You don't need a weatherman: famines, evolution, and intervention into aging

Michael J. Rae

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Abstract Calorie restriction (CR) is the most robust available intervention into biological aging. Efforts are underway to develop pharmaceuticals that would replicate CR's anti-aging effects in humans ("CR mimetics"), on the assumption that the life- and healthspan-extending effects of CR in lower organisms will be proportionally extrapolable to humans (the "proportionality principle" (PP)). A recent argument from evolutionary theory (the "weather hypothesis" (WH)) suggests that CR (or its mimetics) will only provide 2–3 years of extended healthy lifespan in humans. The extension of healthy human lifespan that would be afforded by intervention into aging makes it crucial that resources for therapeutic development be optimally allocated; CR mimetics being the main direction being pursued for interventive biogerontology, this paper evaluates the challenge to the potential efficacy of CR mimetics posed by the WH, on a theoretical level and by reference to the available interspecies data on CR. Rodent data suggest that the anti-aging effects of CR continue to increase in inverse proportion to the degree of energy restriction imposed, well below the level that

would be expected to be survivable under the conditions under which the mechanisms of CR evolved and are maintained in the wild. Moreover, the same increase in anti-aging effects continues well below the point at which it interferes with reproductive function. Both of these facts are in accordance with the predictions of evolutionary theory. Granted these facts, the interspecies data—including data available in humans—are consistent with the predictions of PP rather than those of the WH. This suggests that humans will respond to a high degree of CR (or its pharmaceutical simulation) with a proportional deceleration of aging, so that CR mimetics should be as effective in humans as CR itself is in the rodent model. Despite this fact, CR mimetics should not be the focus of biomedical gerontology, as strategies based on the direct targeting of the molecular lesions of aging are likely to lead to more rapidly developable and far more effective anti-aging biomedicines.

Key words aging/drug effects · calorie restriction · evolution · longevity/drug effects · models · biological

Abbreviations

AL	<i>ad libitum</i>
CR	calorie restriction
LS	lifespan
PP	proportionality principle
WH	weather hypothesis

M.J. Rae (✉)
Calorie Restriction Society,
Society Cohort Study Team,
1827 W. 145th St, Suite 205,
Gardena, CA 90249, USA
e-mail: michaelrae@cadvision.com

Introduction

Calorie restriction—the selective reduction in energy intake, without compromise of essential nutrients—is the most robust non-germline intervention into biological aging known to gerontology (Weindruch and Walford 1988; Masoro 2002). In a wide range of experimental animals—most notably laboratory rodents, but also including yeasts, roundworms, fruit flies, spiders, fish, and (preliminary evidence suggests) cattle (Pinney et al. 1972), dogs (Kealy et al. 2002), and nonhuman primates (Lane et al. 2002)—CR has been demonstrated to extend both mean and maximum lifespan (LS); to reduce the incidence or progression of age-associated diseases; and to preserve physiological function and molecular fidelity with age. In recent years, optimism that the phenomenon will translate to human application has grown considerably, to the point that it appears to be the consensus view in the biogerontology community. As a result, many researchers in the private sector and in academe are now enthusiastically pursuing the development of “CR mimetics” (Lane et al. 2002; Miller 2002; Longo and Finch 2003): pharmaceuticals designed to extend youthful LS by exploiting CR’s fundamental mechanism(s) of action. It is therefore initially surprising to learn that so bullish an advocate of the prospects for genuine anti-aging medicine as Dr. Aubrey de Grey is in fact among the most pessimistic of biogerontologists in his assessment of the potential of CR (or its pharmaceutical simulation) to retard biological aging in humans (de Grey 2005a).

Within rodent species, it is clear that the extension of healthy lifespan is a linear inverse function of calories consumed. Recently, and somewhat counterintuitively, it has been found that this anti-aging effect is fully available even at relatively advanced ages, after substantial molecular aging damage has already accumulated (Dhahbi et al. 2004; Rae 2004). The standard extrapolation of these findings, which available interspecies CR data seem *prima facie* to uphold, is that a given degree of CR imposed on an animal of a given species leads to a similar extension of LS expressed as a proportion of the species maximum LS: what de Grey terms “the proportionality

principle” (PP). Against this, de Grey advances what we may call the “weather hypothesis” (WH): a theoretical argument, rooted in the dominant understanding of the role of evolution in aging and the CR phenomenon (Kirkwood and Austad 2000; Holliday 1989; Harrison et al. 1984; Edney and Gill 1968). The WH proposes that because the evolutionary basis for the CR effect is believed to be the value of the capacity to change life-history “strategy” in response to the threat of starvation, and that

“starvation of a given duration necessarily occurs at broadly the same frequency, on average, for all terrestrial animals—whatever their *ad libitum*-fed life expectancy ... each species will experience periods of famine, and that periods up to a year or so will be of roughly comparable frequency, but periods longer than that will be rarer. ... [Therefore] all such species ‘know’ how to live several months longer when starved than they do when food is plentiful ... because this is a useful facility often enough to be maintained during evolution. Conversely, it is proposed that ... no such species ... knows how to live 5 years longer than normal [because] this is too seldom useful to have been evolved or retained” (de Grey 2005a: 77).

Thus, the WH suggests that since the selective pressure which creates and maintains the capacity to extend lifespan in response to CR is the result of the need to survive famines, and that since famines will last for the same *absolute* length of time for all species irrespective of the *relative* proportion of the LS of a given organism which such famines consume, therefore the force of selection will drive all organisms to retain the capacity to extend LS by a broadly similar absolute, rather than proportional, amount, because this adaptation exists in order to preserve life through famines of similar absolute, rather than proportional, duration. Thus, no species—irrespective of its *ad libitum* (AL) LS—will gain more than 2–3 years of LS from CR (or CR mimetics) (de Grey 2005a).

de Grey then presents an interpretation of the available data on the interspecies variation in LS extension achieved via CR, which de Grey states

to be “strikingly in line with” the “broadly quantitative prediction of that departure” from PP made using the WH (de Grey 2005a). While the interspecies data summarized in the paper’s Table 1 and in the text does superficially appear to support this contention, further analysis leads to the opposite conclusion.

Methods

The present paper will outline the flaws in the WH, and establish on a firmer footing than has been done in the past that both evolutionary theory and the empirical data support the PP over the WH.

