabolic energy. Metabolic rate (B) usually scales with body mass allometrically, $B = B_0 m^{\alpha}$, where B_0 is the normalization coefficient, and α is the scaling power.

Assumption 2. Scavenging radicals and repairing the cellular damage requires metabolic energy. The rate of scavenging/repair, R, is proportional to the energy available for maintenance (repairing damage), B_{maint}, with a coefficient η, i.e., $R = \eta B_{\text{maint}}$, where η is also a constant within a taxon.

The net damage, $H - R$, accumulates. The accumulated damage can be estimated as a function of time, i.e., $\int_0^t (H-R)\,d\tau$. Using the relationships, $B = (f-1)B_{\text{act}} = B_{\text{maint}} + B_{\text{sys}} + B_{\text{act}}$, $B_{\text{sys}} = E_{\text{m}}\text{dm/dt}$, and $B = B_0 m^{\alpha}$, we obtain the net mass-specific cellular damage (per body mass),

$$D(t) = \int_0^t \left(\delta \times B - \eta \times B_{\text{maint}}\right) d\tau / m(t) \approx (1-\epsilon) B_{\text{m}} \times t + \epsilon E_{\text{m}} (M - m_0)/m(t) \quad (1)$$

where $B_{\text{m}} = B_0 M^{\alpha}/m(t)$ is the mass-specific metabolic rate; $\epsilon = \eta/(f\delta)$ is the protective efficiency, indicating the ratio of scavenging/repair and gross damage for one unit of energy, M and m_0 are adult and birth mass respectively, and E_{m} is the energy required

Table 1
The empirical results on the relationship between energy metabolism and longevity that are reconciled by the model presented in this paper.

Empirical results that support the RLT and the OST

Empirical evidence	Tests of the model
Cellular damages increase with age	Semi-quantitative
Damage increases faster during growth, and then slows down during adulthood	Semi-quantitative
Lowering body temperature extends lifespan of free-feeding animals	Qualitative
Lifespan scales with body mass across species within a taxon	Quantitative

Empirical results that seemingly contradict the RLT and the OST

Empirical evidence	Tests of the model	Model's explanation
Diet restriction and genetic modification extend lifespan without changing metabolic rate	Quantitative	Energy tradeoffs
Under diet restriction, higher metabolic rate leads to longer lifespan	Qualitative	Energy tradeoffs
Intra-specifically, larger animals with higher metabolism live shorter	Quantitative	Energy tradeoffs
Cold exposure and mild exercises increase energy expenditure, but not alter lifespan in mammals.	Semi-quantitative	Alterations of protective efficiency and energy tradeoff
Increasing antioxidant levels extends lifespan in *Drosophila*, but not in mice	Qualitative	Alterations of protective efficiency
Down-regulating genes for antioxidants failed to shorten lifespan in mice and nematodes	Semi-quantitative	Alterations of protective efficiency

to synthesize one unit of biomass The detailed calculation of the integral is available in (Hou, 2013) and (Hou et al., 2011a). The first term in Eq. (1) is the mass-specific metabolic rate, B_m, multiplied by a constant $(1-\epsilon)$. The second term is the mass-specific biosynthetic energy spent during growth, $E_m(M-m_0)/m(t)$, multiplied by the protective efficiency ϵ. Based on the first principle of biochemistry and fitting of empirical data, the protective efficiency ϵ has been estimated to be in the neighborhood of 0.99 (Hou, 2013; Hou et al., 2011a).

Assumption 3. The damage estimated by Eq. (1) is a mass-specific quantity, which allows us to make comparisons between animals with different body mass. We assume that when the mass-specific damage level reaches a threshold, animals die, i.e., $D(LS) = C$, where LS is the lifespan. The existence of thresholds of oxidative damage for losses of functions or mortality has also been assumed by Sohal and his colleagues (Sohal and Forster, 2014; Sohal and Orr, 2012). We assume that the threshold C is a constant within a taxon. Note, here we only assume the existence of the constant threshold. As shown below, it is not necessary to know the exact values of the threshold to make quantitative and qualitative predictions.

Several theoretical models have been proposed, based on the disposable soma theory (Kirkwood, 1990; Kirkwood and Holliday, 1979), which considers the energy tradeoffs in aging study (Abrams and Ludwig, 1995; Cichon, 1997; Drenos and Kirkwood, 2005; Kowald and Kirkwood, 1994; Shanley and Kirkwood, 2000). In the broad sense, the model presented here also has roots in disposable Soma theory. However, previous models focused on the tradeoff between energy associated with reproduction and protection, which can explain some empirical results from female animals, but not males, whose reproductive cost is minimal in the absence of male–male competition. Moreover, previous models cannot explain the physiological basis for the tradeoff between protection and reproduction. For example, previous models suggest that reproduction is suppressed by diet restriction, so animals end up with more energy for protection. But this raises the question of why, when the total energy from food is decreased, the energy allocated to protection and reproduction do not decrease simultaneously. Most of these models (e.g., Shanley and Kirkwood, 2000) take an evolutionary approach, and successfully optimize energy allocation strategies under diet restriction for the maximal Darwinian fitness. However, due to the lack of a physiological foundation, they fail to quantitatively estimate the energy reshuffling induced by diet restriction, and therefore cannot make quantitative predictions. Finally, some of the models, such as (Kowald and Kirkwood, 1994) are mathematically complex, heavily depending on parameters that are difficult to link to real physiological measurements. The quantitative model presented here is simple, incorporating only five physiological parameters, but captures the salient features of the energetics of the cellular damage production processes. The proposed model explicitly reveals that the indirect cost of biosynthesis, rather than the direct cost, plays the central role in altering the protective efforts. The quantitative predictions of the model are strongly supported by the empirical data (Hou, 2013). In the rest of the paper, we will apply Eq. (1) and Assumption 3 to explain a series of empirical results (summarized in Table 1), and illustrate the complex relationship between lifespan and longevity.

3. Results and discussion

3.1. Empirical results that support the RLT and the OST

3.1.1. Cellular damage increases with age

Equation (1) predicts the cellular damage curve as a function of age. A typical curve is plotted in Fig. 2A, using the physio-

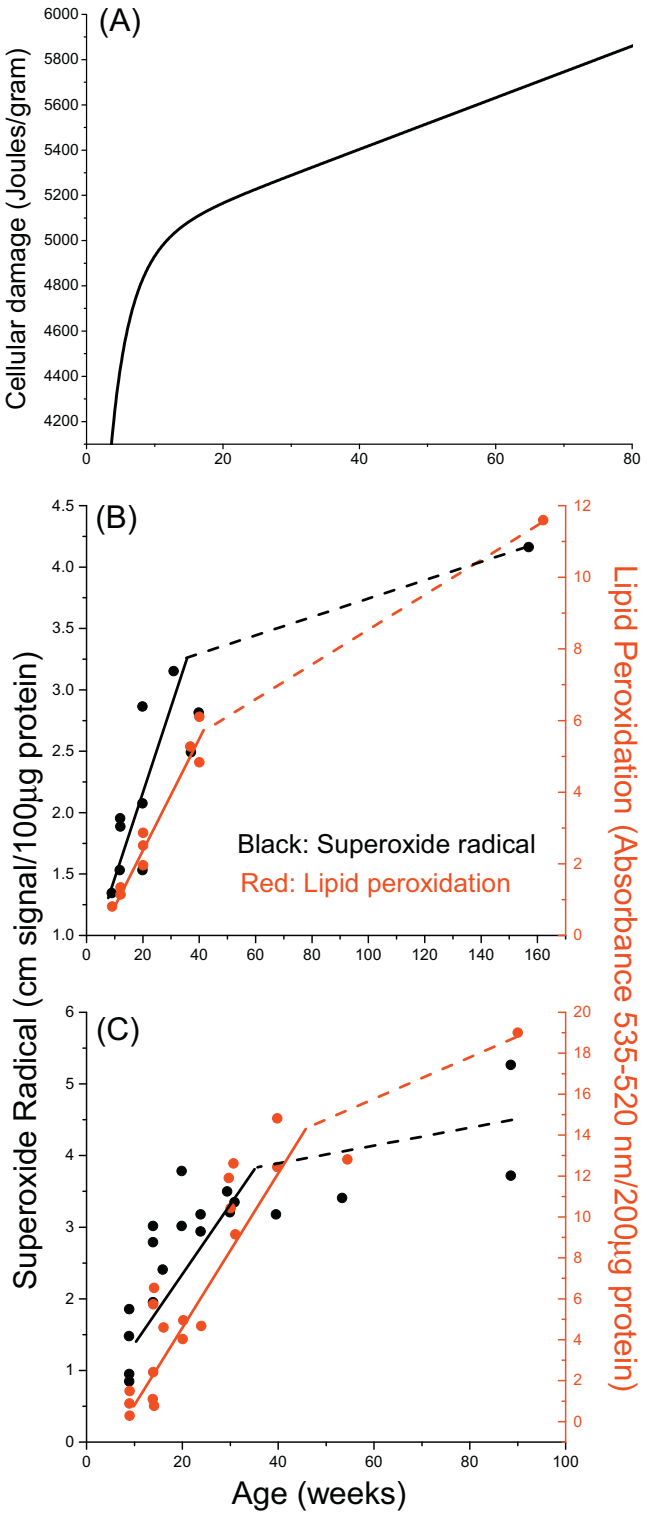

Fig. 2. Theoretical and empirical cellular damage increases as a function of age. (A) The damage curve is calculated based on Eq. (1) and physiological data of a typical mouse, i.e., $M = 30$ g, $B_0 = 3.4$ W/kg$^{3/4}$, $E_m = 6000$ J/g, and $\epsilon = 0.998$ (Hou, 2013). (B) Empirical lipid peroxidation (red) and superoxide radical level (black) in brain tissue of mice reared on a standard diet; (C) Lipid peroxidation and radical level in brain tissue of mice reared on high protein diet. In (B) and (C), the solid and dash lines visualize the slopes of damage and radical levels during growth and adulthood, respectively. Due to the lack of data points between age of 40 weeks and older (160-week in B, and 90-week in C), the exact slopes of the curves during adulthood cannot be estimated. But even the steepest adulthood slopes (the dash lines) are shallower than those during growth, in agreement with our prediction. (Data is from Rollo et al., 1996). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

