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Review

Caloric restriction and longevity: Effects of reduced body temperature

Andres E. Carrillo[a], Andreas D. Flouris[a,b,*]

[a] FAME Laboratory, Institute of Human Performance and Rehabilitation, Centre for Research and Technology Thessaly, Trikala, Greece
[b] Department of Research and Technology Development, Biomnic Ltd., Trikala, Greece

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ABSTRACT

Caloric restriction (CR) causes a reduction in body temperature (T_b) which is suggested to contribute to changes that increase lifespan. Moreover, low T_b has been shown to improve health and longevity independent of CR. In this review we examine the connections between CR, T_b and mechanisms that influence longevity and ageing. Recent findings regarding the overlapping mechanisms of CR and T_b that benefit longevity are discussed, including changes in body composition, hormone regulation, and gene expression, as well as reductions in low-level inflammation and reactive oxygen species-induced molecular damage. This information is summarized in a model describing how CR and low T_b, both synergistically and independently, increase lifespan. Moreover, the nascent notion that the rate of ageing may be pre-programmed in response to environmental influences at critical periods of early development is also considered. Based on current evidence, it is concluded that low T_b plays an integral role in mediating the effects of CR on health and longevity, and that low T_b may exert independent biological changes that increase lifespan. Our understanding of the overlap between CR- and T_b-mediated longevity remains incomplete and should be explored in future research.

1. Introduction

Organismal senescence, or deterioration, is characterized by progressive entropy, a gradual increase in molecular disorder that ultimately increases an organism's risk for mortality (Waters, 2007). Somatic deterioration, however, does not follow an orchestrated age-associated progression. It has been suggested that only 25–30% of lifespan variation can be attributed to genetic factors (Ljungquist et al., 1998; McGue et al., 1993). Thus, the accumulation of molecular disorder that contributes to somatic deterioration is subject to considerable plasticity. This phenomenon has been targeted by scientists with the expectation that advancements could compress morbidity, as well as lower disease and mortality risk. Attempts to understand the rate of human deteriorative processes, along with the exploration of longevity determinants substantially contributes to current biogerontology research (Braeckman et al., 2002; Conti et al., 2006; Houthoofd et al., 2002).

Reports highlighting the biological advantages of caloric restriction (CR) have been circulating since 1935 (McCay et al., 1935). However, the mechanisms responsible for the benefits observed during CR have been difficult to isolate because CR exerts pleiotropic effects that influence several biological systems [reviewed in Walford et al., 1987]. For example, biogerontologists continue to consider the importance of thermal biology and its influence on longevity that stems from the observation that CR reduces body temperature (T_b) (Duffy et al., 1990b; Lane et al., 1996). Indeed, it has been suggested that part of the longevity conferred by CR depends on a reduction in T_b (Tabarean et al., 2010). Ageing thermobiology research has crept away from CR since the suggestion that a low T_b may independently benefit lifespan (Conti et al., 2006; Kent, 1978). Yet, our understanding regarding the mechanisms responsible for this phenomenon remains limited. Relevant findings suggest that the influence of low T_b on longevity in homeotherms may act through mechanisms similar to those that mediate CR (Conti et al., 2006). Conversely, high ambient temperatures, such as that experienced during the August 2003 heat wave in Western Europe, have significant adverse health implications (Klenk et al., 2010). Given the global concerns of increasing ambient temperatures and its important relationship with population ageing (Costello et al., 2009), knowledge of potential mechanisms that expose the inner workings of the connections between T_b with health and longevity is becoming increasingly important. With a focus on mammalian models, we review the relationship between T_b and CR and their influence on ageing and longevity.

2. The role of cold exposure and low body temperature in modulating lifespan, health, and disease

The effects of reduced T_b on health and longevity have been circulating since 1917 [reviewed in South et al., 1972]. In poikilo-

* Corresponding author at: Institute of Human Performance and Rehabilitation, Centre for Research and Technology Thessaly, Karies, Trikala, 42100 Greece.
Tel.: +30 24310 63190; fax: +30 24310 63191.
E-mail address: aflouris@cereteth.gr (A.D. Flouris).