Results and discussion

“False Echoes” in the forecast

Both in arguing for the WH from an evolutionary standpoint and in interpreting the relevant empirical evidence, de Grey confounds the anti-aging effects of CR, which lead to extended healthy lifespans in animals held in a protected (laboratory) environment, with the ability to simply survive famine. Clearly, there is no purely physical reason why the two must be directly linked. In principle, an organism might well have adaptations that allow it to avoid simply starving to death from reduced energy availability, but which do not affect the rate of escalating loss of molecular fidelity that underlies biological aging. Perhaps less obviously, the anti-aging effects of CR may likewise involve adaptations independent of those required to survive the immediate “energy crisis” of a famine.

This confounding leads de Grey to often argue from the related implicit assumption—erroneous, as will be demonstrated—that the “thermostat” by which an organism adjusts the rate of aging in proportion to the restriction of energy availability must have a lower threshold, and that this threshold is the same as that which exists for the organism’s ability to survive famine.

A close connection between the survivability of a given degree of CR and the organism’s incapac-

ity to continue to retard aging below this level of CR is in fact a key prediction of the WH, which is presented as though it were an evolutionary argument in favor of it. For if the “thermostat” which determines how much the organism will retard aging in response to a given level of CR has its range determined by the frequency, severity, and absolute duration of survivable famines which the organism has “encountered” in its evolutionary history, then famines to which the organism has not had sufficient exposure to evolve the capacity to simply survive will also be famines which will not have exerted sufficient evolutionary pressure to drive an anti-aging response. In short, there is no evolutionary logic to retarding aging in response to conditions which are lethal for other, more immediate reasons.

When this concept is evaluated against the existing rodent data on CR, we find that the evidence refutes this prediction, thereby providing a *modus tollens* refutation of the WH itself. For whereas ~60% CR can be survived by animals in the sheltered environment of the laboratory, and whereas pushing the organism down to this same level of CR leads to an inversely proportional extension of healthy LS relative to milder CR, it is clear that the resulting phenotype would be incompatible with survival in the wild. For example, severely CRed animals display inhibited wound healing (Harrison et al. 1984), reduced sprinting capacity (Hopkin 2003), and a combination of reduced body fat and lowered core temperature which would greatly increase vulnerability to cold—a cause of death which already claims an extremely high percentage of mice. Longitudinal field studies in Wales and Scotland report 80–90% mortality in typical winters, increasing to 95% in colder years, on islands where death from predation is only a very minor cause of death (Berry and Scriven 2005; and personal communication, RJ Berry). Indeed, even AL-fed rodents in the climate-controlled conditions of the laboratory are vulnerable to death from hypothermia resulting from simple wetness following cage-flooding consequent to water-bottle accidents (Armed Forces Institute of Pathology 2001).

If the core arguments leading to the WH were sound, organisms would not be able to retain the ability to further decelerate biological aging

beyond a level of CR that the organism should not be statistically able to survive in the field. And considering the high level of extrinsic mortality to which wild-living *M. musculus* is already exposed (field studies find that the median LS of such mice is ~130 days, and 90% mortality is suffered by ~280 days (Phelan and Austad 1989), the reasoning supporting the weather hypothesis must deny that there is any evolutionary purpose served by specifically retaining the ability to reduce their *intrinsic* mortality rate when the resulting phenotype will make death due to *extrinsic* causes a near certainty.

Yet evolution has equipped rodents not only with the ability to survive, under laboratory conditions, reductions in caloric intake well below what could be survived in the wild, but to continue, in monotonic fashion, to reduce their rate of aging as calories are reduced to such levels. It may be argued that the ability to survive such severe levels of CR is retained to deal with occasional severe downward fluctuations in energy availability during a period when average caloric intake is higher. This seems unconvincing, both because existing adipose and other tissue could still be cannibalized for such brief periods without special adaptation, and because it is unclear how a further deceleration of the rate of aging for so transient a period could appreciably increase fitness during or after the famine's end. Indeed, de Grey points out that such an interpretation is not, in fact, reconcilable with the "weather hypothesis": "Famines that are long and severe enough to elicit the organism's maximal life extension response may not in fact be all that rare in terms of *average* severity measured over their entire duration, but most such famines may contain periods of substantially worse food shortage, sufficient to kill the organism quickly. Only famines that do not contain such periods are relevant to the selective pressure to survive" (de Grey 2005a).

Instead, what these examples suggest is that the machinery that decelerates the aging rate in response to CR is a single, simple thermostat, which upregulates defenses against aging damage in linear, proportional fashion to reduced energy intake. There is no check in place to monitor against excessive investment in anti-aging mech-

anisms in response to the realistic survivability of famine, as the WH would seem to require. One alternative hypothesis, which would conform PP with evolutionary theory, would be that it is "thrifter" for the genome to invest in a *potentially* needless capacity to activate robust anti-aging mechanisms than to create a second level of biological machinery to regulate, check, or limit the extent to which the "thermostat" can be "turned down," since if the organism is going to die in any case, the extra resources wasted in retarding its rate of aging are unlikely to lead to survival if redirected elsewhere.

At most, the original evolutionary analysis presented in arguing for the WH explains the failure of rodents to evolve the capacity to *survive* CR that is more severe than ~60% as being due to a lack of selective pressure to do so. However, even this reasoning is not ironclad, as we shall see in analyzing the first of de Grey's interspecies case studies in aging and CR. In this, as in other cases reviewed in the original article (de Grey 2005a) we shall see that the data not only do not support the WH, but that they do not do so even on the argument advanced. What we are instead given is *predictions* about the results of yet-to-be-performed experiments, while the data to hand actually weigh in against it in favor of the PP.

Baby, it's cold inside: C. elegans and drastic metabolic arrest

In his first interspecies case study on the effects of caloric intake on longevity, de Grey notes that in *Caenorhabditis elegans* "A reduction in food availability initiated at the last larval moult extends LS by about 60% relative to "ad libitum" food density, very much the same proportional extent as the maximum resulting from caloric restriction (CR) in mice"—a finding which is *prima facie* in line with the PP. But, he goes on to argue, there is still a "stark non-proportionality of the *maximum* life extension elicited (by any directly or indirectly starvation-related means) in different species" because

"*C. elegans* exhibits a much more dramatic phenotype, however, when subjected during early larval life to a more severe [CR] ... a

state known as dauer, a developmental arrest in which energy utilisation is drastically reduced. Dauer larvae can survive for three months without harm ... Numerous other poikilotherm species are also capable of surviving complete absence of nutrients for similar or even longer periods. Conversely, this degree of starvation of mice is, of course, rapidly fatal ...”