logical data from a rat. The predicted cellular damage increases with age, as seen in many empirical studies (Bokov et al., 2004). An interesting and novel prediction of Eq. (1) is that the damage increases rapidly during growth, and then slows down after adult mass is reached. This switch stems from the high efficiency of protection, ϵ, and the energy tradeoffs between biosynthesis during growth and protection. Due to the high ϵ, most of ROS and the subsequent damage can be scavenged and repaired, if there is no energy requirement for other life history traits, such as growth or reproduction. In other words, without growth or reproduction Eq. (1) reduces to $D = (1-\epsilon) \times B_m \times t + 0$, and damage accumulates slowly due to the fact that ϵ is very close to 1. However, during growth, a considerable amount of energy is required for biosynthesis. Thus, unrepaired damage accumulates fast during growth in spite of the high efficiency. After growth ceases, more energy is allocated to protection, and therefore the accumulation of damage slows down due to the high efficiency. Data available to test this prediction is limited, and only a very few empirical studies have measured the damage profiles over ontogeny. Rollo et al. (1996) assayed the superoxide radical and lipid peroxidation in several tissues of normal mice (F_1 hybrids from C57BL/6J male and SJL female) as functions of age. We re-plot their data of brain tissue in linear scales in Fig. 2B and C. Clearly, both lipid peroxidation and radical rapidly increase during growth up to ~30 weeks, and slow down during adulthood, in agreement with our prediction. Another line of evidence that supports our prediction, although qualitative, comes from a series of landmark studies, which showed high frequencies of advanced coronary atherosclerotic lesion in young people (Joseph et al., 1993; McMahan et al., 2005). It was concluded that "atherosclerotic changes appears in the coronary arteries years and decades before the age at which coronary heart disease becomes a clinically recognized problem" (Strong, 1986), indicating most of the damage is accumulated before adulthood is reached. Moreover, a few studies shows that in maturing rats and cattle calves, the anti-oxidative protective mechanisms are not well developed, compared to those in the adult, probably "due to the rapid growth after birth" (Gaál et al., 2006, 1996; Gupta et al., 1999). Although, the scavenging capability should not be considered the index of cellular damage level, the observed immature protective mechanisms may lead to a rapid increase in cellular damage during development.

3.1.2. Lowering temperature reduces the damage slope and extends lifespan

Although slowly, the damage in adulthood still increases with age with a slope proportional to the adult mass-specific metabolic rate, B_m (Eq. (1)). Metabolic rate changes with body temperature, as $B_0 \sim e^{-E_0/(KT)}$, where E_0 is the activation energy of metabolism (c. 0.65 eV), K is Boltzmann's constant (8.62×10^{-5} eV/Kelvin), and T is body temperature in Kelvin) (Gillooly et al., 2001). Thus, the lower body temperature will result in a shallower damage slope in adulthood, as shown in Fig. 3A. Based on Assumption 3 (animal dies, when the damage level reaches a threshold, C), we have $D_{normal}(LS_{normal}) = D_{cool}(LS) = C$, where LS_{normal} and LS_{cool} are the lifespans of animals with normal and lower body temperature. Using the physiological data of a typical mouse (given in the caption of Fig. 2), this equation predicts that if the normal average lifespan is 600 days, (LS_{normal} = 600 d), lowering body temperature by 1 °C will extend the lifespan by ~50 days (LS_{cool} ~ 650 d) (Fig. 3A), qualitatively agreeing with the empirical results from (Conti et al., 2006). Several studies on invertebrate species (Klass, 1977; McArthur and Sohal, 1982; Miquel et al., 1976) also showed the effects of temperature on lifespan. The negative correlation between body temperature and lifespan has long been considered a support to the rate of living theory (Bokov et al., 2004).

In Section 3.2.2, we will show that this negative correlation only holds when animals are free feeding. When there is a restriction on food availability, metabolism tradeoffs with growth, i.e., lower temperature and metabolic rate will lead to relatively high growth rate, which subsequently results in weakened health protection. Hence, under food restriction, metabolic rate is positively correlated with health and longevity, opposite of what RTL predicts,

3.1.3. Lifespan scales with body mass across species within a taxon

The strongest support for the rate of living theory (RLT) comes from the allometric scaling relationships between lifespan, metabolic rate, and body mass (Speakman et al., 2002). The theory suggests that the mass-specific energy expenditure during a lifetime, which is the product of metabolic rate and lifespan divided by body mass, is independent of body mass. It has been well-documented that the whole organismal metabolic rate, i.e., energy expenditure, scales with body mass to a power, α, between 0.66 and 0.8 with a canonical value of 0.75 (Brown et al., 2004). Thus, RLT predicts that the scaling power of lifespan on body mass should vary between 0.2 and 0.33 (=$1-\alpha$). Data from 249 mammals and 164 bird species show that the lifespan scaling powers are 0.209 and 0.216, respectively (Speakman, 2005). The mammalian dataset combined by Hamilton et al. (2011) confirmed the scaling powers of ~0.21 for both placentals and marsupials. However, there are two problems with the prediction and empirical evidence. First, the prediction of RLT is merely a phenomenological observation. No theory based on the first principles of physiology was able to quantitatively explain why mass-specific energy expenditure is negatively correlated with lifespan. Second, the metabolic rate of mammals and birds generally scales with body mass to a power of 0.75 (Brown et al., 2004). Taking this power of metabolic rate, the lifespan scaling power should be 0.25 (=1 − 0.75), larger than the empirical value, ~0.21.

The model presented here gives solutions to both problems, quantitatively and qualitatively. Equation (1) estimates two predicted damage curves of two species from the same taxon with body masses, M_1 = 30 g and M_2 = 3000 g (Fig. 3B). The damage increases faster in the smaller species, indicating a shorter lifespan in the smaller species. When lifespan (LS) is reached, the damage level reaches a threshold, i.e., $D(LS) = (1-\epsilon) B_m LS + \epsilon E_m (1-\mu) = C$ (Eq. (1)), where C is a constant within a taxon, μ is the ratio of birth and adult body mass, $\mu = m_0/M$, and $B_m \left(= B_0 M^{\alpha-1}\right)$ is the mass-specific metabolic rate. Note, when lifespan is reached, the body mass $m(t)$ in Eq. (1) reaches the adult size, so $m(t) = M$, and $(M - m_0)/m(t) = 1 - \mu$. If μ does not depend on adult mass, i.e., birth mass is linearly proportional to adult mass across species within a taxon, then we have;

$$B_0 M^{\alpha-1} \times LS = (C - \epsilon E_m (1-\mu)) / (1-\epsilon) = \text{constant}. \quad (2)$$

Since the right-hand side of Eq. (2) is a constant that is independent on body mass, this equation offers a theoretical foundation to the RLT, predicting that $LS \propto M^{1-\alpha}$. In Fig. 3B, we assumed that the metabolic scaling power α = 3/4, and the large and small species have the same birth/adult mass ratio, μ. Thus, if the small species (M = 30 g) has an average lifespan of 1000 days, the large one (M = 3000 g) will live up to ~3000 days (Fig. 3B), i.e., $LS_{large}/LS_{small} \approx 3 = \left(M_{large}/M_{small}\right)^{1-3/4}$.

