J. Anat. (2000) **197**, pp. 587–590, with 2 figures Printed in the United Kingdom

Review

Evolution, stress, and longevity

THOMAS B. L. KIRKWOOD, PANKAJ KAPAHI AND DARYL P. SHANLEY

Biological Gerontology Group, University of Manchester, Manchester, UK

(*Accepted 3 July 2000*)

ABSTRACT

The disposable soma theory suggests that longevity is determined through the setting of longevity assurance mechanisms so as to provide an optimal compromise between investments in somatic maintenance (including stress resistance) and in reproduction. A corollary is that species with low extrinsic mortality are predicted to invest relatively more effort in maintenance, resulting in slower intrinsic ageing, than species with high extrinsic mortality. We tested this prediction in a comparative study of stress resistance in primary skin fibroblasts and confirmed that cells from long-lived species are indeed more resistant to a variant of stressors. A widely studied example of within-species variation in lifespan is the rodent calorie restriction model. Food-restricted animals show elevations in a range of stress response mechanisms, and it has been suggested that this is an outcome of natural selection for life history plasticity. We have developed a theoretical model for dynamic optimisation of the allocation of effort to maintenance and reproduction in response to fluctuations in food availability. The model supports the suggestion that the response to calorie restriction may be an evolutionary adaptation, raising interesting questions about the hierarchy of genetic control of multiple stress response systems. The model identifies ecological factors likely to support such an adaptation that may be relevant in considering the likely relevance of a similar response to calorie restriction in other species. Comparative and theoretical studies support the role of somatic maintenance and stress response systems in controlling the rate of ageing.

Key words: Stress response systems; ageing.

INTRODUCTION

The disposable soma theory (Kirkwood, 1977, 1981; Kirkwood & Holliday, 1979; Kirkwood & Rose, 1991) recognises that under pressure of natural selection, organisms can afford only limited investments in the maintenance and repair of somatic cells and tissues. Maintenance systems are often costly, and a significant portion of basal metabolism is concerned with various aspects of maintenance and repair. In the wild, death usually occurs from extrinsic causes (accident, cold, starvation, etc). The organism requires only enough maintenance to keep somatic cells in good condition through the typical survival period, plus probably some modest reserve. For example, few wild mice survive beyond 1 y and probably none reach the 3 y of which they are capable in protected conditions. There would be little or no advantage for the mouse in having somatic maintenance systems that could provide longer survival, and to do so would incur extra metabolic costs that might reduce reproductive fitness. It is therefore predicted that intrinsic ageing is caused by progressive accumulation of unrepaired cellular damage, resulting eventually in frailty, disease, and finally death.

This concept leads to a number of testable predictions (Kirkwood & Franceschi, 1992). First, ageing is not programmed in the sense that there exists any active mechanism to cause death. Instead, lifespan is influenced by 'longevity assurance' genes that control the levels of somatic maintenance and repair. Secondly, longevity of different species is determined by optimising the level of somatic maintenance according to the degree of extrinsic hazard in the species'

Correspondence to Professor Thomas Kirkwood, Department of Gerontology, Institute for the Health of the Elderly, Wolfson Research Centre, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE, UK. Tel.: +44 (0) 191 273 8811 ; e-mail: gerontology-@ncl.ac.uk

ecological niche. An adaptation that reduces hazard should result in selection pressure to increase maintenance, which would explain the greater longevity of animals that have evolved flight (birds, bats) compared with those that must remain on the ground. Thirdly, there are many maintenance mechanisms, so ageing and longevity are likely to be under highly polygenic control. The levels at which individual genes are set will influence the average rate at which damage accumulates or is repaired. Fourthly, at a fine scale the mechanisms of ageing are intrinsically stochastic. Therefore chance, as well as genetic and environmental factors, may play a significant role (Finch & Kirkwood, 1999). The role of chance in ageing is clearly indicated by the variance in lifespans observed for genetically identical populations (e.g. inbred mice, nematodes) maintained in homogeneous environments.

COMPARATIVE STUDY OF CELL STRESS RESISTANCE

We tested the prediction that cells from long-lived species are indeed better protected by somatic maintenance and repair in a comparative study of stress resistance in primary cultures of mammalian skin fibroblasts (Kapahi et al. 1999). To minimise possible confounding variables, cells were derived using a carefully standardised protocol. Fibroblasts from rat, hamster, marmoset, rabbit, sheep, pig and cow were grown from biopsies obtained from the inner side of the forelimb of young (aged less than 20% of the species maximum lifespan) individuals, using 2–4 donors per species. Human fibroblasts were grown from foreskins provided from routine elective surgery. Primary cell cultures were established and tested for stress resistance before the cells had undergone a maximum of 10 doublings in vitro. Replicate cultures were exposed to a dose of one of the following stressors (period of stress shown in brackets): hydrogen peroxide (2 h), paraquat (24 h), tert-butyl hydroperoxide (2 h), sodium arsenite (6 h), or sodium hydroxide (6 h). For each stressor, a range of doses was used to establish a dose-response relationship plotting percent cell survival against the dose (concentration) of the stressor that was used.