“A traditional gerontological interpretation of the observations mentioned thus far is as follows: moderate CR induces a moderate increase in lifespan. In simple organisms ... metabolism is sufficiently plastic that a much more severe reduction in food supply can be tolerated, by adopting a drastically altered metabolic state. In higher animals, by contrast, the metabolic state is more tightly constrained by the structure and function of various tissues, so no comparably fundamental metabolic shift is possible. Teleologically speaking, evolution has come closer to “running out of ideas” to increase lifespan of already long-lived species” (de Grey 2005a: 74).

Here, we should note that the last sentence is rather a *non sequitur*, based on the previously noted confounding of the ability to survive a given level of CR with the organism’s anti-aging response to famine. The inability of mice to survive truly extreme starvation may well be “more tightly constrained by the structure and function of various tissues”, without any implication regarding the limits to the ability of evolution to build the capacity to retard aging into those organisms in response to the same degree of starvation (or via other means and in response to different evolutionary pressures). Indeed, de Grey is careful to make this distinction in arguing for the possibility that existing data on dauer life extension in *C. elegans* may underestimate this species’ actual capacity for retarded aging in response to CR: “in the original study, post-dauer lifespan of individuals restored from dauer after two months had the same remaining life expectancy as ones that had been in dauer for only five days, whereas a truly maximal period in the dauer

state should intuitively entail a certain amount of aging. This indicates that *dauers may simply starve to death rather than ageing “only” 3–4 times slower than fed worms*” [emphasis added](de Grey 2005a: 74).

That the further restriction of calories beyond a limit of ~60% leads to death in rodents does not necessarily demonstrate that this same reduction does not further decelerate the processes of biological aging, nor that evolution has “run out of ideas” on how to do so: these are instead *predictions* of the WH. The contrary hypothesis, that this limit may simply reflect the fact that the organism’s basic physiological functions cannot be maintained below a certain minimal threshold of energy, irrespective of its rate of aging, is not refuted by its analysis, although it is against its predictions. Fortunately, data to hand give us reason to believe that the question can be settled empirically, with minimal recourse to purely theory-based reasoning.

Mice, unlike *C. elegans*, are homeotherms, and their basic biological processes are dependent on maintaining a regulated internal body temperature, which in turn requires a minimum rate of metabolism (a design specification that does not apply in poikilothermic species). Indeed, this can be seen by examining the phenomenon most closely resembling the dauer state in mammals: hibernation.

Hibernation is a particularly useful comparison, because it is not only an example of a mammalian state of considerably repressed metabolism, but may be an anti-aging metabolic state as well. Data on the effect of time spent hibernating and the aging process in Turkish hamsters strongly suggest that hibernation in small mammals does retard biological aging, in proportion to the amount of time spent in the hibernating state (Lyman et al. 1981). It is commonly assumed that small hibernating mammals remain torpid throughout their hibernation period. Instead, hibernators exhibit repeated cycles of torpor and arousal throughout it, marked by dramatic changes in metabolism, including wide—but carefully orchestrated—fluctuations in body temperature (Carey et al. 2003). While the reasons for this phenomenon are not fully understood, it is clear that much of it can be attributed to the fact that key physiological

functions simply cannot be maintained at the nadir of torpor. To preserve basic life processes, small endothermic hibernators undergo periodic arousals to temporarily restore core metabolic functions, from transcription and translation to digestive, renal, and immune function and mitochondrial respiration (Carey et al. 2003).

All of these data suggest that the “traditional gerontological explanation” of the capacity of *C. elegans* to more drastically retard aging via the dauer state, with the absence of a parallel in mammalian species, is essentially correct. The kind of radical shift in metabolic state exemplified in the *C. elegans* dauer state (and other cases of metabolic arrest noted elsewhere by de Grey, such as diapause in *Drosophila*) may well be incompatible with homeothermic life, even in organisms adapted to enter into similar states, not because of a lack of evolutionary pressure to retard aging in response to severe famine but because such a radical slowing of metabolism would depress essential metabolic functions in ways ruled out by the central design features that separate them from ectotherms.

Moreover, even if we agree that the original evolutionary analysis presented in arguing for the WH explains the failure of rodents to evolve the capacity to survive CR more severe than ~60% (or hibernation more sustained than is observed in the field) as being due to a lack of selective pressure to do so rather than some inbuilt aspects of the organism itself, that does not represent evidence against the PP. Rather, it shows that the PP is incompatible with the reasoning underpinning the WH. As already argued above, in this case the conclusion to draw is that the WH fails.

But in any case, the entire line of attack against the PP begins from a straw man: that the “stark non-proportionality of the *maximum* life extension elicited (by any directly or indirectly starvation-related means) in different species” (de Grey 2005a) would in any way constitute evidence against it. The PP itself does not predict *limits* of the degree of CR that an organism can survive, but only that a given degree of CR will result in a similar proportional extension of the AL LS across species. The fatality of >60% CR in rodents is in itself not relevant to this prediction. Indeed, it may even be that in response to more

severe CR, rodents undergo gene expression modulations that still further retard the accumulation of molecular disorder in their bodies, which *would* lead to delayed loss of physiological function and slower acceleration in vulnerability to disease and death, so that such animals *would* live longer, were it not for the unfortunate intervention of death from starvation.

“Relative humidity” makes foggy windows?

Another argument advanced by de Grey to undermine PP is the suggestion that the striking parallel in relative LS extension at similar levels of CR between *C. elegans* and the laboratory mouse may actually underestimate the capacity of the former to extend life in response to famine, because the relative LS extension observed is in comparison to an artificially short AL lifespan in the roundworm controls. This is argued on the basis of such findings as the much greater LS “achieved by a combination of genetic and surgical interventions whose gene expression effects substantially overlap the dauer profile [and that] these original experiments, like most using *C. elegans*, involved feeding standard *E. coli*, which has been shown to reduce nematode LS relative to bacteria with a lipid composition more similar to typical soil bacteria” (de Grey 2005a: 74).