However, the data from placentals show that the birth mass is not linearly proportional to the adult mass across species, and μ scales with adult mass to a power of −0.07 (Hamilton et al., 2011). Thus, Eq. (2) becomes $LS = C_1 M^{1-\alpha} + C_2 M^{1-\alpha-0.07}$, where C_1 and C_2 are two constants, determined by the damage threshold, C, protective efficiency, ϵ, biosynthetic energy, E_m, and metabolic

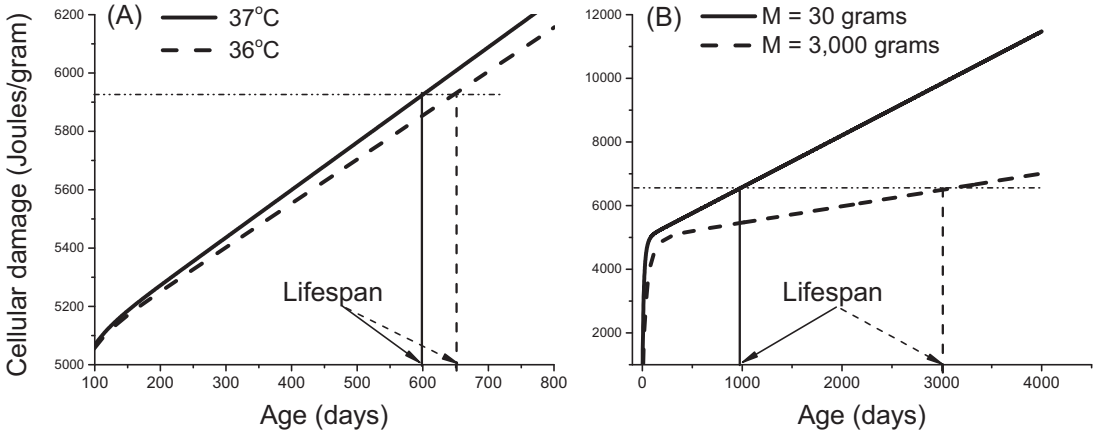

Fig. 3. Cellular damage and lifespan. (A) Lower body temperature and metabolic rate result in lower damage level and a longer lifespan; (B) the larger species has lower damage level and longer lifespan, compared to the smaller species in the same taxon. The horizontal lines in (A) and (B) are the damage threshold, which is assumed to be a constant within a taxon. The damage curves are estimated based on Eq. (1) and physiological data of a typical mouse given in the caption of Fig. 2.

coefficient, B_0. Taking the metabolic scaling power, $\alpha = 0.75$, we obtain $LS = C_1 M^{0.25} + C_2 M^{0.18}$, which means that the scaling power of lifespan lies between 0.18 and 0.25. It leans towards 0.25, if $C_1 \gg C_2$, and leans towards 0.18, if $C_2 \gg C_1$. Unfortunately no data is available to estimate C_1 and C_2 across species within a taxon. Nonetheless, the weak-scaling of birth/adult mass ratio (μ) can qualitatively explain why the scaling power of lifespan is smaller than what is predicted by the RLT.

The relationship between lifespan and body mass discussed above does not hold; however, when comparison is made within a species. Numerous intra-specific studies have shown that the smaller animals live longer, opposite of the inter-specific patterns. The variation of body mass within a species can be induced by diet and genetic modification (Hou, 2013; Rollo, 2002), or through selections, such as in diverse breeds of domestic dogs (Greer et al., 2007; Speakman et al., 2003). In these cases, negative correlations between lifespan and body mass have been observed. The RLT also loses its predictive power when comparing species across taxon. For example, with a higher metabolic rate, birds usually live longer than mammals with the same body mass (Hulbert et al., 2007). In the following sections, we will show that the intra-specific correlation between body mass and lifespan can be quantitatively explained, if the energy tradeoff between health protection and biosynthesis is considered (Hou, 2013), and the across-taxon puzzle can be potentially explained by the different protective efficiencies in different taxon (Hulbert et al., 2007).

3.2. Explanation of controversial empirical results with the concept of energy tradeoffs

In the following three sections, we will show that the energy tradeoffs between growth and protection is the key to understanding how diet restriction and genetic manipulation of growth hormones extend lifespan without largely reducing metabolic rate, and why breeds with larger body mass and lower energy expenditure live shorter. The correlation between growth rate and longevity has been observed since the era of Pearl (1928) and McCay et al. (1935). Here, using the quantitative model based on the first principle of energy conservation, we make novel predictions and provide the mechanistic foundation for the statistic and descriptive studies that attempted to link growth and longevity (McCay et al., 1935; Pearl, 1928; Ross et al., 1976).

3.2.1. How does diet restriction and genetic manipulation of growth hormones extend lifespan without reducing metabolic rate?

The negative correlation between lifespan and energy expenditure predicted by the RLT has been questioned by the studies of diet restriction (DR) and genetic modification (GM) of growth hormone, two interventions that largely extend lifespan but only induce mild or no change in metabolic rate (Brown-Borg, 2003; Hou et al., 2011b; Masoro, 2005; McCarter et al., 1985; Merry, 2002; Westbrook et al., 2009). Under severe DR, the body temperature of small rodents drops by 1–2 °C, and metabolic rate decreases only by ~15%, disproportional to the lifespan extension by DR (see review in Hou et al., 2011b). In one study, metabolic rate even increased in DR mice (Faulks et al., 2006b) (see detailed analysis of the changes in metabolic rate under DR in Appendix B). Studies on ectothermic species also found that while extending the lifespan of fruit flies and nematodes, DR does not lower metabolic rate after body mass is corrected (Houthoofd et al., 2002; Hulbert et al., 2004; Mair et al., 2003; Partridge et al., 2005; Walker et al., 2005). These findings indicate that lowering metabolic rate, including torpor and quiescence, is not crucial for DR to extend lifespan (Stuart and Brown, 2006), and they have been considered the major challenges of the RLT and OST (e.g., Brys et al., 2007; Speakman et al., 2004; Stuart and Brown, 2006).

In this section, we show that the energy tradeoffs between health protection and biosynthesis, as suggested in Fig. 1, is the key to understanding the correlations between energy expenditure, body mass, and lifespan in the cases of DR and GM. Fig. 4 describes the basic idea. When animals are fed ad libitum (AL) the energy assimilated from food (black framed box in Fig. 4A) is partitioned between the metabolic energy (red framed box) and energy deposited in the new biomass (green framed box). The metabolic energy is further partitioned between energy for activities, biosynthesis, and protecting existing biomass. The energy deposited in the new biomass is the combustion energy content of tissues. To deposit energy in new biomass requires energy for biosynthesis, which includes the metabolic work, such as assembling monomers into polymers and folding polypeptides into functional proteins. The energy deposited in biomass is the direct cost of growth, and the energy for biosynthesis is the indirect cost of growth. These two energy costs are proportional to each other (Brody, 1945; Hou et al., 2008).

When animals are under diet restriction (DR) (Fig. 4B), the total energy from food decreases (black framed box). If the metabolic

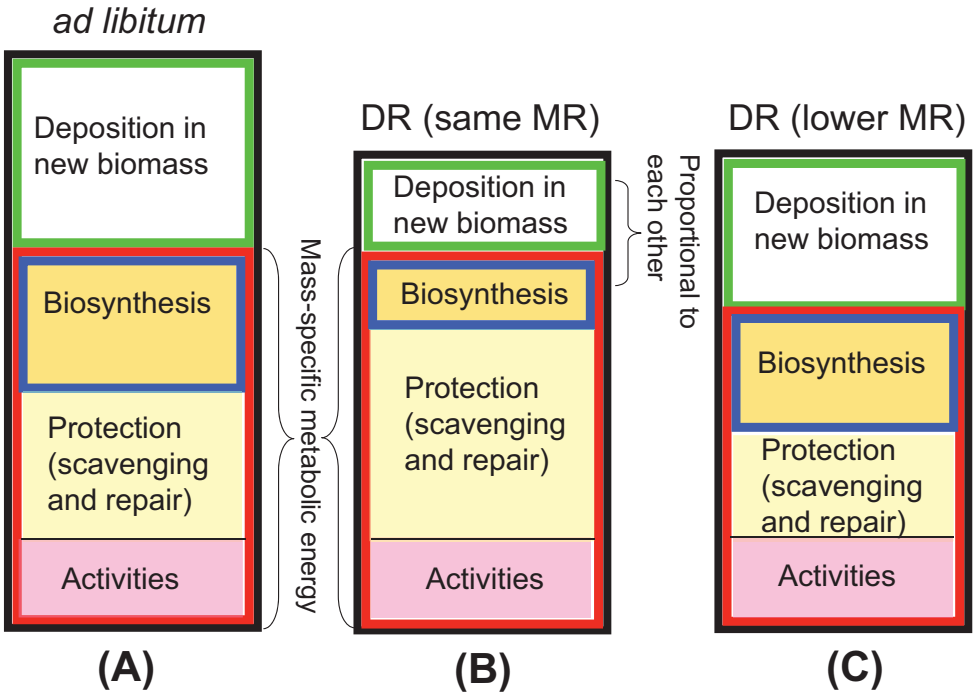

Fig. 4. Energy partition under (A) ad libitum (free-feeding); (B) diet restriction with unchanged metabolic rate (MR); and (C) diet restriction with lower metabolic rate. See detailed explanation in the text. (Panel (A) and (B) are re-drawn from Hou, 2013). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

rate (red framed box) remains roughly unchanged, then the deposition in new biomass (green framed box) must be suppressed. This means that the amount of molecules that are available from food to be synthesized into new biomass is reduced. Since the energy deposition in new biomass is proportional to the energy for biosynthesis (blue framed box), when fewer molecules are available to be synthesized, the energy for biosynthesis decreases automatically. Again, the metabolic rate remains the same, so the decreased biosynthetic energy means more energy for protection (light yellow box). As a result of enhanced protection, the damage level is lower, and the lifespan is extended. Here, without optimizing the Darwinian fitness for energy budget or making any assumptions, this model gives a physiological explanation for how diet restriction induces the tradeoff between growth and protection. The model shows that the tradeoff is inevitable under DR, if metabolic rate remains unchanged. Later we will discuss the cases where DR does reduce metabolic rate. Similarly, animals under genetic modification (GM) do less work for biosynthesis due to growth hormone deficiency or receptor resistance. Since their metabolic rate also stays the same as the wild type controls, extra energy therefore is channeled from biosynthesis to health protection.