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therms, early studies showed that fish living in cool water (15 °C) lived significantly longer than fish living in warm water (20 °C) (Liu and Walford, 1966). Temperature-mediated longevity has also been examined in homeotherms, which has been difficult to assess due to innate physiological thermoregulatory mechanisms (Holloszy and Smith, 1986; Vaanholt et al., 2009). A number of relevant associations, however, have been documented (Doblhammer and Vaupel, 2001; Flouris et al., 2009; Moore et al., 1997). For example, we have reported that being born during the colder seasons of the year is associated with increased birth weight, gestational age, and longevity, as well as with lower risk of foetal growth restriction, and premature birth. In contrast, being born during the warmer seasons is negatively associated with birth weight, gestational age, and longevity (Flouris et al., 2005, 2009). These findings are supported by European and African data showing that birth weight and longevity are positively associated with being born during the autumn and winter seasons but negatively associated with being born during the remaining periods of the year (Doblhammer and Vaupel, 2001; Moore et al., 1997; Vaiserman et al., 2002). These reports support the notion that the rate of ageing may be pre-programmed in response to environmental influences at critical periods of early development (Flouris et al., 2005, 2009; Vaiserman et al., 2002). Indeed, the fragile biochemical equilibrium of the human intrauterine environment is significantly associated with environmental factors (Flouris et al., 2005, 2009; Hille et al., 2007; Murray et al., 2000). Although stimuli in early postnatal life also play an important role, such insults may be linked to permanent structural, functional, and metabolic changes, which in turn can lead to physiological or metabolic 'programming' of the newborn with negative effects on longevity (Flouris et al., 2005, 2009; Hille et al., 2007; Murray et al., 2000).

To date, only one experimental model of a transgenic mouse has shown that a sustained reduction in core temperature can influence lifespan in homeotherms. Conti et al. (2006) performed an elegant experiment in which a transgenic mouse model with a reduction in core temperature was developed by hypothalamic over-expression of uncoupling protein (UCP) two in hypocretin neurons. Hypocretin neuron over-expression of UCP2 generated heat in the hypothalamus that mimicked an increase in core temperature. Thus, the hypothalamus activated thermoregulatory mechanisms to reduce core temperature by ~0.5 °C. Prolonged reduced core temperature resulted in a 12 and 20% increase in median lifespan from birth, independent of CR in male and female mice, respectively (Conti et al., 2006). The mechanisms responsible for lifespan extension in hypocretin neuron over-expression of UCP2 mice remains to be determined; but it is suggested that the mechanisms may be similar those that mediate longevity by means of CR (Conti et al., 2006). Sections 2.1, 2.2, and 2.3 provide a review of the potential mechanisms that have been suggested to contribute to temperature-mediated longevity.

2.1. Brown adipose tissue and oxidative stress

Evidence suggests that in homeotherms, brown adipose tissue (BAT) functions to generate heat for the purpose of maintaining T_b in cold environments (Jastroch et al., 2008). BAT is located in the thorax in quantities inversely proportional to the size of the animal, is highly vascularized, and consists of brown adipocytes and adipocyte progenitor cells (Jastroch et al., 2008). The interesting contention that BAT can be targeted for the prevention and/or treatment of age-associated diseases in humans was discussed in a recent review (Mattson, 2010).

UCPs, specifically UCP1, are mitochondrial transporters located in the inner mitochondrial membrane of BAT that enhance proton conductivity, which uncouples adenosine triphosphate (ATP) production from substrate oxidation resulting in heat production [reviewed in Rial and Zardoya, 2009]. When maintained in a standard housing temperature (23 °C), UCP1 deficient mice developed an age-associated increase in diet-induced obesity that was more evident in female mice (Kontani et al., 2005). In humans, positive genetic associations were reported between a polymorphism in the UCP1 promoter, which reduces UPC1 gene expression, and obesity (Sramkova et al., 2007). Interestingly, disruptions of the mitochondrial electron transport chain, which lead to impaired UCP functioning and increased reactive oxygen species (ROS) production, observed in obesity and type 2 diabetes have been linked to the inflammatory burden associated with increased adipose tissue [reviewed in Martinez, 2006]. Indeed, autoimmune diabetes was exacerbated in UCP2 knock-out mice characterized by increased macrophage infiltration of islets, enhanced interleukin (IL)-1β and nitric oxide production from macrophages, and increased ROS-induced molecular damage compared to wild type controls (Emre et al., 2007). Several other proteins in the UCP family have been described, such as UCP2, UCP3, UCP4, and UCP5, but although some evidence suggests a role in thermogenesis, ROS elimination, and longevity, their exact physiological functions have been difficult to define (Andrews and Horvath, 2009; Larkin et al., 1997). For example, UCP3 mRNA is up-regulated in rat BAT following treatment with triiodothyronine (T3) or cold exposure, supporting a role for UCP3 in thermogenesis and energy expenditure (Larkin et al., 1997). Indeed, during cold exposures and low T_b, norepinephrine (NE) and thyroid hormones stimulate thermogenesis in BAT through UCP activation, a mechanism supported by reports showing an increase of UCP expression in BAT in cold exposed rodents (Florez-Duquet et al., 1998; Freeman et al., 1989). In turn, the acceleration of respiration resulting from UCPs uncoupling of oxidative phosphorylation from ATP production for the purpose of heat production reduces the production of ROS [reviewed in Rial and Zardoya, 2009]. Based on the above, UCPs are now widely considered to play a role in the antioxidant defence system of eukaryotes [reviewed in Rial and Zardoya, 2009]. In light of these observations, enhanced UCP activity may explain, at least in part, the positive health outcomes observed with a low T_b and thus, provides a potential mechanism for improved health and increased longevity associated with a low T_b.