The first counter to this line of argument is that it is simply a case of *argumentum ex silentio*, not data to hand in support of the WH (de Grey 2005a). Just as importantly, we can largely dispense with this concern by reference to the existing data from the rodent model. Improvements in feed quality that reduce pathology and/or extend LS in AL rodents either exert additive benefits when fed under CR conditions (King and Visscher 1950; Ross and Bras 1973), so that no *disproportional* increase is gained, or yield no additive benefit to CR (Davis et al. 1983; Tapp et al. 1989; Kemi et al. 2000).¹ Likewise, the

¹ An important exception to this rule is frank malnutrition. While juvenile rodents respond robustly to the simple feeding of reduced portions of the AL diet, this protocol has been repeatedly demonstrated to fail in adult and older organisms; full provision of essential nutrients appears to be essential for effective anti-aging CR in these animals (Weindruch and Walford 1982; Dhahbi et al. 2004; Rae 2004).

objection that the finding “has apparently not been repeated under a range of conditions” (de Grey 2005a) may be countered by the observation that other environmental manipulations that favorably impact LS in AL-fed rodents have a relatively blunted effect in the CR cohort (eg. Snyder et al. 1990).

De Grey advances a similar argument regarding the capacity of genetic manipulation to extend lifespan in these organisms, first by suggesting that the effects of such manipulations are comparable to the effects of CR, making an across-the-board interspecies comparison of maximal LS extension obtained from both types of intervention cogent, and then introducing examples such as the “maximum lifespan of 6 months ... achieved by a combination of genetic and surgical interventions whose gene expression effects substantially overlap the dauer profile” in *C. elegans*, to suggest that the larger proportional LS extensions afforded by these “CR-like” genetic interventions in lower organisms vs laboratory mice refute the PP (de Grey 2005a: 74).

There are two major objections to this line of reasoning. The first is the one already mentioned: that the capacity for an even greater relative LS extension in response to these interventions, as in the case of more severe CR, does not in fact refute the Principle, for the defense of which we only need to show that a *given* degree of CR (or, if we accept de Grey’s equation of these interventions, “CR-equivalent genetic manipulations”) leads to a similar proportional extension of the LS on an interspecies basis—not that the organism can survive any *particular* level of CR or CR-equivalence.

The second is to contest the analogy of the interventions. While there is indeed substantial overlap between the gene expression shifts elicited by CR and some life-extending genetic manipulations, it is clear in the rodent case that the two are not isomorphic, and may well work in part through distinct mechanistic pathways (Tsuchiya et al. 2004; Miller et al. 2002). Interestingly, one gene expression profile study “revealed divergent responses of dwarf and normal animals to CR [itself,] raising an interesting possibility that CR may affect longevity of normal and dwarf mice by different mechanisms”

(Masternak et al. 2004). And while there are reasons to be cautious regarding the interpretation of the results (Barger et al. 2003), preliminary evidence in rodents suggests that CR has additive LS-extending benefits to these genetic manipulations (Shimokawa et al. 2003; Bartke et al. 2001). One possible reading of these data is that their mechanisms of life extension are not identical, and that greater maximal level of LS extension generated by the latter need not imply anything about the relative interspecies efficacy of the former. To question whether it may mischaracterize the very nature of the genetic (including both actual genes, and gene expression) coordination of the anti-aging response elicited by CR is an additional, though more speculative, objection.

All of these arguments suggest that optimization of husbandry or even modulation of genetic pathways related to CR within a species may not lead to a significant *relative* increase in the extension of LS by CR itself, in which case the parallel increase in LS from roundworm to rodent would remain intact under the conditions which de Grey proposes. In short, the original, *prima facie* reading of the *C. elegans* data as supporting PP appears to be the correct one.

The remaining interspecies data discussed by de Grey also seem to be more parsimoniously interpreted in terms of the PP rather than the WH.

Like a dog in the rain

The next example cited in the “Weather” paper is the results of Kealy et al. (2002) in dogs. de Grey summarizes the experiment as follows:

Labrador retrievers were given a diet reduced by 25% in calories from the age of 8 weeks. Their maximum lifespan was about 14 months longer than that of control animals. The authors of this study did not discuss why they chose a CR level of 25%, rather than the 40% more typical of rodent CR experiments; the hypothesis presented here predicts that a 40% CR regime would not extend the dogs’ lifespan further (and might be harmful) [de Grey 2005a: 79].

However, this summary fails to capture important aspects of the study design. While it reflects the description given in the publication of the LS result (Kealy et al. 2002), an earlier publication gives more complete details:

“Labrador retrievers were paired ... at 8 weeks of age. One dog of each pair was fed ad libitum. The limit-fed pairmate was fed 75% of the amount eaten the previous day by the ad libitum-fed counterpart. All dogs received the same diet ... When the dogs were 3.25 years old ... the amount of food [given to the AL animals] was reduced and fed at a constant amount to prevent insidious development of obesity ... Limit-fed dogs continued to receive 75% of the amount fed [on the previous day] to the corresponding ad libitum-fed pairmate” [Kealy 1999: 34].

In personal communication, one of the investigators, Dennis Lawler, specified that this constituted a 17% reduction in caloric intake. What is important about this additional information is that, as de Grey notes, the current standard protocols for LS studies in CR, pioneered by Weindruch and Walford (1982), evaluate LS in the CR group “relative to non-obese controls” (de Grey 2005a: 74). This is achieved because the control group is not allowed to eat a genuinely “ad libitum” diet, but is restricted by 10–20%. It is relative to *this* “AL” control diet that a given amount of CR is compared. As we can see from the more detailed description of the protocol (Kealy 1999; and personal communication, Dennis Lawler), “CR proper” was imposed only at 3.25 years of age—well into adulthood (which in dogs begins within the 2nd year of life), and >24% into the AL cohort maximum LS.