The reduction of adult body mass can be considered the index of the suppression on biosynthesis by DR and GM. Based on this model, we derived a quantitative relationship between lifespan extension and body mass reduction from Eq. (1) (detailed derivations are available in Hou, 2013),

$$B_{\mathrm{m,D/G}} \times \mathrm{LS}_{\mathrm{D/G}} - B_{\mathrm{m}} \times \mathrm{LS} = \frac{\epsilon E_{\mathrm{m}} \mu}{1-\epsilon} \left(\frac{M}{M_{\mathrm{D/G}}} - 1 \right), \quad (3)$$

where $\mathrm{LS}_{\mathrm{D/G}}$ and LS are lifespans, $B_{\mathrm{m,D/G}}$ and B_{m} are mass-specific metabolic rate, and $M_{\mathrm{D/G}}$ and M are body masses of animals under treatments of DR or GM and free feeding respectively, and μ is the normal ratio of birth and adult mass of a given species. Compared to the free feeding controls, DR and GM animals have smaller adult mass, $M_{\mathrm{D/G}}$. Thus, the right-hand side of Eq. (3) can be considered the reduction in body mass induced by DR or GM, $(M/M_{\mathrm{D/G}} - 1)$, which is proportional to the extension in lifespan on the left-hand side of the equation with a constant coefficient $\epsilon E_{\mathrm{m}} \mu / (1-\epsilon)$.

Equation (3) suggests a powerful way of plotting data that reveals a universal pattern of the tradeoff between biosynthesis and lifespan. Noting that the birth/adult mass ratio, μ, is a constant for a given species, we can normalize the lifespan extension on the left-hand side of Eq. (3) by correcting for the species-specific constant $\frac{\epsilon E_{\mathrm{m}} \mu}{1-\epsilon}$. If we plot the normalized lifespan extension against the body mass reduction, i.e., $(1-\epsilon)/(\epsilon E_{\mathrm{m}} \mu) \times (B_{\mathrm{m,D/G}} \mathrm{LS}_{\mathrm{D/G}} - B_{\mathrm{m}} \mathrm{LS})$ against $M/M_{\mathrm{D/G}} - 1$, we will obtain a straight line with a slope of 1. Data from DR rats, DR mice, and GM mice ($N = 246$) shown in Fig. 5 strongly supports this prediction. Regardless of the interventions, diet restriction or genetic modification, the lifespan extension is

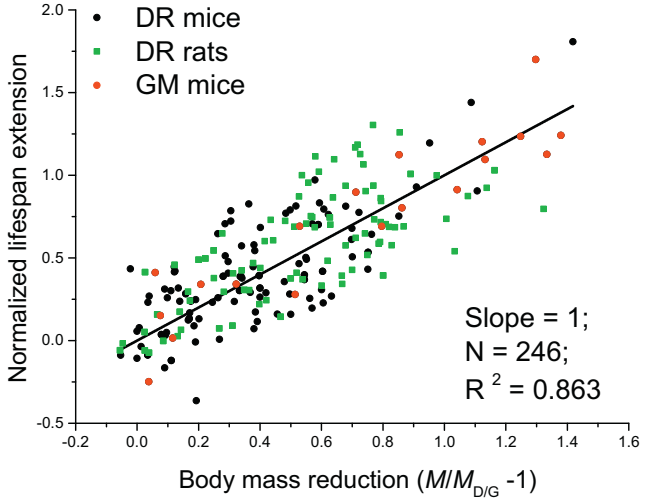

Fig. 5. The normalized lifespan extension induced by diet restriction (DR) and genetic modification of growth hormone (GM) is linearly proportional to the body mass reduction in mice and rats (slope = 1). (Data is collected in Hou, 2013).

proportional to the body mass reduction, and the patterns from different species—rat and mouse—are identical in Fig. 5. It is also important to point out that the DR data shown in Fig. 5 are collected from studies that applied a wide variety of DR protocols, including different DR severities, different DR onset ages, different DR period lengths, and different diet composition. Despite the complexity of these protocols, the tradeoff between biosynthesis and protection revealed by this model explains 86% of the lifespan extension ($R^2 = 0.86$ in Fig. 5).

Some researchers assumed that DR extends lifespan through its ability to reduce ROS generation (e.g., Kowald and Kirkwood, 1994). However, as emphasized in Fig. 1, ROS generation may not necessarily be proportional to net cellular damage level. Our model suggests that even if the ROS generation (and the oxygen consumption) stays the same, the suppression of biosynthesis induced by DR can channel more energy to enhance the protection efforts, and reduce the net damage level.

3.2.2. Why is metabolic rate positively correlated with lifespan in some studies of diet restriction?

Many diet restriction (DR) studies showed that mass-specific metabolic rate of rodents under DR stays roughly the same, or only slightly decreases; (see details in Appendix B). These studies also reported no correlation between metabolic rate and lifespan. However, a few studies on mice (Liao et al., 2011), houseflies (Cooper et al., 2004), parthenogenetic insects (Roark and Bjorndal, 2009), and yeasts (Lin et al., 2002) showed that metabolic rate was significantly altered by DR in these cases, and it was positively correlated with lifespan. This paradox can be qualitatively explained by Fig. 4C.

Because energy assimilated from food (black framed box) is partitioned between energy deposited in new biomass (green framed box) and metabolic energy (red framed box), DR induces a tradeoff between these two partitions. The effects of DR on metabolic rate in species with different life history are different (Jiao et al., 2014; Shanley and Kirkwood, 2006). Most mammals prioritize metabolism and maintenance over growth, because as long-living species, they can resume growth after the low-food availability season is over. This strategy will lead to delayed maturation and reproduction, but will ensure low mortality and better quality in the animals and their offspring. Thus, the loss of overall fitness caused by DR can be minimized. However, some species, especially the short-living ones, may sacrifice metabolism and maintenance in order to keep a relatively fast growth rate under DR (Jiao et al., 2014). Our laboratory has shown that in *Manduca sexta* caterpillars, lower metabolic rate leads to higher growth rate under DR (Hayes et al., 2015). Discussion on detailed life history strategies under DR stress is beyond the scope of this paper. Here we focus on the consequences of alteration in metabolic rate under DR. When metabolic rate is lowered in DR animals, animals will have relatively more energy to deposit in the new bio-tissues (green-framed box in Fig. 4C), which means that the suppression of growth will be diminished, or even reversed (Fig. 4C). With more molecules from food to be synthesized into new biomass, animals need to allocate more energy to biosynthesis—the indirect cost of growth (blued framed box). Consequently, the protective effects of DR (light yellow box) will be weakened (Fig. 4C). Thus, when food availability is limited, low metabolism leads to relatively high energy deposition, which in turn entails high biosynthetic energy, and the price that animals need to pay is the low effort for health protection. Note, under free-feeding conditions, to maintain a high growth rate, animals can simply increase the uptake of food (to a certain degree confined by the ingestion capability), and do not need to lower metabolic rate.

However, although lowering metabolic rate will reduce the protective effort under DR, it will also reduce the gross production of ROS and cellular damage. So, a question arises. Does the net damage (gross damage minus protection) decrease or increase, when metabolic rate decreases? This question can be answered by a quantitative analysis of Eq. (1), $D = (1-\epsilon) \times B_m \times t + \epsilon \times E_m(M - m_0)/m(t)$, where D is the accumulated cellular damage over the period of interest, B_m is the mass-specific metabolic rate, $E_m(M - m_0)/m(t)$ is the mass-specific biosynthetic energy spent on growth, and ϵ is the protective efficiency. As we discussed above, under DR, if B_m (in the first term) decreases, biosynthetic energy (in the second term) will increase, so the opposite changes in them may cancel each other and lead to an uncertain change in the overall damage, D. However, in most species, the protective efficiency, ϵ, is remarkably high. Estimates from empirical data gave that the value of the protective efficiency ϵ is 0.998 for mice, 0.999 for rats, and 0.976 for *M. sexta* larvae (Amunugama et al., 2015; Hou, 2013), which are in the range of 0.964–0.999, estimated based on the bioenergetics of breaking and repairing polymer linkages (Hou, 2013; Hou et al., 2011a). With such a high ϵ, the first term in Eq. (1), $(1-\epsilon)B_m \times t$, is much smaller than the second term, $\epsilon \times E_m(M - m_0)/m(t)$. Using the physiological parameters of rats, the first term of a rat at age $t = 200$-day was estimated to be about 60 J/g, whereas the second term is more than 5000 J/g (Hou, 2014). This means that the cellular damage level is more sensitive to the changes in biosynthetic energy (the second term) than to the changes in metabolic rate (the first term). Thus, although lowering metabolic rate will lead to less gross damages, the increase in growth rate will result in increase in overall damage level. The model and Fig. 4C qualitatively explained why under DR metabolic rate is positively correlated with lifespan, opposite of the correlation predicted by the RLT. Our lab is currently conducting dedicated experiments to quantitatively test this theory. We have induced a broad range of variations in lipid peroxidation, metabolic rate, and growth rate in *M. sexta* larvae by simultaneously adjusting ambient temperature and food supply levels. Our preliminary results show that the lipid peroxidation is insignificantly correlated with metabolic rate, but the correlation with growth rate is significant. Moreover, when regressing damage level on metabolic and growth rate, the coefficient of the former is much smaller than the latter, as Eq. (1) predicts, $1 - \epsilon \ll \epsilon$ (Amunugama et al., 2015).