It was found that cell stress resistance, expressed in terms of the dose of the stressor required to kill 90% of the cell population, correlated positively with species lifespans (Fig. 1). Care must be taken in comparative studies to minimise, as far as possible, the effects of confounding variables. We controlled for 2 known confounding variables affecting the fibroblast model of cell ageing by restricting donor age and cell doubling level. We also ensured that all samples were from sites not regularly exposed to sunlight (foreskin of human; inside forelimb of other species). An additional control was included for possible confounding by phylogeny (a major issue in comparative studies). The data were independently analysed by M. Pagel using a generalised least squares (GLS) model that takes account of phylogeny (Pagel 1998). The GLS analysis showed that the positive correlations were statistically significant ($P < 0.001$) for all stressors except tert-butyl hydroperoxide (for which the correlation was positive but nonsignificant).

The fact that similar correlations were obtained for

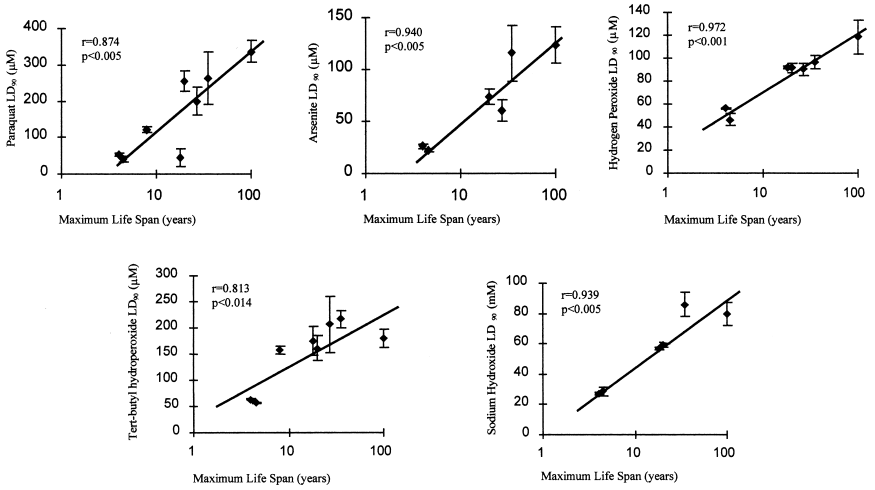

Fig. 1. Correlations between cell stress resistance and species' maximum lifespan. The vertical axis indicates the dose (LD90) of each stressor required to cause 90% decrease in DNA synthesis, as measured by the uptake of radiolabelled thymidine. The horizontal axis indicates species lifespan. Data are shown as mean (standard error of the LD90 determinations from replicate cell cultures grown from separate individuals (see text). Species and lifespans (years) were as follows: human (100), cow (35), pig (27), sheep (20), rabbit (18), marmoset (8), rat (4.5) and hamster (4). The r values in each panel indicate Pearson product moment correlation coefficients, and the *P* values denote levels of statistical significance. From Kapahi et al. (1999), by permission.

a variety of cellular stresses that damage cells in different ways supports the idea that multiple stress response mechanisms are involved in the determination of species-specific lifespans. Investigation of genes that mediate the cellular response to stress may therefore reveal some of the mechanisms that determine species' longevities.

CALORIE RESTRICTION

It has long been known that lifespan in laboratory rodents is extended, typically by 20–30 %, by reducing food intake (Weindruch & Walford, 1988; Sprott, 1997). Long-term calorie restriction results in a small, lean animal, with impaired fertility, but which is otherwise healthy and active. Almost without exception, somatic maintenance functions are upregulated (Masoro, 1993; Yu, 1994; Merry, 1995). Identifying whether there might be an evolutionary basis for the response to calorie restriction in rodents will help not only to understand this phenomenon, with its complex effects on the ageing process, but also to assess whether it might also work in long-lived species, like humans.

At first sight, it seems paradoxical that an organism with less energy available can upregulate physiological processes, which must have an associated metabolic cost. This requires that metabolic savings be made elsewhere. The most obvious saving is in reproductive effort. Not only is reproduction costly in direct physiological terms but behaviours associated with reproduction can also be expensive. Rodents typically invest a large fraction of their energy budget in reproduction. Calorie restriction results in animals that are mostly infertile, although there is some variation depending on the species, the sex, the degree of restriction and the age at which restriction is first applied (Weindruch & Walford, 1988).