This means that at 25% adult-onset CR the dogs gained an 11% increase in median and an ~8% increase in maximum LS (Kealy et al. 2002), although this latter figure was nonsignificant in this small ($n=24$) CR cohort. These are figures roughly in line with the effects of careful 25% adult-onset CR relative to nonobese controls in rodents (Pugh et al. 1999). Moreover, these results if anything likely *underestimate* the ability of this level of CR to retard aging and extend LS

in dogs, because “All dogs received the same diet” in differing proportions (Kealy 1999: 34), so that the CR animals may have been marginally malnourished. As previously noted, a generous supply of essential nutrients appears to be essential for a robust result in adult-onset CR. This level of CR also protected the dogs against *age-related* losses of lean tissue and bone mass, and considerably reduced the incidence of osteoarthritis and hip dysplasia, and the age of onset of chronic conditions requiring treatment including malignant and benign neoplasia, hepatic disease, cystic endometrial hyperplasia, pyometra, hypothyroidism, and seizures (Kealy et al. 2002).

So the data of Kealy et al. (2002) seem to be “strikingly in line with” the “broadly quantitative prediction” of the PP, and not the WH as de Grey suggests (de Grey 2005a). As a preemptive counterargument, de Grey adds the suggestion that “The authors of this study ... chose a CR level of 25%, rather than the 40% more typical of rodent CR experiments; the hypothesis presented here predicts that a 40% CR regime would not extend the dogs’ LS further (and might be harmful)” (de Grey 2005a: 79). Again, however, this is a *prediction* of the WH, and not *evidence* in support of it. And it seems unlikely that this hypothesis is correct, on at least two grounds.

First, a photograph that compares a CR to an AL dog from this cohort (Pickrell, 2002) does not seem to show the CR animal to be badly emaciated, or the AL dog to be morbidly obese, although the CR animals were fed 25% less than “the estimated maintenance requirement [of “ideal body weight”] for large-breed dogs” given to their AL pair-mates (Kealy et al. 2002: 1315). The CR animal looks trim, lean, and healthy, and seems likely able to lose substantially more body weight before it would literally starve.² The evolutionary reasoning underpinning the WH itself would seem to rule out an imposed “off

² Lawler (personal communication) indicates that the reason for the very mild level of CR in these animals was wide-ranging interindividual variability in energy requirements, which may be as much as 40%: the investigators were concerned that more severe CR might have led to starvation or malnutrition in some animals even though it was tolerable by most.

switch” or regulatory limit to the degree of additional CR which would continue to confer a proportional retardation of aging *within* the limits of energy intake required to sustain life. On the basis of the anti-aging response of rodents subjected to a level of CR that is not survivable under wild conditions, as well as the reasoning outlined above, it seems unlikely that such a regulatory floor exists even beyond this limit.

A second, and stronger argument for the prediction of the ability of dogs to further extend their LS in response to more severe CR flows from an evolutionary explanation for aging and its modulation by CR on which the WH depends (Kirkwood and Austad 2000; Holliday 1989; Harrison and Archer 1989; Edney and Gill 1968). This hypothesis is predicated on a life-history analysis, in which under conditions of restricted energy availability, under which reproduction is rendered an ineffective fitness “strategy” due to the unlikelihood of the survival of the young, organisms have adapted the capacity to strategically reallocate resources, shifting investments away from reproduction and into enhanced somatic maintenance, thus maintaining the biological youth of the organism against the forces of reproductive (and other) senescence in order that it might “live to breed another day.” And indeed, animals (including, but not limited to, rodents) subjected to CR exhibit downregulated reproductive function.

As one would expect from the analysis of levels of CR that are not survivable in the wild, this shutdown occurs significantly before the degree of CR which induces the maximal anti-aging effect in the rodent model. In mice the arrest of estrus occurs around 20% CR (Nelson et al. 1985), while in rats CR does not lead to amenorrhea but dramatically delays menarche (Holehan and Merry 1985). The continuum of decrements in reproductive function in response to energy restriction should thus provide a guideline as to the parallel continuum in life extension. In rodents, as noted, the first signs of shutdown of reproductive function occur far before the degree of CR that elicits maximal anti-aging benefits, and the above analysis predicts that a similar significant interruption of reproductive function should be present at levels

of CR which are submaximal for lifespan extension. The fact that “Mean annual frequency of estrous cycles and duration of interestrus intervals did not differ between feeding groups” (CR vs AL) amongst the bitches of this cohort (Lawler et al. 1999)³ and that this level of CR was similarly insufficient to reduce middle-aged males’ serum testosterone levels, either at baseline or in response to a gonadotropin-releasing hormone challenge (personal communication, Dennis Lawler), thus strongly implies that the 25% CR dogs must still have significant “room” with which to reallocate investments in reproductive function to yet more enhanced age-retardation.

A cow with its tail to the east ...

I agree with de Grey (2005a) that the very preliminary nature of the CR data in cattle (Pinney et al. 1972) forbids any strong reliance on its results. To the extent that he attempts to use these results in support of the WH, de Grey chooses to de-emphasize the average LS data (which are available) in favor of an attempted extrapolation of maximum LS figures (which figures are not available, as not all animals had been culled by the termination of the study), which de Grey suggests implies an extension of just 6 months of maximum LS in the CR cohort. While one might be tempted to quibble with de Grey’s extrapolation (the same data might be read to give 1 year of extended maximum LS), it seems tenuous to engage in any such analysis, not only because the number is itself little more than conjecture, but also because (a) the cohorts were so small (90 cattle, divided into 6 cohorts, such that the 10% survival for even the combined isocaloric cohorts would embrace just 3 animals), and (b) the maximum LS for the entire study

³ In personal correspondence, Lawler adds the caveat that this breed of dogs often fails to exhibit visible signs at first estrus, and may undergo a “split estrus” during its youth, involving intermittent cycles of pituitary hormone secretion and a preliminary surge of estrogen production and associated proestrus symptomatology, yet without ovulation.

cohort would have been no more than 16 years on any analysis, whereas species maximum LS for cattle have been reported as being 20–30 years (Altman and Dittmer 1972) and Rockstein et al. 1977 respectively).

The mere fact that significant average, and some cohort maximum, LS extension was observed under only ~17% integrated CR per annum (and not, NB, ~40% CR as de Grey's Table 1 indicates, as CR was maintained for less than half of the year) seems, however, to be more readily interpreted as evidence for PP than for the WH—particularly as it accords with previous, albeit still weaker, studies cited by Pinney et al. (1972). So does the considerable reduction in ocular squamous cell carcinoma (one, six, and six cows were removed from the study for this reason in the under-, adequately-, and overfed groups respectively. This accords with previous observations in another study (Anderson 1959)).

