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Visions & Reflections (Minireview)

Considerations on Temperature, Longevity and Aging

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Abstract. A modest reduction in body temperature prolongs longevity and may retard aging in both poikilotherm and homeotherm animals. Some of the possible mechanisms mediating these effects are considered here with respect to major aging models and theories.

Keywords. Temperature, aging, longevity, calorie restriction, energy.

Introduction

Temperature is one of the parameters of thermodynamics influencing virtually every chemical and biological process. Longevity was demonstrated to be increased by temperature reduction in poikilotherms as well as homeotherms. A modest temperature reduction increased longevity and retarded senescence in the fruit fly *Drosophila melanogaster* [1], the nematode *Caenorhabditis elegans* [2], and in vertebrates as demonstrated by the fish *Cynolebias adloffi* [3]. In homeotherms, a reduction in core body temperature (CBT) was found to be associated with calorie restriction (CR). A controlled and balanced dietary regimen is, to date, the most effective way to prolong lifespan and retard aging (Reviewed in [4]). The effects of CR on CBT were observed in rats, mice, monkeys and humans [5–7]. Although considered secondary to CR, CBT was also hypothesized to contribute to the beneficial effects of CR on longevity and aging [8–10]. This hypothesis remained difficult to test in the absence of an appropriate model. While reducing the body temperature of poikilotherms can easily be achieved by lowering the ambient temperature at which they are maintained, a similar approach was not possible in homeotherms. When exposed to cold, homeotherms activate physiological thermoregulatory mechanisms to maintain a constant CBT, including reduced heat dissipation and increased thermogenesis, with increased energy expenditure. Experiments in which homeotherms were exposed to cold demonstrated that such a regimen reduced lifespan and activated adaptive mechanisms, including the extent of antioxidant protection, but did not represent a model of reduced CBT [11–16]. Recently, a transgenic mouse model with a modest but prolonged CBT reduction was generated by hypothalamic over-expression of uncoupling protein 2 in hypocretin neurons (Hcrt-UCP2) [17]. UCP2 over-expression generated heat that elevated hypothalamic temperatures mimicking increased CBT. As a consequence, the hypothalamic "central thermostat" responded by activating thermoregulatory mechanisms, resulting in permanently reduced CBT. With a 0.5–0.6 °C reduction of CBT, Hcrt-UCP2 mice showed up to a 20% increase in the median life expectancy in the absence of CR [17]. This, together with the observation that CR-induced lifespan extension in poikilotherms is additive to the effects of temperature [10], indicated that the CR and temperature may influence lifespan independently. Although CR and CBT can influence longevity independently, whether they act through

similar or distinct mechanisms, and the nature of those mechanisms, remains to be demonstrated.

Thermodynamic effects and specific pathways

In addition to death, Leonard Hayflick distinguished three fundamental phenomena describing the finitude of life: aging, the determinants of longevity, and age-associated diseases [18]. In the absence of definitive evidence that aging is actively driven by a genetic program, Hayflick described it as a stochastic process resulting in loss of molecular fidelity (or an increase of molecular disorder) bringing increased susceptibility to age-related diseases. Thus, adhering to the interpretations of the Second Law of Thermodynamics, which does not include the necessity of a closed system, he proposed that aging can be seen as an increase of molecular entropy. In this scenario, the effects of temperature on longevity and aging may be interpreted as effects on molecular entropy. But is it possible that temperature affects longevity and aging through specific pathways?

Comparison of the mortality curve trajectories of animals under CR or temperature reduction suggested the possibility that the mechanisms mediating the effects of temperature on lifespan may differ in poikilotherms and homeotherms. In poikilotherms the effects of CR and temperature on longevity can be distinguished by the slopes of the mortality curves [19–23]. In *Drosophila*, chronic CR results in a delay in the onset of a detectable, aging-related increase in mortality. However, once detected, the mortality increase proceeds at roughly the same rate in calorie restricted and control flies [19,24]. This differs from the effects of lowered temperature, which increases life-span in *Drosophila*, but also reduces the slope of the mortality trajectory [19,20]. In contrast, mice with a reduced CBT show a reduced 'frailty' parameter on the Gompertz curve, similar to that observed during CR. These observations suggest that the effects of temperature reduction on longevity may differ in poikilotherms and in homeotherms and that, in the latter, it may act through specific pathways that may be similar to those mediating the effects of CR.