In future studies, it may also be relevant to examine whether early life environmental temperatures influences the rate of ageing through changes in BAT activity, especially since the uncoupling or thermogenic activity of BAT can be increased with intermittent cold exposures (Freeman et al., 1989). For example, six month old Fischer 344 rats demonstrated a significant chronic cold-induced increase in interscapular BAT mass, cell proliferation, and UCP1 content, whereas the aged mice were unresponsive to chronic cold exposures (Florez-Duquet et al., 1998). These findings provide support to the aforementioned hypothesis according to which the rate of ageing may be pre-programmed in response to environmental influences at critical periods of early development (Flouris et al., 2009; Vaiserman et al., 2002).

2.2. Metabolism and body composition

Early cold exposure experiments, which have been cited as support for the "rate of living" theory (described in Section 3.1), were aimed at determining whether a reduction in T_b could influence longevity (Holloszy and Smith, 1986). In these studies, rats kept at 6 °C or 9 °C ambient temperatures resulted in a chronic increase in metabolic rate and a significant decrease in lifespan (Heroux and Campbell, 1960; Johnson et al., 1963). It was later suggested that the chronic cold exposure was an unremitting stress, which may have exacerbated the effects of chronic infections that ultimately resulted in a shortened lifespan (Holloszy and Smith, 1986). Later studies introduced intermittent cold exposures and produced dif-

fering results. For example, Holloszy and Smith gradually increased cool water (23 °C) exposure of male specific pathogen free Long-Evans rats until the rats were standing in the cool water for four hours per day (5 days/week) (Holloszy and Smith, 1986). Cold exposed rats experienced no change in core temperature (during or after cold exposures), a 44% increase in food intake, and had significantly lower body weights and higher levels of energy expenditure compared to controls. Interestingly, no differences in longevity were detected, but cold exposed rats had a significantly reduced incidence of malignancies, particularly of sarcomas. In a more recent study, Vaanholt and colleagues assessed metabolism and longevity in mice maintained continuously at either 22 °C or 10 °C during their adult life (Vaanholt et al., 2009). Mice exposed to cold had significantly higher mean daily energy expenditure (48%), and significantly reduced body mass and fat mass, but a similar median lifespan compared to mice maintained in neutral temperatures. Findings from Vaanholt and Holloszy directly contradict the "rate of living" theory, and instead, reveal health benefits that may be attributed to cold exposure such as reduced body fat mass and tumour incidence (Holloszy and Smith, 1986; Vaanholt et al., 2009). Vaanholt et al. (2009) suggested that the low incidence of tumours reported by Holloszy and Smith may be linked to the low fat content of cold exposed animals and thus, provides an interesting avenue for future work.

Reduced fat content in cold exposed animals is a relevant consistency that has significant health implications. For example, increased adiposity has been linked to high levels of inflammation which in turn has been associated with several chronic diseases (Schlitt et al., 2004; Vachharajani and Granger, 2009). Specifically, increased adiposity is associated with enhanced production of pro-inflammatory cytokines. Secreted adipokines interact with immune cells to release inflammatory proteins and recruit immune cells into adipose tissue that subsequently produces and releases pro-inflammatory mediators [reviewed in Gutierrez et al., 2009; Shah et al., 2008]. To date, however, data regarding the direct long-term effects of cold exposure and low T_b on inflammation are limited, but will be discussed further in Section 2.3. It is important to mention here an interesting concept recently introduced based on environmental observations at the other end of the temperature spectrum. According to Hansen and colleagues, continuous exposure to neutral or high ambient temperatures contributes, at least in part, to the obesity epidemic (Hansen et al., 2010). The authors suggest that lowering ambient temperatures in the home and/or workplace could counteract global obesity trends through similar mechanisms that are proposed to influence temperature-mediated longevity. This novel contention is timely and warrants further study to determine any existing parallels with disease. Currently, however, the physiology responsible for this phenomenon remains unclear but is becoming increasingly important, particularly because of increasing global ambient temperatures and population ageing (Costello et al., 2009).