Finally we address the variation in activity levels of animals under DR. Table B.1 (in Appendix B) shows the changes in activity level from 15 studies of rodents and monkeys under DR. In seven of them the activity level stayed the same, four of them slightly increased, three largely increased, and in one exception the activity even slightly decreased (Table B.1). Considering the facts that in most cases activity only slightly increases or stays the same, and that most studies did not report the changes in energy expenditure caused by the changes in activity, we assume activity level to be a constant under DR to keep the quantitative model simple without losing generality.

However, the model can be generalized to include the variation in activity level. As shown in Fig. 4, with a limited food supply, an increase in activity would further suppress growth. Thus, depending on the degree of the increase in activity, the adult mass of DR animals (M_{DR}) will be even smaller. In Eq. (3), lifespan extension is proportional to the body mass reduction ($M/M_{DR} - 1$). So, if M_{DR} is smaller due to the increase in activity, the lifespan extension will be larger.

There is no empirical data to test this prediction directly and quantitatively, because most studies did not measure the energy cost of the increased activity level. But the results from Holloszy (1997) support this prediction indirectly by showing that the major determinant of lifespan extension is the body mass reduction even if activity level varies. Holloszy (1997) reported the lifespan, food consumption, and body mass of four groups of male Long-Evans rats reared at different levels of food supply and exercises: ad libitum (AL)-runner, AL-sedentary, DR-runner, and DR-sedentary. The peak

body mass (M and M_{DR}) of these four groups rank in such an order: AL-sedentary (597 g) > AL-runner (420 g) > DR-runner (333 g) = DR-sedentary (330 g).

Equation (3) predicts that their lifespan will be in the opposite order. The data supports this prediction: AL-sedentary (858 days) < AL-runner (973 days) < DR-runner (1058 days) = DR-sedentary (1051 days). Note, in this study, although they are both under DR, DR-runners consumed more food (13.4 g/day) than DR-sedentary group (10 g/day). So the runner and sedentary groups ended up with the same body mass (~330 g). The interesting result is that despite the different exercise and food levels, the same body mass led to the same lifespan (~1050 days) in these two groups, exactly as our model predicts.

We postulate that if DR-runner and DR-sedentary were fed with the same level of food, then the runners will be have a smaller body mass, and therefore a longer lifespan.

3.2.3. Why do breeds with larger body mass and lower energy expenditure live shorter?

When comparing two animals with different birth and adult masses, Eq. (3) can be re-written in such a way that the right-hand side of it is proportional to $\left(m_{0,1}/M_1 - m_{0,2}/M_2\right)$, where m_0 is the birth mass, and the subscripts 1 and 2 indicate two animals. Thus, the difference in lifespan between animal 1 and 2, which is the left-hand side of the equation, is proportional to the difference in the mass ratio, m_0/M, of them. This offers an answer to the question of why larger dog breeds with lower mass-specific metabolic rate live shorter. We used data from Speakman et al. (2003) as an example to illustrate this point. The authors measured mass-specific energy expenditure, body mass, and lifespan of three dog breeds, Papillons, Labrador retrievers, and Great Danes. The daily average energy expenditure per kg lean body mass in the first 8 years of life of these three breeds are 337 kJ, 231 kJ, and 228 kJ, respectively, and the adult body masses are 3.0 kg, 29.8 kg, and 62.8 kg, respectively. However, their lifespans are 14 years, 12.6 years, and 8.4 years (Speakman et al., 2003). These findings are contrary to the findings across species, and therefore challenge the RLT. Nonetheless, when we take the birth mass into account, the correlation between body mass and lifespan can be predicted by Eq. (3). From web-based resources primarily generated by dog breeders, we estimate that the birth mass of these three breeds are 0.115 kg, 0.45 kg, and 0.735 kg, respectively. Thus, the birth/adult mass ratios, $\mu = m_0/M$, which can be considered the index of the amount of energy allocated to growth, are 3.83%, 1.51%, and 1.17% for Papillons, Labrador, and Great Danes, respectively. This means that larger dog breeds allocate relatively more energy to growth, and less energy to health protection. Thus, Eq. (3) predicts that the breed with larger birth/adult mass ratio will have a longer lifespan, i.e., if $m_{0,1}/M_1 < m_{0,2}/M_2$, then $LS_1 < LS_2$.

It is worth pointing out that if the birth/adult mass ratios (m_0/M) of two groups of animals are the same, as seen in many inter-specific comparisons, Eq. (3) reduces to $B_{m,1}LS_1 - B_{m,2}LS_2 = 0$, which is exactly the prediction of the RLT, i.e., the mass-specific metabolic rate (B_m) is negatively correlated with lifespan (LS).

In summary, Eq. (3) predicts that the difference in the lifelong mass-specific energy expenditure (left-hand side of the equation, $B_m \times LS$) between two group of animals depends on the difference in the amount of energy allocated to growth (indexed as the birth/adult mass ratio, $m_{0,1}/M_1 - m_{0,2}/M_2$). We have discussed three comparisons. First, in inter-specific comparison, the birth/adult mass ratio is roughly the same in a taxon, so $B_m \times LS$ is roughly a constant, as predicted by the RLT. Second, in comparison between DR/GM and the controls, the birth masses, m_0, are the same, but adult masses are smaller in DR/GM animals, so the control animals spend more energy on growth and live shorter. Third, when comparing different breeds of the same species, both birth and adult mass are different, so the breed with a smaller ratio spends more energy on growth and have a shorter lifespan.

The widely cited statement of RLT concerns the relationship between longevity and metabolic rate (e.g., Speakman, 2005). In terms of metabolic rate, the RLT does not hold intra-specifically. However, in one of his books on RLT, Pearl (1928) tried to connect longevity to growth rate, instead of metabolic rate. Pearl's efforts are pertinent to our model. In the last three sections, we have offered an energetic explanation to the well-known correlation between growth and longevity—because of the tradeoff between growth and protection, animals with slow growth rate may have more energy for protection, thereby have longer lifespan. We need to emphasize that the correlation between growth and lifespan does not merely stem from the temporal lengths of ontogeny, as McCay et al. (1935) suggested. It is generally true that animals with longer growth periods also live longer. But besides the length of growth period, the initial and ultimate body masses also play important roles from the energetic viewpoint, because the total energy allocated to biosynthesis during growth is proportional to the difference between these two masses. It has been shown in at least seven studies on rodents, dog, and quail that under constant diet restriction, it takes the same time period for the AL controls and the DR counterparts to reach the same percentage (e.g., 70% or 90%) of their own ultimate body masses (Hou et al., 2011b). Studies on rats (Engelbregt et al., 2000) also showed that if the degree of DR is a constant (percentage of AL food supply), then DR animals reach puberty at the same age as the AL controls. These studies indicate that DR with a constant severity does not elongate growth period. So, how does DR extend lifespan? The explanation from our model is that DR animals have the same initial body mass as the AL controls, but their ultimate mass is smaller, and therefore they allocate less energy to biosynthesis.

3.3. Explanations of controversial empirical results with the concept of protective efficiency

Facing constant oxidative insults, organisms have evolved remarkably high protective efficiencies, ϵ. As the result of natural selection, the efficiency is robust under normal conditions (Sohal et al., 2002). However, in some cases a low dose of stress can improve the efficiency, a phenomenon known as hormesis (Masoro, 2005; Rattan, 2004; Ristow and Zarse, 2010; Sinclair, 2005). It has been hypothesized that some protection-related gene expressions can be up-regulated, when organisms are under short-term mild stress (Rattan, 2004). In the following sections we will discuss a few cases, where the empirical results against the RLT and OST cannot be explained by the energy reshuffling, but can be understood by the alteration in protective efficiency. Unlike the previous sections, in which the analyses of the energy tradeoffs and the effects on lifespan were quantitative, the discussions on the efficiency are more qualitative, due to the lack of data to estimate the exact alterations in the efficiency induced by the empirical interventions.

3.3.1. Why do cold exposure and mild exercise increase energy expenditure, but not alter lifespan in mammals?

Cold exposure costs energy for thermoregulation in endotherms, and induces substantial increases in energy expenditure, especially in small mammals (Barnett, 1965), which, according to the RLT, would presumably shorten animals' lifespan. However, studies on mice (Vaanholt et al., 2009), voles (Selman et al., 2008), and rats (Holloszy and Smith, 1986), showed that while increasing metabolic rate, cold exposure failed to make significant differences in lifespan or oxidative damage levels. One possible explanation is that cold exposure induces suppression of biosynthesis, and the extra energy channeled to protection offsets the extra damage caused by increased metabolic rate.