To address these and similar issues, the effects of temperature will need to be evaluated at the molecular, cellular, as well as physiological levels. Temperature can influence protein structure, which is maintained through a large number of weak non-covalent interactions, and affects enzymatic stability and activity. The relevance of temperature dependence on enzymatic activity in biochemistry and physiology across species has been measured and demonstrated by studies of physiological adaptation to different ambient temperature in poikilotherms [25,26]. Yet the biological significance of temperature changes similar to those observed in mice with reduced CBT or during CR (0.5 to 2°C) on enzymatic stability and activity within one species and in homeotherms remains to be demonstrated [27]. It may be argued that such minor changes are unlikely to have any influence at all. However, seemingly small changes can have large effects when maintained over a long period of time, especially over a lifetime. For instance, it can be hypothesized that a modest reduction of temperature results in a reduction of free radical-mediated damage believed to be a major contributor to aging. Such an hypothesis can be evaluated by determining and comparing the amount, and possibly the quality, of free radical species produced at different temperatures, as well as the level and activity of endogenous antioxidants including superoxide dismutase, catalase and glutathione. These measurements may indicate which, if any, of the components regulating free radical production and degradation are mediating the effects of temperature.

The biology of aging has recently advanced formidably thanks to the identification of genes that influence longevity. Experiments with hetero or homozygous mutants in *C. elegans*, *Drosophila,* and in the mouse have identified pathways and genes that influence lifespan. The most extensively studied include growth hormone (GH), the insulin/insulin-like growth factor (IGF-I) (IIS) pathway [28,29], the sirtuins, [30] but also the isoform p66 of the locus encoding for the proto-oncogene *Shc* $p66^{shc}$ [31] and type 5 adenylil cyclase [32.]. The relevance of the role of IIS in regulating longevity in mammals is mounting, including the demonstration of life extension in mice with heterozygote null mutation of the IGF-I Receptor ($Igf1r^{+/-}$) as well as of the insulin receptor substrate 1 ($Irs1^{+/-}$), and in mice lacking insulin receptors exclusively in the adipose tissue (FIRKO mice) [33–35]. These findings demonstrated that longevity can be regulated by specific pathways and suggested that targeting them may become a possible means to effectively increase lifespan and possibly retard the aging process. They also opened new avenues to investigate mechanisms of aging, introducing the concept that longevity and/or aging are subject to endocrine regulation and can be influenced by genomic regulation and stability.

Interestingly, some of these long-lived mutant mice also have reduced CBT. These include Ames as well as Snell dwarf mice that carry a mutation in the *Prop-1* and *Pit-1* locus, respectively, both leading to a deficit in thyroid stimulating hormones, prolactin, and growth hormone; they also include mice with targeted disruption of the GH receptor/GH binding protein

gene [28,36]. These mice present some features also found in calorie restriction including reduced size, lowered insulin and glucose levels, and reduced CBT. The extent to which reduction of CBT contributes to the increased longevity in these animals and what the mechanisms of reduced CBT are remains to be determined. One possibility is that lowered CBT is a consequence of the reduced thyroid stimulating hormone (TSH) levels or is adaptive to the lower metabolic rate, at least as demonstrated in GH deficient mice [37]. No direct indication exists, to date, that the IGF-I pathway can modulate CBT, although it has been hypothesized that reduced insulin signaling may be one of the signals inducing hypothermia during hibernation [38]. Since the IGF-I pathway can regulate lifespan in poikilotherms, the insulin pathway is likely to act independently of CBT in controlling longevity and aging. Similarly, no data are available to determine either whether $p66^{shc}$ or type 5 adenylyl cyclase null mice have a reduced CBT or if sirtuins regulate CBT.