2.3. Inflammation and immune function

Low T_b (usually achieved through cold exposure) has been shown to induce changes in both cellular and humoral aspects of immune function in different mammalian models, including a reduction in lymphocyte proliferation (Goundasheva et al., 1994; Shu et al., 1993), and a decrease in natural killer (NK) cell count and cytotoxic activity (Aarstad et al., 1983; Won and Lin, 1995). In humans, the small number of available experiments show that low T_b may favourably influence immune function [reviewed in Walsh and Whitham, 2006]. For instance, a 0.8 °C reduction in core temperature does not alter total leukocyte numbers (Cross et al., 1996), while a 1 °C core temperature drop during surgery results in a reduction in lymphocyte proliferation and IL-2 production 24 and 48 h post surgery (Beilin et al., 1998). Moreover, core temperature reductions of 0.5 °C (Lackovic et al., 1988) and 0.6 °C (Brenner et al., 1999) result in elevated NK cell activity. These findings are supported by *in vitro* experiments showing that monocytes incubated for one hour at 34 °C killed a greater number of *E. coli* compared with incubation for one hour at 37 °C (Roberts and Steigbigel, 1977), a finding considered favourable for host defence (Walsh and Whitham, 2006). More importantly, chronic reductions in T_b, similar to those observed during CR, have been shown to evoke improved immune responsiveness, specifically an increased T-lymphocyte, T-helper, and T-suppressor cell number as well as an increased expression of activated T- and B-lymphocytes (Jansky et al., 1996).

The potential mechanisms of altered immune response due to low T_b include the secretion of NE and the production of heat shock proteins (HSPs) [reviewed in Shephard and Shek, 1998]. However, if the cold exposure is sufficiently severe enough to induce an unremitting stress, similar to Heroux and Campbell's protocol, an increase in cortisol production and immunosuppressive response will result (Heroux and Campbell, 1960). Thus, the immune response to cold exposure appears to be context dependent and may be suppressed at long durations of exposure at lower temperatures in non-acclimated individuals, characterized by increased levels of circulating cortisol [reviewed in Shephard and Shek, 1998]. Indeed, acclimation to cold temperatures has been associated with a suppression of the cortisol response (Izawa et al., 2009) and an increase in immune system activation (Jansky et al., 1996). Chronic reductions in T_b through repetitive cold exposures for prolonged periods induce HSPs in mice BAT, with increased binding of heat shock transcription factors to DNA that may contribute to the development of cold tolerance (Matz et al., 1995, 1996; Shephard and Shek, 1998). The transcription of HSPs is initiated by NE after binding to BAT adrenergic receptors. In turn, the HSPs facilitate the translocation and activity of the enzymes involved in heat generation, and may interfere with the inflammatory response mediated by tumour necrosis factor alpha (Jaattela, 1993; Matz et al., 1995, 1996). Thus, although not completely understood, the connections between inflammation and immune function with low T_b appear to depend on the severity of the stress imposed by the extent and duration of the cold exposure, as well as the acclimation level of the individual (Heroux and Campbell, 1960; Holloszy and Smith, 1986; Izawa et al., 2009). Future work in this area or interventions that include cold, such as cryotherapy, should include measurements of cortisol to assist with monitoring levels of biological stress.

3. The role of low body temperature in modulating the effects of CR on longevity, health, disease prevention

In 1935, McCay and colleagues reported that when food intake was restricted in rats it prolonged their life (McCay et al., 1935). Since this first report, a number of similar observations have been published that support CR as the only known intervention to consistently prolong lifespan in animal models (Anderson et al., 2009; Gerritsen, 1982; Wang et al., 2007). CR involves a shift from a state of growth and proliferation to maintenance and repair (Walford et al., 1987; Weindruch et al., 1988; Yu et al., 1985) and is most effective when the caloric intake of animals fed *ad libitum* is reduced by 20–40% without malnutrition (Anderson et al., 2009; Gerritsen, 1982; Wang et al., 2007) Health benefits and subsequent lifespan extension in response to CR have been reported in diverse organisms including yeast, worms, flies, and rats [reviewed in Fontana et al., 2010]. In humans, although CR has been shown to reduce risk factors for diabetes, cardiovascular disease, and cancer, its influence on lifespan is not clearly established (Fontana and Klein, 2007).