Here, as in other examples, de Grey suggests that this is close to the *maximum* degree of CR-induced life extension of which these animals are capable. At the risk of repetitiveness, however, it must be emphasized that this is a *prediction* of the WH, and not *evidence* available in support of it. And as in the case of the dog data discussed above, the fact that the animals were still successfully breeding suggests (again on evolutionary grounds) that there was yet plenty of energetic “room” in which to shift resources away from reproduction and into somatic maintenance, so that more extreme CR would be predicted to lead to further increases in LS, bringing the results further in line with the PP. De Grey suggests to the contrary that “Since the CR level imposed was too mild to impact fertility severely, we may extrapolate that perhaps a year of life extension could have been achieved if CR at the same level (40%) had been imposed year-round” (de Grey 2005a), but it would seem unreasonable to suggest that, if animals are already shifting some of their resources from reproduction into anti-aging mechanisms at an “integrated” 17% CR, yet are still able to reproduce, they are not equipped to considerably further shift such resources in response to the doubling, trebling, or even quadrupling of that level of energy restriction.

In short, while the results of this study (Pinney et al. 1972) are far from definitive, the overall picture that they present is more compatible with the PP than with the WH.

Clams digging in

One might also mention briefly a 1939 report, briefly summarized by Weindruch and Walford (1988) in their landmark review, on the effects of CR in the common limpet: “Fischer-Piette (1939) reported that when food is abundant, the mollusc *Patella vulgata* only lives 2.5 years, whereas in waters poor in organic material its survival may reach 16 years.” So dramatic a LS extension would be powerful support of PP; however, current sources (Hill 2005) place the LS of this species at 10–20 years, making this finding not terribly robust. It does seem, however, to add some limited weight to the thesis that restricted energy intake can have considerable effects on LS in relatively longevous species.

Brass monkey weather

I again agree with de Grey that the results he cites from one of the three CR studies being carried out in nonhuman primates (Bodkin et al. 2003) cannot be taken as credible evidence for PP—although they certainly do not support the WH. The study is indeed deeply flawed, for reasons going well beyond those cited by de Grey (Lane et al. 2004; Rae 2003), deterring us from relying upon it. Additional evidence is available, however, from studies underway at the University of Wisconsin, Madison and the National Institutes on Aging, which are closer in design to classic rodent CR studies. While maximum LS data, which would provide powerful evidence on the PP vs WH debate and even for the extrapolability of the CR phenomenon to our own species, are not yet available, these researchers have already reported considerable reductions in mortality in CR vs AL squirrel (33% vs 54%) and rhesus monkeys (13 vs 23%) respectively (Lane et al. 2002). Preliminary results are also available for a variety of chronic age-related diseases, apparently showing reduced cardiovascular dis-

ease, diabetes, ulcers, cataracts, and especially proliferative diseases, including malignancies (Lane et al. 2001a; Black et al. 2000). Again, while not definitive, so large a difference in age-related mortality and morbidity in these better-husbanded colonies must be taken as evidence in favor of PP.

Moreover, it should be noted that available evidence suggests that these animals are consuming significantly more energy than would elicit the maximal anti-aging effect. Female CR Rhesus monkeys 7–27 years old from this study exhibit no differences in menstrual cycling or sex steroid levels relative to AL controls (Lane et al. 2001b). As argued above in the cases of dogs and cattle, based on both rodent data and evolutionary theory, significant alterations in these parameters should occur well before the maximally life-extending degree of CR. Thus, evolutionary theory predicts that both the present results, and the ultimate effect on maximal lifespan, represent only a fraction of the full potential anti-aging effects of CR in nonhuman primates.

The face of the sky and the signs of the times

Of course, the ultimate organism of concern for de Grey, as for all but the most curiosity-driven biogerontologist, is our own species. There are, of course, no definitive answers to the question of the human translatability of the rodent CR data. If there were, then the question of the WH vs PP would be both moot and presumably resolved. However, it would be remiss to ignore the existing data, however tentative, suggesting that CR will significantly retard aging in our species. Much of this has recently been reviewed, although far from exhaustively (Heilbronn and Ravussin 2003; Hursting et al. 2003). Not only do humans respond to CR with metabolic changes parallel to those seen in rodents and nonhuman primates (Fontana et al. 2004; Walford et al. 2002; Velthuis-te Wierik et al. 1994; unpublished data, Rae, Delaney, and Walford), but there is considerable evidence of reduced cancer and other chronic disease.

The effects of CR on mortality in humans have also been the subject of a controlled trial. Vallejo

1957 assigned 120 nonobese residents of an elder care home on either a control diet of 2300 kilocalories, or an intervention in which days on this diet were alternated with an 885 kilocalorie menu of fruit and milk, for an average of 1593 kilocalories/day for 3 years. In that time period, the control group spent a total of 219 days in the infirmary, and 13 deaths occurred, while the corresponding figures in the CR group were 123 days and 6 deaths respectively. These results are all the more striking when one considers the reasonable expectation that an elderly, presumably frail study cohort might have adapted poorly to CR (as is seen under suboptimal conditions in older rodent populations (Weindruch and Walford 1982; Rae 2004).

Finally, the Okinawan longevity phenomenon is strikingly consistent with the hypothesis of the human translatability of the rodent CR data, and thus of PP. Okinawa enjoys the highest life expectancy in Japan, and an exceptionally high incidence of centenarians, which may constitute an operational definition of extension of maximum LS in humans, as is 10th-decile survivorship in rodents (an endpoint chosen to avoid outlier effects). In 1977, the incidence of centenarians per 100,000 population over the age of 65 in Okinawa was 37, vs ≤ 9.9 in most prefectures and ≤ 1.9 in Japan's north (Kagawa 1978). In 2000, Okinawa continued to enjoy an advantage in extreme longevity, with 202.8 centenarians per 100,000 elderly, with the runner-up prefecture (Kochi) at 113.4 and the national average at approximately 58.2 (Shibata 2004). Okinawans also enjoy markedly reduced incidence of atherosclerosis, ECG abnormalities, and hormone-dependent cancer mortality (Suzuki et al. 2001) and delayed age-related incidence of dementia (Willcox et al. 2001), relative to the rest of Japan.