The evolutionary question of why a calorie-restricted organism should retard ageing may help throw light on the physiological basis of this phenomenon. Harrison & Archer (1988) suggested that the primary role of calorie restriction is to postpone reproductive senescence. If a famine lasts longer than the normal reproductive lifespan of the animal, any female that delays reproductive senescence and is able to breed after the famine has passed will experience a selective advantage. Harrison & Archer (1988) predicted that calorie restriction should have greater effect on species with shorter reproductive lifespans, and suggested a comparison between the house mouse, *Mus musculus*, and the longer-lived white-footed mouse, *Peromyscus leocupus*. They also predicted that calorie restriction should have little effect on long-lived species such as humans. A more explicit

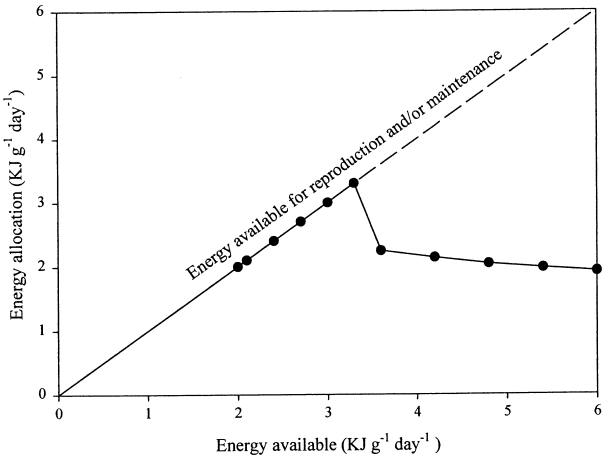

Fig. 2. Variation in the optimum allocation of metabolic resources (energy) to somatic maintenance, according to the level of abundance in the environment. The horizontal axis shows energy availability, the left hand end representing conditions of starvation and the right hand end representing conditions of plenty. The diagonal line indicates the maximum amount of energy that is available for maintenance (i.e. all of the energy which is available in the environment). The data points show the optimum predicted by the model, taking account of the need in most circumstances to allocate some energy to reproduction. For a 6-mo-old mouse exposed to conditions of reduced energy availability (around $3\,KJ\,g^{-1}\,day^{-1}$) for a period of 3 mo, the results show that the optimal strategy is to increase the allocation to maintenance above that which is optimal in conditions of plenty and to suspend reproduction.

hypothesis was proposed by Holliday (1989), based on the disposable soma theory of aging. Under conditions of plentiful food supply, a constant amount of energy is allocated to maintenance. As food supply diminishes, reproduction is reduced and eventually curtailed. Holliday suggested that at famine levels where, in principle, a small amount of energy could be allocated to reproduction, it may in fact be better temporarily to increase the investment in somatic maintenance. The potential benefit is that the animal gains an increased chance of survival with a reduced intrinsic rate of senescence, thereby permitting reproductive value to be preserved for when the famine is over. We tested this idea with a dynamic programming model to examine whether the physiological responses to calorie restriction observed in the laboratory might reflect an adaptive resource allocation strategy that has evolved to maximise fitness under conditions of intermittent food stress in the wild (Shanley & Kirkwood, 1999). The model was developed for *M. musculus*, for which extensive physiological and life history data have been recorded both in the wild and in the laboratory (Berry & Bronson, 1992), and was based on a resource allocation rule governing the partition of energy between maintenance functions and reproduction. Resources allocated to reproduction are used to produce pro-

geny, whereas resources allocated to maintenance are used to conserve state. At any age, the change in state is determined by the amount of energy invested in maintenance. With a large investment in maintenance, the state remains relatively unchanged, corresponding to a slow rate of ageing. Conversely, with a small investment in maintenance, the state change is large, corresponding to a rapid rate of ageing.

To test the hypothesis that the physiological response to calorie restriction reflects adaptation to periods of famine, a temporary reduction of food level was introduced in an otherwise abundant environment.

It was found that as long as 2 assumptions were made, the model clearly predicted an increase in the allocation of metabolic resources to somatic maintenance during periods of famine (Fig. 2). These assumptions were (1) the presence of a 'reproductive overhead', i.e. a minimum investment in reproduction that must be made in order to initiate the fertile state, and (2) the presence of an adverse effect of famine directly on juvenile survival. In the absence of either a reproductive overhead or an effect on juvenile survival, calorie restriction had little effect on the optimal allocation of energy to maintenance. Reproduction declined as food availability diminished, but it continued as long as there was sufficient energy to support any reproduction at all.