In mammals undergoing CR a reduction in T_b consistently occurs as a maintenance strategy in response to reduced nutrient intake (Duffy et al., 1990a; Lane et al., 1996; Redman et al., 2008). This is because metabolic rate, which is synonymous with the rate of heat production, is reduced in order to conserve energy (Argyropoulos and Harper, 2002). However, a primary physiological objective of mammalian species is to maintain a constant T_b that is normally higher than the ambient temperature, which requires a considerable amount of energy to sustain (Flouris, 2010; Flouris and Cheung, 2009a, 2010). Thus, animals undergoing CR appear to tolerate a low T_b in order to oxidize less quantities of fuel that is evident from a reduction in metabolic rate (Lane et al., 1996). Indeed, preliminary results from a group of human participants on CR for six months showed a ~0.2 °C reduction in core temperature (Redman et al., 2008) that is consistent with observations in nonhuman primates (Lane et al., 1996). Subtle genetic variation, however, can lead to substantial differences in core temperature reductions during CR, as shown in six strains of inbred mice (Rikke et al., 2003). Results from mice undergoing CR show that the tumour preventative effects of CR are impaired when the animals are maintained in a high temperature (30 °C) environment (Koizumi et al., 1996), and as mentioned above, rodents exposed to cold ambient temperatures have reduced tumour incidence (Holloszy and Smith, 1986). Indeed, it has been suggested that part of the benefits induced by CR depend on T_b (Tabarean et al., 2010). The mechanisms, however, that explain the effects T_b during CR on longevity have not been clearly identified by previous authors. In Sections 3.1, 3.2, and 3.3, with a focus on mammalian models, we review the relationship between CR and T_b and their influence on ageing and longevity.

3.1. Metabolism and oxidative stress

In 1977, Sacher hypothesized that reduced metabolic rate and a subsequent decrease in free radical production during CR is responsible for the CR-induced increase in lifespan (Sacher, 1977). The potential link between energy expenditure and the rate of organismal deterioration was an attractive and highly relevant connection that characterized the "rate-of-living" theory proposed in 1928 (Pearl, 1928). The "rate-of-living" theory corresponds with the age-associated production and accrual of ROS during oxygen consumption that induce macromolecular damage, as proposed by the free radical theory of ageing by Harman in 1956 (Harman, 1956). For example, the concentration of 8-hydroxydeoxyguanosine, an indicator of DNA oxidative damage, has been shown to increase with age in mammalian tissue (Ames et al., 1993). Although a close connection exists between the "rate of living" theory and the free radical theory of ageing, their levels of soundness have diverged. Specifically, recent discoveries of differences in longevity, free radical production rates or in free radical defence and repair mechanisms observed in animals of different species with varying metabolic rates refute the "rate-of-living" theory (Holmes et al., 2001; Speakman et al., 2004).

In the year 2000, Brand proposed a theory where uncoupling oxidative phosphorylation from ATP synthesis, by enabling protons to leave the intracellular space via UCPs, reduces free radical production at the expense of ATP (Brand, 2000). Thus, metabolic rate, on its own, is not a good predictor of lifespan. For instance, T_b and energy homeostasis intrinsically depend on each other (Flouris et al., 2008b). As mentioned above, a high priority among mammalian species is to maintain a constant T_b that is regulated at the cost of a considerable amount of energy (Flouris, 2010; Flouris and Cheung, 2006). In response to low T_b, UCPs enhance proton conductivity in mitochondria that leads to heat production and a reduction in ROS [reviewed in Rial and Zardoya, 2009] (Florez-Duquet et al., 1998). In addition to a lower T_b and reduced metabolic rate, rodents undergoing CR show an attenuation of age-associated increases in rates of mitochondrial superoxide radical and hydrogen peroxide generation, slower accumulation of oxidative damage, decreased alkane production, and a delayed loss of membrane fluidity (Sohal and Weindruch, 1996). Cold exposure and low T_b elevate metabolic rate (Flouris and Cheung, 2009b; Holloszy and Smith, 1986) and increase UCP expression (Freeman et al., 1989; Larkin et al., 1997). Given that UCP expression is associated with a reduction in ROS production [reviewed in Rial and Zardoya, 2009], evidence is accumulating in support of Brands' uncoupling theory.