While these data are ecologic in nature, there is evidence that the phenomenon is primarily of environmental rather than genetic origin. The incidence of centenarians in Okinawan expatriate communities is far lower than that on the island itself, e.g., 5.32-fold lower in Brazil (Moriguchi 1999), and successive generations of Okinawans born during and after the period of rapid Westernization consequent to American military occupation of the island after World War II have

increasingly displayed the expected poor health (Todoriki et al. 2004; Willcox 2005).

The only credible explanation of the Okinawan longevity phenomenon which has been advanced is that it is a natural experiment in CR. Okinawa is a striking exception to the general finding that life expectancy follows social gradient. It has been the poorest prefecture in Japan, yet enjoys the highest life expectancy of a nation leading the world in this parameter (Cockerham and Yamori 2001). This has imposed a considerable constraint on energy intake. Amongst Okinawan children in 1967, energy intake was estimated to be only ~62% of Japanese recommended intake (which, in turn, is lower than that in North America). Okinawan adults also appeared to be restricted (Kagawa 1978), and centenarians in Okinawa and across Japan appear to be unusually short (Kagawa 1978) and to consume considerably less energy (Suzuki et al. 2001) than the rest of the Japanese population, although caloric intake is typically lower in older persons.

Crucially, compared to most environmentally-imposed or even poorly self-selected energy-restricted diets, intake of essential nutrients appears to be relatively good (Suzuki et al. 2001; Shibata et al. 1992; Mimura et al. 1992), although deficiencies in several nutrients (especially vitamins B₁₂ and D (the latter being uncompensated by sun exposure, as evidenced by signs of early rickets in the present centenarian cohort) still exist (Willcox 2005). This relative lack of malnutrition is due in large part to a larger proportional intake of animal, fish, and soy protein, and to an unusually nutritious staple food (the Satsuma sweet potato, which is far more nutrient-dense than the conventional potato, or the rice, wheat, corn, or other grains on which so many other impoverished populations depend for the bulk of their calories) (Willcox 2005; Sho 2001; Mimura et al. 1992).

On the other hand, it is clear that the imposition of energy restriction on the Okinawan population has been relaxed considerably in the postwar period with rising standards of living and the effects of American military occupation (Todoriki et al. 2004; Willcox 2005), so that the high incidence of centenarians and low incidence

of age-related disease seen in Okinawa may be the result of CR imposed for only the first half of the lifespan. Thus, whereas energy intake amongst Okinawan children was only ~62% of Japanese recommended intake in 1967, a study of Japanese centenarians in 1989 (Akisaka et al. 1996) and a similar comparison in 1994 (Suzuki et al. 2001) found that Okinawan and non-Okinawan centenarians had approximately the same energy intake. Estimates of caloric intake in the present centenarian cohort suggest that energy consumption has risen by ~200 kilocalories since 1960 (Willcox 2005). These limitations make the Okinawan longevity phenomenon yet more impressive. Had energy restriction been continuous, and nutrition fully adequate, and accompanied by better sanitation, medical treatment, and the benefits of higher socioeconomic status, we would expect to see an even more remarkable longevity phenomenon in Okinawa, providing even stronger support for the PP.

There is another such cross-sectional datum connecting extreme survivorship to CR in humans. Whereas lifestyle variables explain a great deal of survival to average old age, “no particular environmental trait, such as diet, economic status, or level of education has been significantly correlated with the ability to survive to extreme old age” (Perls 2002). Informally, Perls has extended this observation to smoking, and yet his group has encountered no male, and few female, obese centenarians (personal communication). Likewise, a smaller study confined to supercentenarians (Coles 2004: 581) reports that “These extreme ages are achieved in spite of what we would call poor lifestyle choices (such as heavy smoking, heavy drinking, or both, failure to exercise on a regular basis, and no conscious effort to eat nutritiously). *None of our participants, however, were obese* [emphasis added].” This may, at the very least, indicate that leanness, which is of course partly an inverse function of caloric intake, is an extremely common contributing factor to the achievement of extreme longevity. Such an interpretation seems especially credible in light of Perls’ report of a more consistent finding of a lack of obesity in *male* centenarians, as female centenarians outnumber males severalfold in all populations, suggesting

that their survivorship may be less dependent on environmental factors such as energy intake.

Earlier, in discussing the results of CR in dogs and cattle, I argued that the widely-accepted understanding of the role of evolutionary pressures in creating and maintaining the anti-aging response to CR upon which the WH depends necessarily predicts that the maximally effective level of life-extending CR will be significantly greater than that at which reproductive function is clearly impaired, as is observed in the rodent model. The human case is susceptible to the same analysis. Human women undergoing high-quality “undernutrition without malnutrition” can be under substantial CR (as demonstrated by food records and significant weight loss from a healthy initial body mass index (BMI)) without entering amenorrhea (Walford et al. 2002; unpublished data, Rae, Delaney, and Walford); and in turn women with anorexia nervosa are amenorrheic at levels of energy intake which do not lead to actual starvation (Boyar et al. 1974). Of 214 women with long-term anorexia nervosa with a BMI 16.8 ± 1.4 (13.2–20.8), 69% experienced menarche later than the 12.88-year mean age for white American females (note that this is not the large percentage that it might seem to be: in a bell-shaped distribution, one expects this of close to 50% of girls in any case), including only 15% who had menarche at ages ≥ 15.28 years (two standard deviations later than the normal mean) (Miller et al. 2005). As in the dog and cow case, the lack of a profound effect of CR at these levels on menstruation suggests that evolution has allowed for a substantial range of degrees of human CR, to which it will respond by deprioritizing a breeding strategy in favor of somatic maintenance.

Similarly, as discussed above, rodent data indicate that the anti-aging response to CR extends to the point of frank starvation, and indeed beyond the level at which death from causes related to energy restriction (hypothermia, vulnerability to predation) would be expected to make survival a near-impossibility. The WH would predict CR of 5–10% to be the maximal survivable CR in humans in order to also be the maximal level of anti-aging CR, because it assumes that evolutionary pressure will either

ensure the capacity to survive a given degree of famine and to retard aging proportionally in response to it, or will even prioritize the latter, in order to ensure that the few organisms that survive a very severe famine are able to carry on with reproduction and other fitness-salient activities, since insuring a more widespread survival might be impossible under such extreme conditions (de Grey 2005a, and personal communication, de Grey). Yet humans clearly can survive CR considerably more severe than the 5–10% thereby predicted in the paper. Thus, even if we accept de Grey’s evolutionary reasoning, this suggests that the degree of anti-aging metabolic flexibility in response to CR available to humans will be considerably greater than that predicted in the article.