Temperature and energy homeostasis

Reduction of CBT observed during CR can be considered as resulting from adaptive mechanisms that permit survival in conditions of limited resources, as lower CBT means reduced metabolic demand for its maintenance. If so, genetic determinants for this adaptation may exist similar to those demonstrated recently for the existence of the two genes PHA-4 and SKN-1, both encoding for transcription factors that mediate the effects of CR in the poikilotherm *C. elegans* [39,40]. PHA-4 is very similar to mammalian FOXA proteins that, in adult life, regulate glucose metabolism; SNK-1 is similar to the mammalian NRF2 and helps protect against oxidative stress. Remarkably, SNK-1 dependent regulation of lifespan during CR was central and specific to the role of this transcription factor in neurosensory cells that participate in the regulation of energy homeostasis, most likely via hormonal secretion. Specifically, CR increased mitochondrial activity in an SKN-1 dependent manner. It is not yet known whether these genes also contribute to mediating the effects of lowered temperature on longevity.

In the mouse, some genes determining the response of CBT to CR may be located on chromosomes 9 and 17, since quantitative traits loci were mapped on these two chromosomes [41]. Genes that may control CBT reduction are likely to be those involved in the central modulation of the temperature setpoint and those regulating hibernation. Their identification is hampered by the lack of molecular characterization of the central thermostat, and its components are primarily focused on the electrophysiological characterization of temperature sensitive neurons in the preoptic area of the hypothalamus [42,43]. The situation is only slightly better for the molecular biology of hibernation, which is in its infancy. Importantly, studies on hibernation emphasized the close correlation between energy metabolism and CBT and identified some important mediators or regulators of this seasonal adaptation. These include the thyroid hormone derivative 3-iodiothyronamine, the 5'-adenosine monophosphate, the hormone grhelin, and hydrogen sulfide gas, all of which are endogenous compounds that can induce hypothermia in mice (Reviewed in [38]). It is likely that further development in the field will identify molecules and pathways that are directly involved in the modulation of CBT.

The correlation between CR and CBT is one example of the intrinsic interdependence between CBT and energy homeostasis. Thus, to understand the effects of temperature on longevity it is important to take into consideration its relevance with respect to metabolic rate and the 'rate of living' theory. This theory, proposing that longevity and aging are inversely regulated by metabolic rates, ([44,45]Reviewed in [46]) is supported by several kinds of evidence, including the reduction of energy expenditure and increased energy efficiency in CR rodents, and is challenged by others [47–49]. Results also vary depending on whether metabolism is measured as resting metabolic rate (at thermoneutral temperatures and in a post absorptive state) or as daily energy expenditure. As mentioned above, energy and temperature homeostasis are dependent, one reason being that homeotherms have a constant CBT value that is higher than that of the environment and must spend a considerable amount of energy for its maintenance. Accordingly, compared to their wild type littermates, the long lived transgenic mice with reduced CBT have higher energy efficiency, losing a smaller percentage of body weight during food deprivation, when energy is utilized for the maintenance of temperature homeostasis [17], and consuming less oxygen per kilogram per hour (unpublished data). In addition, since these mice maintained the same calorie intake as their wild type littermates they showed a 10% increase in body weight. Investigating the contribution of CBT to metabolism, particularly, may shed new light on the correlation between metabolism and longevity. Such analysis will be especially interesting since temperature homeostasis is likely to be achieved through mechanisms already demonstrated to influence longevity and aging. These may include the degree of mitochondrial uncoupling, the overall cellular metabo-