It has been suggested that, in homeotherms, a reduction in T_b may influence longevity through mechanisms similar to those which mediate the effects of CR (Conti, 2008). As described above, a reduction in metabolism, which is proposed to attenuate ROS production during oxidative phosphorylation, is observed during CR (Redman et al., 2008) and has been documented as a key mechanism by which CR extends lifespan (Sacher, 1977). Similarly, it has been proposed that a reduction in T_b may influence longevity, at least in part, through a reduction in ROS production [reviewed in Conti, 2008]. Support for this contention stems from the finding that, despite significant increases in metabolism, lifespan was similar in rodents intermittently exposed to low ambient temperatures compared mice kept in a thermoneutral environment (Holloszy and Smith, 1986). Thus, longevity-associated health benefits have been documented when metabolic rate was either low [i.e., CR (Redman et al., 2008)] or high [i.e., cold exposure (Holloszy and Smith, 1986)]. It is, however, important to mention that the metabolic responses of CR and the potential of decreased metabolism as a potential mechanism of CR-induced health and longevity remains inconclusive and controversial (Houthoofd et al., 2002).

3.2. Body composition

Reductions in fat free mass and fat mass, including both visceral and subcutaneous fat depots, are reported during CR (Redman et al., 2008). For example, results from the eloquent CR experiments conducted at the Pennington Centre show that after six months of 25% CR, participants lost ~24% of fat mass and ~4% of fat free mass (Heilbronn et al., 2006; Redman et al., 2008). This is largely due to the overall decrease in energy intake but also to a shift in substrate oxidation towards lipid oxidation in order to preserve carbohydrate (Weindruch et al., 1988). The benefits of reduced adipose tissue are extensively documented [reviewed in Forsythe et al., 2008] (Koutedakis et al., 2005). Excessive adiposity and its associated metabolic alterations are implicated in the pathogenesis of many chronic diseases, such as diabetes, hypertension, and cardiovascular disease (Flouris et al., 2008a; Mokdad et al., 2003; Wilson et al., 2002). However, sustained weight loss in obese individuals significantly reduces mortality, primarily from a reduction of deaths caused by diabetes, heart disease, and cancer (Calle and Kaaks, 2004; Poirier et al., 2006). Moreover, low-level inflammation, which occurs with increased adiposity, is substantially lessened with fat loss that ultimately, decreases the risk of developing several chronic diseases [reviewed in Andersson et al., 2008; You and Nicklas, 2006]. The anti-inflammatory effects of CR are discussed in more detail in Section 3.3.

Despite the well known benefits of reduced adipose tissue, low adiposity alone is not the key factor in regulating ageing and lifespan [reviewed in Fontana, 2009b]. It may be, however, that the combination of reducing adiposity with other CR-induced biological changes (i.e., low T_b) is responsible for the extension in lifespan associated with CR. For example, maximum lifespan is longer in genetically obese mice undergoing CR than in genetically normal lean mice fed *ad libitum* despite that fat mass levels in the CR genetically obese mouse are significantly higher than the *ad libitum* fed genetically normal lean mouse (Harrison et al., 1984). It appears, therefore, that more work using mammalian models is needed to

determine the influence of fat loss and its involvement with the differential effects of CR.

A potential mechanism induced by CR that may explain the benefits observed in body composition is the reduction in T_b which has been shown, through cold exposure, to activate non-shivering thermogenesis by BAT (van Marken Lichtenbelt et al., 2009). In humans, BAT levels are plentiful during embryonic and early postnatal development, but tend to show a steady age-associated decline and are absent or present in small amounts in adults [reviewed in Mattson, 2010]. Similarly, BAT responsiveness to reductions in T_b is diminished with age. This was recently shown by Saito et al. (2009) who found that a 2-hour exposure to cold (19 °C) increased radiolabeled glucose uptake into supraclavicular and paraspinal adipose tissue in 53% of young subjects but only in 8% of older subjects. Interestingly, in addition to stimulating a reduction in T_b, CR has been shown to retard the age-related decline in mitochondrial function of BAT (Valle et al., 2008). Furthermore, evidence suggests that individuals with low levels of BAT are more susceptible to developing obesity, insulin resistance, and cardiovascular disease, compared to individuals with higher levels of BAT which demonstrate low body weights, and superior health [reviewed in Mattson, 2010]. Indeed, recent studies have shown that BAT activation in healthy men following cold exposure correlated with metabolic rate but was reduced with increasing obesity (van Marken Lichtenbelt et al., 2009). Scientists continue to investigate BAT in search of practical interventions and/or pharmacological means that may be useful for the expansion or activation of this tissue in the development of novel strategies for tackling obesity (Nedergaard and Cannon, 2010).