The model has been quantified with data on house mouse physiology and ecology. The conditions for life extension to be an adaptive response to calorie restriction will not necessarily be the same for other species. Activity levels are affected differently in rodents and primates by short-term fasting (Masoro & Austad, 1996), although recent data indicate that primates, like rodents, may upregulate activity levels when subjected to long-term calorie restriction (Weed et al. 1997). Nevertheless, the model suggests that there may be an adaptive basis for fluctuations in the allocation of metabolic resources to somatic maintenance and stress response systems, according to changes in environmental factors such as food availability. The fact that an intervention like calorie restriction has widespread effects on diverse aspects of the ageing process has long been a puzzle. Our evolutionary analysis suggests that there may be adaptive value in having a mechanism to effect coordinate control of multiple stress response systems.

CONCLUSION

The studies reviewed above support the concept that natural selection has a major influence on how organisms optimise their levels of somatic maintenance and stress response systems in relation to their einvironments. Although there are many stress response systems, reflecting the diverse stresses that challenge cells, long-lived species appear generally superior to short-lived species in the ability of their cells to survive a given dose of stress. The calorie-restricted rodent invokes a coordinate upregulation of stress response systems, for which there is a plausible evolutionary basis. The evolutionary and comparative perspectives on the links between stress resistance and longevity support the idea that interventions designed to enhance stress responses offer a possible means to alter fundamental aspects of the biology of ageing.

REFERENCES

BERRY RJ, BRONSON FH (1992) Life history and bioeconomy of the house mouse. *Biological Reviews of the Cambridge Philosophical Society* **67**, 519–550.

FINCH CE, KIRKWOOD TBL (1999) *Chance, Development, and Aging*. New York: Oxford University Press.

HARRISON DE, ARCHER JR (1988) Natural selection for extended longevity from food restriction. *Growth Development and Aging* **52**, 65.

HOLLIDAY R (1989) Food, reproduction and longevity—is the extended lifespan of calorie-restricted animals an evolutionary adaptation? *BioEssays* **10**, 125–127.

KAPAHI P, BOULTON ME, KIRKWOOD TBL (1999) Positive correlation between mammalian lifespans and cellular resistance to stress. *Free Radical Biology and Medicine* **26**, 495–500.

KIRKWOOD TBL (1977) Evolution of aging. *Nature* **270**, 301–304.

KIRKWOOD TBL (1981) Repair and its evolution: survival versus reproduction. In *Physiological Ecology: An Evolutionary Approach to Resource Use* (ed. Townsend CR, Calow P), pp. 165–189. Oxford: Blackwell Scientific.

KIRKWOOD TBL, HOLLIDAY R (1979) The evolution of aging and longevity. *Proceedings of the Royal Society of London Series B: Biological Sciences* **205**, B531–B546.

KIRKWOOD TBL, ROSE MR (1991) Evolution of senescence-late survival sacrificed for reproduction. *Philosophical Transactions of the Royal Society of London Series B* **332**, B15–B24.

KIRKWOOD TBL, FRANCESCHI C (1992) Is aging as complex as it would appear? *Annals of the New York Academy of Sciences* **663**, 412–417.

MASORO EJ (1993) Dietary restriction and aging. *Journal of the American Geriatrics Society* **41**, 994–999.

MASORO EJ, AUSTAD SN (1996) The evolution of the anti-aging action of calorie-restriction: a hypothesis. *Journals of Gerontology Series A: Biological Sciences and Medical Sciences* **51**, B387–B391.

MERRY BJ (1995) Effect of calorie-restriction on aging—an update. *Reviews in Clinical Gerontology* **5**, 247–258.

PAGEL M (1998) Inferring evolutionary processes from phylogenies. *Zoologica Scripta* **26**, 331–348.

SHANLEY DP, KIRKWOOD TBL (2000) Natural selection, calorie restriction and aging: a life history analysis. *Evolution* **54**, 740–750.

SPROTT RL (1997) Diet and calorie restriction. *Experimental Gerontology* **35**, 205–214.

WEED JL, LANE MA, ROTH GS, SPEER DL, INGRAM DK (1997) Activity measures in rhesus monkeys on long-term calorie restriction. *Physiology and Behaviour* **62**, 97–103.

WEINDRUCH RH, WALFORD RL (1988) *The Retardation of Aging and Disease by Calorie-Restriction*. Springfield. Ill.: Charles C. Thomas.

YU BP (1994) How diet influences the aging process of the rat. *Proceedings of the Society for Experimental Biology and Medicine* **205**, 97–105.