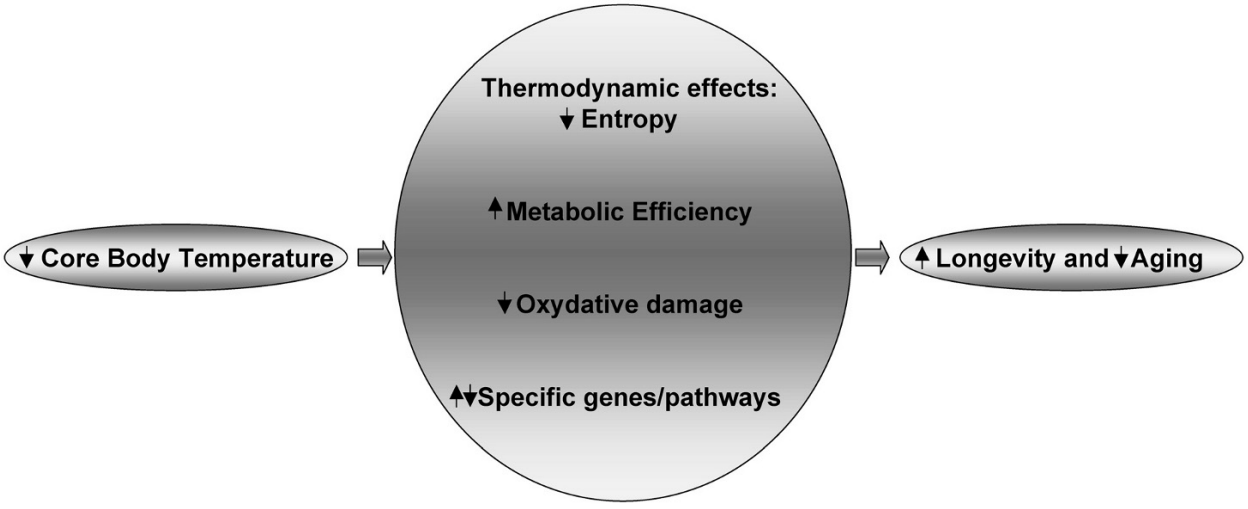

Figure 1. Schematic summary of the topics presented. Modest reduction of core body temperature (CBT) prolonged longevity and possibly slowed the aging process in both poikilotherms and homeotherms independently of calorie restriction (CR). The mechanisms mediating the effects of reduced CBT on longevity remain to be determined and include: 1. Thermodynamic effects. These influence the kinetics of biochemical reactions and can ultimately be seen as a reduction of molecular entropy. These effects are believed to be the primary mediators of the effects of temperature reduction on the lifespan and aging in poikilotherms and to contribute to increased longevity in homeotherms. 2. Effects on energy homeostasis. Reduced CBT may contribute to increased lifespan by reducing metabolic rate or increasing energy efficiency. Indeed, higher energy efficiency was observed during CR as well as in transgenic mice with reduced CBT. This correlation is in accordance with the 'rate of living' theory of aging, hypothesizing that longevity is inversely correlated with metabolic rate. 3. Reduced oxidative damage. Reduced metabolic rate could result in a reduction of free radical mediated damage due to their lowered production or increased degradation; 4. Specific genes and pathways. Analysis and comparison of survivorship trajectories suggested that thermodynamic effects may not be the only factors contributing to increased longevity by CBT reduction in homeotherms. CBT reduction may be contributing to increased longevity by modulating specific pathways through the products of specific genes, similar to what has recently been demonstrated for CR.

lism, and the endocrine regulation of energy homeostasis.

Summary and conclusion

Reduction of CBT increased lifespan and possibly retarded aging in poikilotherms as well as in homeotherms, independent of CR. The mechanisms mediating the effects of CBT on life expectancy are not known and could differ in poikilotherms and homeotherms, due simply to thermodynamics, or modulated by specific pathways or genes (Fig. 1). These factors may influence the extent of oxidative damage and/or be similar to those influencing the endocrine regulation of lifespan, including the GH/IGF-I pathways. Importantly, CBT reduction in homeotherms is associated with reduced metabolic activity and is observed during CR. Since CBT reduction during CR can be considered an adaptive mechanism to a condition of limited resources, the existence of specific genetic determinants for this regulation can be hypothesized. Their identification will shed light on the mechanisms of temperature and energy homeostasis, and they may represent targets for modulating lifespan and energy metabolism.

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