3.3. *Inflammation and immune function*

In the year 2000, Franceschi coined the term, inflamm-ageing (Franceschi et al., 2000). This paradigm is characterized by the relationship between the concomitant age-associated increase in the risk of chronic diseases and pro-inflammatory cytokines. The belief is that inflammatory cytokines are key players in the ageing process that increase disease risk and thus, decrease lifespan. Indeed, pro-inflammatory cytokines have been shown to increase with age, and systemic inflammatory stress has been linked to the pathogenesis and/or exacerbation of numerous chronic diseases such as type 2 diabetes, hypertension, and atherosclerosis (Dandona et al., 2004; Imakiire et al., 2007; Lechleitner et al., 2002).

Individuals living in a state of chronic negative energy balance elicited by CR experience a significant reduction in systemic inflammation (Spaulding et al., 1997). It is suggested that the anti-inflammatory effect of CR is one of the most important mechanisms responsible for the anti-ageing effects of CR, and for the delay in the development of chronic diseases [reviewed in Fontana, 2009a] (Ershler et al., 1993). Studies in humans undergoing CR report low levels of circulating C-reactive protein (CRP) (Fontana et al., 2004), while lower inflammatory cytokine production was observed in CR mice following lipopolysaccharide injections compared to controls (Matsuzaki et al., 2001). Furthermore, CR has been shown to preserve age-associated changes in immune function that includes immune response capabilities, thymic involution, and shifts in leukocyte and lymphocyte subsets [reviewed in Jolly, 2004]. In non-human primates, long-term CR preserved the maintenance and production of naïve T-cells, improved T-cell function, and reduced inflammatory cytokine production from memory T-cells (Messaoudi et al., 2006).

The CR-induced reduction in inflammation has been ascribed to positive changes in metabolic, hormonal, and gene expression products that repress the inflammatory reaction to potential stimuli that do not require a full response [reviewed in Fontana, 2009a]. Other potential mechanisms responsible for the anti-inflammatory effects of CR include a reduction in adipose tissue and a subsequent change in circulating adipokines (Fontana, 2009a). Indeed, high levels of body fat are associated with increased circulating levels of tumour necrosis factor alpha, IL-6, fibrinogen, and CRP [reviewed in Rajala and Scherer, 2003] (Fontana et al., 2007). Furthermore, weight loss reduces circulating levels of resistin and leptin (pro-inflammatory) and increases circulating levels of adiponectin, an anti-inflammatory and adipocyte-specific secretory protein that modulates glucose regulation and fatty acid catabolism (Fontana, 2009a). The anti-inflammatory effects of CR may also occur due to enhanced glucocorticoid production, reduced glycaemia, increased parasympathetic tone, and increased ghrelin production (Fontana, 2009a). Ghrelin is an orexigenic hormone that is released during CR. Dixit and colleagues have eloquently shown that ghrelin exerts potent anti-inflammatory effects in T-cells, monocytes and endothelial cells through an interaction with the growth hormone (GH) secretagogue receptor (Dixit, 2008; Dixit et al., 2004). Little is currently known regarding the extent to which a low T_b influences immune function during CR. Future work should include the investigation of the connections between a low T_b, CR, and immune function.

4. The metabolic/hormonal adaptations during CR responsible for a reduction in body temperature

Mammals undergoing CR experience a significant and sustained reduction in metabolism that is maintained even when corrected for lean mass (DeLany et al., 1999). Metabolic rate is synonymous with the rate of heat production (Argyropoulos and Harper, 2002; Flouris and Cheung, 2006). Specifically, a primary physiological objective of homeotherms is to maintain a T_b that is higher than the ambient temperature (Flouris, 2010; Flouris and Cheung, 2009a, 2010). Thus, a low T_b is often reported alongside reduced metabolic rate during CR (Redman et al., 2008) that has been described as a survival strategy to cope with the condition of limited nutrients (Tabarean et al., 2010). For example, preliminary results from human participants on CR for six months showed a significant reduction in absolute 24-h energy expenditure and sleeping metabolic rate as well as a ~0.2 °C reduction in core temperature (Redman et al., 2008).