Indeed, the adult masses were reduced in mice and rats under cold exposure. However, the body mass of voles was not affected (Selman et al., 2008). Moreover, as shown below, even in the case of mice (Vaanholt et al., 2009), where data is available for quantitative calculation, the energy channeled from suppressed biosynthesis is insufficient to offset the extra damage. Below, we calculate the damage level in the control and cold-exposed mice using Eq. (1), $D(t) = (1-\epsilon)B_m \times t + \epsilon \times E_m(M-m_0)/M$. Again, B_m is the mass-specific metabolic rate, and $E_m(M-m_0)/M$ is the biosynthetic energy spent on growth. In Vaanholt et al. (2009) study, the body masses of 11-month-old mice reared at warm and cold temperatures are 37.4 g and 32.6 g, respectively. Taking $E_m = 6000$ J/g (Hou, 2013; Hou et al., 2011a), and the typical birth mass of mice, $m_o = 5$ g, the biosynthetic term of the damage equation, $E_m(M-m_0)/M$, is, 5198 J/g in the warm group, higher than that in the cold treatment, 5079 J/g. However, the metabolic term of the equation ($B_m \times t$) in the warm group is much lower than that of the cold group. Vaanholt et al. (2009) estimated the mass-specific lifetime energy expenditure, $B_m \times t_{(warm)} = 1416$ kJ/g and $B_m \times t_{(cold)} = 2115$ kJ/g. Taking the protective efficiency for mice, $\epsilon = 0.998$ (Hou, 2013), the lifetime damage level in the mice reared at warm temperature $D_{warm} = 8030$ J/g, is lower than that of cold treatment, $D_{cold} = 9310$ J/g. This means that if the protective efficiency is the same in both groups, then cold-exposed mice would have a shorter lifespan.

However, cold exposure may exert a hermetic action on these rodents, and therefore enhances the protective efficiency. Using the energetic data calculated above and Eq. (1), it is straightforward to conclude that if ϵ increases from 0.998 to 0.9998, the damage level in both groups will be equal to each other, $D_{normal} = D_{cold}$. This indicates that a 0.2% increase in the efficiency under cold exposure is sufficient to balance the extra damage caused by the increased metabolic rate, and results in the same lifespan.

Similarly, moderate exercise increases energy expenditure, but has no effect lifespan (Goodrick et al., 1983), or in some cases even increase lifespan (Holloszy, 1993; Navarro et al., 2004) despite mild or no growth retardation. Some evidence indicated that physical exercise may induce mitochondrial hormesis (Ristow and Zarse, 2010) and promote metabolic health (Lanza et al., 2008; Warburton et al., 2006). Due to the lack of empirical data on energy expenditure and/or body mass reduction, we are not able to quantitatively compare the damage levels in exercise and sedentary groups in these studies. But the qualitative analysis leads to the same conclusion as that in the case of cold exposure, i.e., a slight increase in efficiency induced by exercises can counteract the increased energy expenditure.

It is important to note that cold exposure and physical exercises may cause antagonistic changes in the three factors in Eq. (1), i.e., higher energy expenditure (B_m) increases damage, higher protective efficiency (ϵ) decreases damage, and suppression on growth (the second term) decreases damage. The levels of the changes in these factors may balance or surpass each other, depending on the severities of the stressors, and species' physiology, behaviors, and life history, so the overall effects of the stressors on lifespan can be neutral, beneficial, or deleterious. Noticing that some of these stressors failed to extend lifespan, Masoro (2005) suggested that that voluntary exercise may not be a hermetic stressor for rats, and the cold exposure protocol in (Holloszy and Smith, 1986) may be too intense to induce hormesis. The semi-quantitative analysis conducted here, nonetheless, suggests that these stressors did induce hormesis, if hormesis is defined as improved protective efficiency, but the hermetic effects were offset by the increases in the damage level due to the increases in energy expenditure. To fully understand the effects of these stressors and the underlying mechanisms, we call for more quantitative experiments that are designed under the guides of the first principle models.

3.3.2. Why did alterations of antioxidant levels fail to alter lifespan?

Feeding animals antioxidants and genetically changing the expressions of antioxidant enzymes are assumed to alter the levels of oxidative damage, and the oxidative stress theory (OST) predicts that these interventions would alter animal lifespan. However, the empirical results are controversial. For the detailed description and discussion of these results, we recommend a few excellent review papers (Bokov et al., 2004; Pérez et al., 2009; Sohal et al., 2002). Here we conduct a semi-quantitative analysis within the framework of the energetic model presented here.

First, we discuss the interventions that pharmacologically or genetically elevate the level of antioxidants, including ROS scavengers and damage repair enzymes. Generally, feeding antioxidants to mammals, nematodes and houseflies, do not have a positive effect on lifespan, and only thiazolidine carboxylic acid extended the lifespan of fruit flies (Bokov et al., 2004; Miquel et al., 1982). Similarly, overexpressing ROS scavengers, such as superoxide dismutases (SOD), failed to extend the lifespan of mice (Pérez et al., 2009), but manifested beneficial effects on lifespan in some studies of fruit flies (Parkes et al., 1998; Pérez et al., 2009; Spencer et al., 2003). Overexpressing methionine sulfoxide reductase A (MsrA), which reduces methionine sulfoxide residues in proteins, also increases lifespan in fruit flies (Chavous et al., 2001; Ruan et al., 2002).

Elevating the levels of ROS scavengers or the repair enzymes presumably increases the protective efficiency, ϵ, and therefore decreases the level of cellular damage. In Eq. (1), the damage level increases as a function of age, t, i.e., $(1-\epsilon)B_m \times t$. When ϵ increases, not only the level of the damage will decrease, but also the slope of the damage level, $(1-\epsilon)B_m$, will be shallower. This prediction has been proven true in fruit flies. Overexpression of Cu/ZnSOD and catalase caused a reduction DNA damage level at every age and also a shallower slope, (Fig. 1 in Sohal et al., 1995), indicating that the damage increases slower in transgenic fruit flies. As predicted by the OST, the lifespan of the transgenic fruit flies was extended too. Using Eq. (1) and the damage slopes given by Sohal et al. (1995), 47 and 37.8 fmol/μg DNA/day for control and transgenic fruit flies, we estimate that the efficiency (ϵ) is ~0.5% higher in the transgenic group, assuming the mass-specific metabolic rates are the same in two groups.

Why did the similar manipulation fail to extend the lifespan of mice? It is important to note that most studies on mice only showed that the transgenic mice are more resistant to external oxidative stress, such as paraquat, but did not compare the damage levels in the wild type and transgenic groups reared under normal living conditions (Pérez et al., 2009). An exception is the study of overexpressing MnSOD by (Jang et al., 2009). In this study, the protein carbonyl levels are the same in wild type and transgenic mice, both young and old, and F_2-isoprostane (one of the indexes of lipid peroxidation) is lower in the old transgenic mice, but are the same in young wild type and transgenic mice. This line of evidence indicates that the enhancement of protective efficiency in mice by genetic manipulation is not as significant as in fruit flies, because the manipulation did not induce significant difference in damage levels. Sohal et al. (2002) gave a reasonable explanation: "the function of antioxidants, resulting in their evolutionary conservation, lies in the response to stress noted above (external oxidative stress). Under stressful conditions the production of ROS may be rapid and widespread, and their elimination by antioxidants would be necessary for the survival of the animal. . .... However, under normal conditions, the rate of production of ROS is sufficiently slow that increasing levels of antioxidants may not lead to further neutralization of ROS". (Sohal et al., 2002). While offering an explanation to the question why transgenic mice do not enjoy longer lifespan, Sohal et al's argument cannot explain why elevated anti-oxidant

generally increases the lifespan of fruit flies. Here, comparing the results from fruit flies and mice, we extend Sohal et al's explanation by linking the energetic model to the evolutionary theory of aging.

Let us consider the situations in different taxon. In the previous section, using the first principle of energy conservation, it has been shown that within a taxon, the lifespan across species is inversely proportional to mass-specific metabolic rate (Eq. (2)). But this energetic consideration cannot explain why the inverse proportionality does not hold when comparison is made across taxons, e.g., birds have higher mass-specific metabolic rate, but live longer than mammals with the same body mass. The explanation from the classic evolutionary theory of aging is that birds have much lower external mortality rate than mammals. Proximately, Hulbert and his colleagues (e.g., see Hulbert et al., 2007) have shown that bird species have evolved a membrane fatty acid composition that is significantly more resistant to peroxidation than mammals. In our model, the higher resistance to peroxidation means a higher protective efficiency, ϵ, and therefore longer lifespan. Comparing fruit flies and mice, it is reasonable to hypothesize that, due to the remarkably lower external mortality rate of mice, the protective efficiency in mice is much higher than that of fruit flies. Natural selection has resulted in such a high efficiency in mice that "under normal conditions," i.e., without external stresses such as oxidant or cold exposure, no significant further enhancement can be achieved by the pharmacological or genetic manipulations. In fruit flies, on the other hand, the efficiency is relatively low, so we postulate that there is still "room" for the interventions to further improve the protection. One way to test this hypothesis is to compare the effects of these interventions between long- and short-lived strains within one species that have been under artificial selection, assuming that long-lived strains have evolved relatively higher efficiency, and therefore will benefit less from the elevation of anti-oxidants. In fact, at least one of such experiments was conducted on fruit flies (Orr et al., 2003). Based on their results, Orr et al. (2003) suggested that transgenes encoding Mn-SOD or thioredoxin reductase "does not decrease the rate of aging in long-lived strains of *Drosophila*, although there may be some effect in relatively short-lived strains". We call for more similar dedicated experiments to test this hypothesis.