While none of these data are definitive, or even fully quantitative, they all seem to point to the ability of CR in humans to significantly retard biological aging. They are therefore an additional stream in the converging evidence upholding PP as against de Grey’s WH.

Discussion

Implications for retarding human aging by modulating nutrient sensing

The preceding defense of the PP against the WH will doubtless be heartening to the small but growing number of men and women practicing CR as the sole intervention currently available which may reasonably be predicted to retard biological aging in humans.⁴ It will also encourage those biogerontologists, whether from within the academic world or from biotech startups, who are advocating and pursuing the development of “CR mimetic” drugs.

Despite the fact that the arguments in this paper appear to support the likelihood that pharmaceuticals based on the CR phenomenon could indeed retard aging in humans, this pursuit,

⁴ The Calorie Restriction Society <<http://www.calorierestriction.org>> advocates and supports the responsible human practice of CR as a potential anti-aging intervention by its membership, and the advancement of human and other CR science.

however, must ultimately be regarded as a strategic distraction in the pursuit of effective biomedical interventions to the aging process. Such agents are, first, likely to have unacceptable side-effects, as they will of necessity induce the dramatic metabolic shift which underlies both the desired and the undesired effects of CR. This is as we would expect from evolutionary theory (Kirkwood and Austad 2000). The signaling pathways in which such agents would intervene are normally set to maximize reproductive fitness, even at the expense of long-term somatic health, and thus exert pleiotropic effects on the health of the organism. If these pathways could be manipulated to retard our rate of aging at no cost to the “normal,” apparently “healthy” functioning of the organism within the parameters of our existing genetic toolkit, such adjustments would already have been made, as they would increase reproductive fitness. Side effects observed in CR rodents and also in a substantial proportion of more dedicated human CR practitioners (unpublished data, Rae, Delaney, and Walford) include lower body temperature, reduced libido, and irritability and other changes in affect, although others (such as loss of bone mass) are so closely tied to actual weight loss as to be unlikely to result from small molecule modulation of key metabolic pathways in the absence of actual reduction in energy intake or absorption.

Secondly, CR mimetics will be extraordinarily difficult to license for clinical use in humans, not only because of the regulatory hurdles facing a pharmaceutical designed to treat “aging,” but because they will (like CR itself) only *retard* the further accumulation of aging damage rather than *reversing* it. Prevention, rather than treatment, of age-related disease is by its nature a longer-term clinical endpoint. Thus, even extrapolating from the surprisingly positive results of the latest work of the Spindler group in older animals (Dhahbi et al. 2004), any such drug will take many times the length of time usually required of existing pharmaceuticals to reveal clinically significant benefits on “licensable” indications in its users.

Indeed, there is a definite irony to the fact that, as essentially *preventive* agents, CR mimetic drugs would at once be most likely to benefit patients if administered while they are still young

and healthy, and yet be all the more difficult to prove effective for any clinical outcome in such patients. And while the multi-decade efficacy trials proceed, such subjects will still be forced to endure the great majority of the drug’s side-effects. Ethical review panels will be especially wary of approving clinical trials using agents whose safety and efficacy will not be clear for decades in young, healthy subjects. Thus, the time lag will be even greater before the full benefits of a true CR mimetic, as envisioned by, e.g., Richard Miller (2002), would be manifested in the clinic, in patient lives, and in reduced burden on public and private medical systems.

While today’s human CR practitioners choose to continue with their dietary regimens despite any side effects that they may encounter, this is possible precisely because adopting the diet is an individual choice. These pioneers are able to prioritize their immediate and projected improvements in health and vitality over the immediate quality of life impacts of the diet, and to adopt the level of CR severity that they find to best balance the benefits (current and anticipated) against the risks. This will not be the case for pharmacological agents whose greatest benefits would accrue to healthy people, and will not be an option in any case until their benefits are *proven*, whereas today’s CR practitioners make an entirely self-directed choice after weighing the balance of probabilities.

The way forward for anti-aging biomedicine

The gloomy prospects for the actual realization of the benefits which would theoretically accrue from the development of CR mimetics is not, however, a reason for abandoning the cause of interventional biogerontology. An alternative approach is to shift the focus away from schemes premised on tinkering with basic metabolic pathways, in favor of making the accruing molecular lesions that underlie the aging process themselves the direct therapeutic target of new therapies (de Grey et al. 2002).

In other words, instead of attempting to perturb the biological processes which at once underlie normal organismal functionality and yet generate the reactive intermediates which de-

grade the organism's molecular fidelity and thus ultimately drive the accelerating functional decay with chronological time which constitutes biological aging, interventions against aging should allow metabolism to proceed normally, but undo or obviate the molecular damage that links metabolism and its waste products to pathology. Such an approach would yield near-term results in major age-related diseases, such as atherosclerosis, neurodegenerative disease, and diabetes, which are in the first instance caused by such molecular lesions, and be more rapidly validated in, and transferred to, younger, healthier cohorts. This would allow the widespread dissemination of genuine anti-aging medicines to occur more rapidly, to a wider cohort, and (because metabolism is not channeled in directions contrary to the parameters favored for normal functionality) with fewer side-effects than a classically preventive approach to biogerontology.

A panel of interventions designed to accomplish just this end has been proposed (de Grey et al. 2002) and updated (de Grey 2003, 2005b; de Grey et al. 2004, 2005). The apparent feasibility of this therapeutic platform led its formulators to the conclusion "that indefinite postponement of aging—which we term 'engineered negligible senescence'—may be within sight" (de Grey et al. 2002). In the face of such a possibility, CR mimetics are seen to be an unpromising diversion from the more direct approach of intervention into aging damage, whose benefits would be both more near-term and ultimately far greater, as such interventions would lead not merely to the deceleration, but to the arrest (and ultimately, the *reversal*) of the accelerating accumulation of molecular entropy in the body. Such interventions hold forth the promise of severing the passage of time from the ever-increasing physiological decay, loss of functionality, vulnerability to disease, and risk of death that are the hallmarks of biological aging.

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