Now, let us switch to the studies on the effects of underexpressing antioxidant enzymes on lifespan. Van Raamsdonk and Hekimi (2012) found that nematodes that completely lack SOD activity (*sod-12345* worms) has a normal lifespan, and the normal levels of the protein carbonyls. The authors concluded that other antioxidant mechanisms, such as catalase and protein repair/turnover, are up-regulated to compensate for the lack of SOD, and contribute to the normal level of oxidative damage. Since neither damage level nor the lifespan was affected by the manipulation in this case, the oxidative stress theory (OST) was neither refuted nor proved. The real challenges of the OST come from the studies on mice. Pérez et al. (2009) reviewed a series of experiments conducted in Arlan Richardson's laboratory, in which knocking out a wide variety of genes encoding ROS scavengers, such as SOD or Gpx, or repair enzyme, such as MsrA, failed to shorten lifespan of mice, except for only one manipulation—the deletion of *Sod1* gene. The authors concluded that "our data calls into serious question the hypothesis that alterations in oxidative damage/stress play a role in the longevity of mice". Unlike most studies of overexpressing antioxidant, which did not compare the cellular damage levels in the wild type and transgenic groups under the normal conditions, many of the knockout experiments did assay the damage levels, and clearly showed that the damage levels in the knockout mice are significantly higher than that in the wild type (Pérez et al., 2009; Van Remmen et al., 2003; Zhang et al., 2009), yet lifespan was not affected, except in the experiment of knocking out *Sod1*. Thus, unlike the overexpressing manipulations, which may fail to alter the protective efficiency in mice as hypothesized above, these knockout manipulations did weaken the efficiency.

These results raise two questions. First, why did $Sod1^{-/-}$ mice have shortened lifespan, but other knockout mice did not? Pérez et al. (2009) gave a semi-quantitative explanation. The nuclear DNA damage level is about ~400–500% higher in the livers of $Sod1^{-/-}$ mice than WT mice, but only 40% higher in the $Gpx1^{-/-}$ or $Sod2^{+/-}$ mice. The authors also observed that the nuclear DNA damage in $Sod1^{-/-}$ mice increases much faster with age than that in $Gpx1^{-/-}$ or $Sod2^{+/-}$ mice. Thus, when the comparison is made between $Sod1^{-/-}$ and other knockout mice, the results agree with the OST, i.e., higher damage level in $Sod1^{-/-}$ mice leads to shorter lifespan. However, this analysis does not answer the second question—why do other knockout mice, such as $Sod2^{+/-}$, have the same lifespan as the wild type, while the damage levels in them are higher?

We combine our Assumption 3 (lifespan is reached when damage reaches a threshold) and the argument from Sohal and Forster (2014) to answer this question. Attempting to explain why the relatively high DNA damage in OGG1-null mice showed no effect on survival, Sohal and Forster (2014) suggested that this is because "high threshold levels of DNA and protein oxidative damage are required for manifest losses in function". Here, we extend Sohal and Forster's argument. We notice that in the data from (Van Remmen et al., 2003), although the DNA damage is higher in the $Sod2^{+/-}$ mice than the wild type at all ages, the increase of damage with age in the knockout mice is slower than that in the wild type controls. Fig. 6A shows the increases in nuclear DNA damage from age of 3–6 months to 26 months in four tissues (re-plotted data from Van Remmen et al., 2003). Except for the spleen, the increases in liver, brain, and heart of $Sod2^{+/-}$ mice are slower than that of wild type mice. The average increases over four tissues from young to old age are 103% in wild type, but only 50.5% in $Sod2^{+/-}$ mice. Taking the DNA damage in the heart as an example, Fig. 6B shows the trend of the increases in damage with age, where the dots are empirical data from (Van Remmen et al., 2003). The dashed lines in Fig. 6B indicate that if the increases in damage keep the same trends as they were during the ages of 3–6 months to 26 months, the faster increase in the wild type (black dashed line) will catch up with the damage in $Sod2^{+/-}$ mice (red dashed line) around age 40 months. Now, we recall the Assumption 3 in our model—animals die when the damage level reach a threshold. It is possible that these two groups of animals die at the same age, if the threshold of the damage is in the neighborhood where the two damage lines meet. Interestingly, in that study of (Van Remmen et al., 2003) the maximum lifespan of both groups is about 40 months, where the two damage lines meet in Fig. 6B.

It should be noted that we do not intend to draw a quantitative conclusion based on Van Remmen's data, which only gave two points at young and old ages (3–6 months and 26 months) for each group of mice. Obviously, two points are far from enough to draw a damage profile and to estimate the exact slope of the increase. Nonetheless, this analysis does give a qualitative explanation—it is possible that the higher damage level in knockout mice can lead to the same lifespan as the wild type mice, because the damage increases more slowly in the knockout mice, so that the threshold is reached at the same age in both groups.

More interestingly, our qualitative analysis can also potentially reconcile the discrepancy in the results from the knockout experiments conducted in different laboratories. Moskovitz et al. (2001) reported a 40% reduction in mean and maximum lifespan in $MsrA^{-/-}$ mice, but Richardson's lab found no difference in the lifespans of $MsrA^{-/-}$ mice. Pérez et al. (2009) noticed that the living conditions used in Moskovitz et al. (2001) is sub-optimum, because the lifespan of WT mice in Moskovitz et al's study was 680 days, whereas that in Richardson's lab was 925 days, indicating a better condition in Richardson's. The semi-quantitative analysis by our

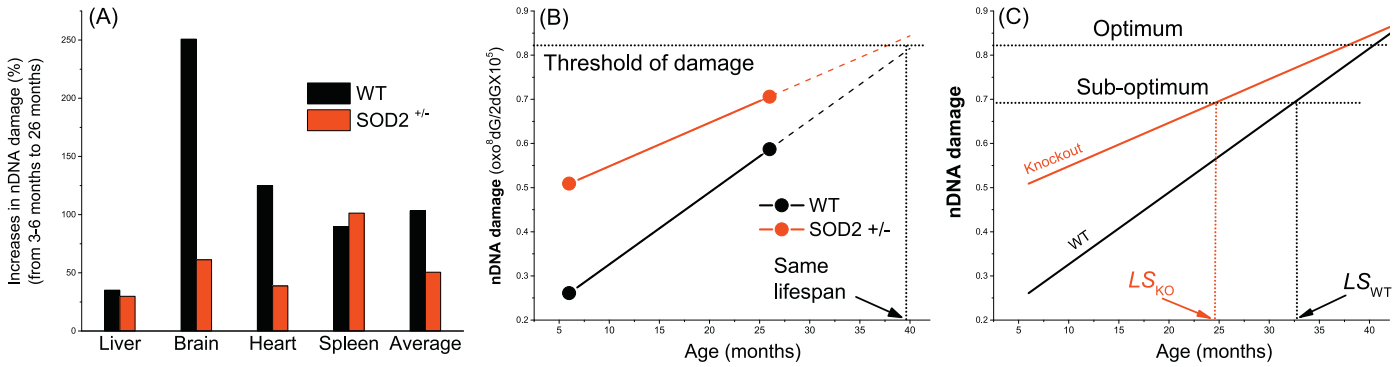

Fig. 6. Nuclear DNA damage increases slower in *Sod2*$^{+/-}$ mice. (A) Percentage increases in nuclear DNA damage from age of 3–6 months to 26 months in four tissues and the average in wild type and knockout mice. (B) Nuclear DNA damage in heart tissue of wild type and knockout mice. The dots are data from (Van Remmen et al., 2003). The dashed lines indicate the trends of the increases in DNA damage after the age of 26 months. The horizontal line indicates the threshold of the damage, and the vertical line indicates the lifespan where the damage reaches the threshold. (C) Same damage profiles of wild type and knockout mice as in (B). Under sub-optimum conditions, the damage threshold line is lower, so that the damage in knockout mice reaches the threshold sooner, and die sooner.

model suggests that the difference in living conditions (indexed by the lifespan of wild type animals) may help to understand the discrepancy between the effects of knockout in these studies. Animals die sooner in the sub-optimum environment, which means that the threshold that the damage level needs to reach at death is lower. As shown in Fig. 6C, with lower damage threshold in the sub-optimum environment, the wild type and MsrA$^{-/-}$ mice died before the two damage lines meet. In other words, under the sub-optimum conditions, the knockout mice reach the lower threshold sooner than the wild type, and die sooner, whereas under better conditions, the threshold is higher so that the damage in the wild type mice with a faster slope has enough time to catch up with that in the MsrA$^{-/-}$ mice, and die at the same age.

Here, two points need clarification. First, in our model the slope of the damage curve depends on the mass-specific metabolic rate, so the slower increase in the damage of the knockout mice may be attributed to the lower metabolism. However, since none of the knockout studies reported metabolic rate, this postulation is yet to be tested. Second, if the damage threshold is even lower than that under the sub-optimum conditions, which can be caused by environmental factors, such as infectious risks, or by genetic factors, such as cancer-promoting genetic background, then the difference in lifespan between the knockout and wild type animals would be even bigger. This model offers a quantitative framework to estimate the effects of living conditions on the knockout treatment. Future studies are called for to test the prediction.

4. Conclusion

We have developed a simple theoretical model based on the first principles for understanding the relationships between energy expenditure, cellular damage, and lifespan. Considering the energy reshuffling between life history traits and the alteration in the protective efficiency induced by empirical manipulations and selection, the model successfully reconciles a series of seemingly contradictory empirical results. Oxidative energy metabolism can affect longevity in different ways in animals with different life history and under different experimental conditions. Qualitative data and the simple proportionality between energy expenditure and lifespan assumed in previous studies are not sufficient to understand the complexity of the relationships. Our model offers a theoretical framework for quantitative analyses. The quantitative predictions of the model are strongly supported in the cases where the quantitative empirical data is available. The qualitative and semi-quantitative analyses conducted in this paper remain to be improved and verified by future quantitative research.

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Appendix A.

Incomplete mitochondrial coupling due to proton and electron leak

Some studies suggested that due to proton leak cross mitochondrial inner membrane and electron leak along the respiratory chain, a fraction of consumed oxygen is not involved in the production of ROS, and therefore metabolic rate (measured as oxygen consumption) may not be proportional to ROS production and cellular damage (Barja, 2013; Brand, 2000; Speakman et al., 2004). However, the incomplete mitochondrial coupling does not offer a complete explanation for the disproportionality between oxygen consumption and net damage level. Under general conditions, the percentage of oxygen consumption that drives proton leak is roughly a constant, ranging from 15 to 30% and clustering around 20%, in a series of tissues from different animals with a wide range of body mass and taxon (Brand, 2000). Thus, the fraction of oxygen consumption that is involved in ROS production is also roughly a constant (~80%). This means that in these cases, the overall oxygen consumption (counting proton leak) is proportional to ROS production.

Variation of percent free radical leak (the percentage of electrons out of sequence) also causes the disproportionality between ROS production and oxygen consumption (Barja, 2013). But, the percent free radical leak varies in small ranges. For example, in heart mitochondria, it varies between 0.6% (pigeon) to 1.3% (rat), and between 0.9% (DR rat) and 1.6% (free-feeding rats) (Fig. 9 in Barja, 2013). This indicates that the non-leak percentage is above 98% across a broad variety of situations. It is quantitatively unclear if this small variation can cause a large disproportionality and a large difference in lifespan. Moreover, empirically measured ROS level from mitochondria may represent only a small fraction of the total ROS generated due to the scavenging mechanisms (Balaban et al., 2005). Thus, the DR-induced decrease in ROS level may be attributed to the enhanced ROS scavenging mechanisms by DR channelling extra energy to protection, as we suggested in Figs. 1 and 4. The percentage of electron leak can also vary during exercises, where the mitochondrial respiration transits from state 4 to state 3 (Barja, 2007, 2013). Under state 4 (resting respiration), oxygen consump-

tion is low, proton-motive force is high, and ROS production is high (Barja, 2013; Harper et al., 2004), whereas under state 3 (active respiration), ROS production reduces rapidly (Boveris and Chance, 1973; Boveris et al., 1972; Loschen et al., 1971). But exercise should not be considered as a general condition that animals live in. In fact, mitochondria generally operate in the states between states 3 and 4, in which ROS production is neither extremely high nor low for a given oxygen consumption (Barja, 1999; Harper et al., 2004). In summary, proton and electron leak cause disproportionality between oxygen consumption and ROS production, especially in some special cases such as exercise bout. However, the leak alone cannot provide the full explanation. Due the two factors discussed in the main text, namely energy allocation and protective efficiency, the net cellular damage may still not be proportional to the non-leak dependent oxygen consumption.

Appendix B.

The changes in metabolic rate and activity level of animals under diet restriction

Metabolic rate (B) scales with body mass (M) as $B = B_0 M^{\alpha}$, where B_0 is the normalization coefficient, and α is the scaling power. Generally, the metabolic scaling power is about 3/4. B_0 changes with body temperature exponentially as $B_0 \sim e^{-E_0/KT}$ (the Boltzmann–Arrhenius factor, equivalent to the Q10 effect), where E_0 is the average activation energy of metabolism (c. 0.65 eV), K is Boltzmann's constant (8.62×10^{-5} eV/Kelvin), and T is body temperature in Kelvin (Gillooly et al., 2001).

Using this allometric scaling law, we have the mass-specific metabolic rate, $B_m = B/M = \left(B_0 M^{3/4}\right)/M = B_0 M^{-1/4}$. Now, we compare the mass-specific metabolic rate of diet-restricted (DR) animals ($B_{m,DR}$) and that of free-feeding (AL) controls (B_m) by estimating their ratio,

$$\frac{B_{m,DR}}{B_m} = \frac{(B_{0,DR} M_{DR}^{-1/4})}{B_0 M^{-1/4}} = \frac{B_{0,DR}}{B_0}\left(\frac{M}{M_{DR}}\right)^{1/4} \quad \text{(B.1)}.$$

Eq. (B.1) shows that the ratio of the mass-specific metabolic rate depends on two factors, $\left(M/M_{DR}\right)^{1/4}$ and $(B_{0,DR}/B_0)$.

To estimate a general body mass ratio, we used the data collected by Hou et al. (2011b) from 47 studies on mice and rats. After excluding two studies, in which the DR degree was too severe (30% of ad libitum level), we found that the ratio of the body mass of AL and DR rodents is about $\left(M/M_{DR}\right)^{1/4}$=1.68±0.31 (N =45, and the DR degree ranges from 45% to 78%). So, $\left(M/M_{DR}\right)^{1/4}$ = 1.138. This means, if the normalization coefficient does not change, i.e., $B_0 = B_{0,DR}$, the reduction of body mass will cause the mass-specific metabolic rate to increase by ~14%, i.e., $B_{m,DR}/B_m = \frac{B_{0,FR}}{B_0}\left(\frac{M}{M_{DR}}\right)^{1/4} = 113.8\%$. Thus, a 68% reduction in body mass will result in a 14% increase in mass-specific metabolic rate, if B_0 stays unchanged.

However, most diet restrictions cause reductions in body temperature, especially in small rodents. Hou et al. (2011b) collected the data from 15 studies on mice and rats, which explicitly gave the reduction in body temperature induced by DR. The temperature drop ranges from 0.8 to 4.5 °C, and the DR degree varies from 40% to 90%. We took the data and regressed the temperature drop on the DR degree (in percentage of the ad libitum). The linear regression shows that temperature drop is weakly correlated to diet restriction degree ($Y = -0.03X + 3.89$, $R^2 = 0.03$). Because the correlation is weak and the slope of the regression is close to zero, we use the mean value of the drop, 2.01 ± 1.25 °C, for the following estimate.

The normalization coefficient (B_0) decreases with temperature exponentially as $B_0 \sim e^{-E_0/KT}$. Using the values of E_0 and K given above, we estimate that when the temperature reduction is about 2 °C, the reduction in B_0 is about 15%: $B_{0,DR}/B_0 = e^{-E_0 \times (1/KT_{DR} - 1/KT)} = 0.852$. Thus, the 14% increase in mass-specific metabolic rate induced by the change in body mass is offset by the 15% decrease induced by the temperature drop. Combining these two factors, we have $B_m = \frac{B_{0,FR}}{B_0} \times \left(\frac{M}{M_{DR}}\right)^{1/4} = 1.137 \times 0.852 = 0.97$, which means that the mass-specific metabolic rate (B/M) is roughly unchanged in diet-restricted animals.

We need to point out that the calculation above is approximate and is based on the rough estimates of the changes in body mass (M/M_{DR} = 1.68) and the changes in temperature ($T - T_{DR}$ = 2 °C). But this rough analysis shows that the drop of body temperature offsets the effect of body mass reduction, so the overall mass-specific metabolic rate of DR animals may slightly increase (Faulks et al., 2006a; McCarter et al., 1985), stay the same (McCarter and McGee, 1989b; McCarter and Palmer, 1992; Rising and Lifshitz, 2006), or slightly decrease (Gonzalespacheco et al., 1993; Rising and Lifshitz, 2006).

Table B.1
Changes in activity level of animals under diet restriction.

Species	Strain	DR level	Activity	Source
Mouse	QS		=	Faulks et al. (2006a)
Mouse	Golden spiny	70%	Slight⇑	Ehrhardt et al. (2005)
Mouse	B6C3F1	60%	73% ⇑	Duffy et al. (1997)
Mouse	CD1	60%	300%⇑	Chen et al. (2005)
Rat	F344	60%	=	Mccarter and Mcgee (1989a)
Rat	FBNF1	65%	=	Evans et al. (2005)
Rat	F344	60%	15% ⇑	Duffy et al. (1997)
Rat	S-D	80%	Slight⇑	Rising and Lifshitz (2006a)
Rat	S-D	70%	= or*arrow*	Rising and Lifshitz (2006a)
Rat	S-D	60%	⇑or*arrow*	Rising and Lifshitz (2006a)
Rat	F344	60%	15% ⇑	Duffy et al. (1989)
Rhesus monkey			=	Ramsey et al. (1997)
Rhesus monkey		70%	Slight*arrow*	Moscrip et al. (2000)
Rhesus monkey	Young	70%	=	Weed et al. (1997)
Rhesus monkey	Old	70%	60% ⇑	Weed et al. (1997)

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