The investigation of longevity through the manipulation of specific pathways in long-lived Ames and Snell dwarf mice has developed into a useful model to study mechanisms that contribute to the ageing process (Bartke and Brown-Borg, 2004). A curious observation among the investigation of endocrine fluctuations that influence longevity is that long-lived Ames and Snell dwarf mice, which are deficient in thyroid stimulating hormone (TSH), prolactin, and GH, present certain features that are consistent with CR (Bartke and Brown-Borg, 2004; Hauck et al., 2001). Specifically, Ames and Snell dwarf mice have lower circulating insulin, glucose, and a reduced T_b. It was recently suggested that the low T_b, reported in these animals, may occur as a consequence of the reduced TSH (Conti, 2008). Thyroid hormones, T3 and thyroxine (T4), are important players in the coordination of vital human processes such as cellular metabolism and are secreted in response to TSH (Moreno et al., 2008). Indeed, the processes that regulate the metabolism of macronutrients are affected by thyroid hormones in almost all tissues [reviewed in Moreno et al., 2008]. In humans undergoing CR, absolute 24-hour and sleeping energy expenditure as well as plasma T3 levels were significantly decreased, compared to baseline, after three and six months of the intervention (Heilbronn et al., 2006). Similar results were reported for plasma T4 levels and T_b. Furthermore, the change in both T3 and T4 levels were associated with the degree of metabolic adaptation in 24-hour energy expenditure after three months of CR (Heilbronn et al., 2006). Another

investigator reported a rapid decrease in serum T3 after only four weeks of CR that was associated with a reduction in metabolic rate (Vagenakis et al., 1975). Thus, the conservation of energy through a reduction of metabolic processes during CR that translates into a low T_b appears to occur, at least in part, through a decrease in the secretion and activity of thyroid hormones T3 and T4.

Alternative suggestions indicate that low GH or insulin may be related to a reduction in T_b in long-lived mice (Conti, 2008). Specifically, GH-deficient mice demonstrate lower basal and resting metabolic rates, and lower T_b compared to wild-type controls (Meyer et al., 2004). This, however, is inconsistent with human CR trials that demonstrate no change in GH levels (Redman et al., 2008). Others report that the induction of hypothermia in hibernating animals occurs partly by a reduction in insulin signalling (Andrews, 2007). More research is necessary to investigate the connections between low T_b and endocrine systems that may influence longevity (i.e., insulin/ Insulin-like growth factor 1) by means of CR.

5. CR, T_b and longevity

Based on available data and the notions presented above, we propose a model describing the links between CR, T_b and longevity (Fig. 1). This model is based on experimental evidence suggesting that CR delays the onset of ageing and extends lifespan in diverse animal species (Fontana et al., 2010). The relationship between CR and lifespan was examined in mice that were fed *ad libitum* (115 kcal/mouse/week) compared to a variety of CR diets (85, 50, and 40 kcal/mouse/week). Mice undergoing CR lived longer than their *ad libitum* counterparts, and lifespan was inversely proportional to the degree of CR (Weindruch et al., 1986). As outlined in Fig. 1, mammals undergoing CR live in a state of chronic negative energy balance, which results in a significant reduction of fat mass (Redman et al., 2008) that causes major changes in circulating levels of adipokines such as adiponectin, resistin, and leptin (Fontana, 2009a), as well as the innate stimulation of appetite induced by the secretion of ghrelin (Yang et al., 2007). Alterations in adipose tissue and the resulting alterations in adipokine and ghrelin levels that occur with CR are proposed to be, at least in part, mechanistically responsible for the reduced levels of circulating pro-inflammatory cytokines observed during CR (Fontana, 2009a). For example, ghrelin has been shown to inhibit leptin-induced pro-inflammatory cytokine expression by monocytes, and caused a dose-dependent inhibition of IL-1β and IL-6 production by incubated monocytes (Dixit et al., 2004).

As shown in Fig. 1, studies of the anti-ageing mechanisms of CR have also included examination of the expression of genes or activation of certain pathways that are proposed to influence longevity (Higami et al., 2006, 2004). For instance, with the use of high-density oligonucleotide microarrays, transcription profiles in white adipose tissue from mice undergoing CR were examined to investigate the influence on gene regulation (Higami et al., 2004). Glucose transporter four, insulin-activated amino acid transporter, and fatty acid transporters were up-regulated by long-term CR (Higami et al., 2004). Similarly, the expression of genes involved in the glycolytic and lipolytic pathways, amino acid metabolism, and mitochondrial energy metabolism (i.e., Krebs cycle, electron transport, and oxidative phosphorylation) were up-regulated by long-term CR (Higami et al., 2004). This suggests that long-term CR in mice enhances the efficiency and uptake capacity of fuels such as glucose, amino acids, and fatty acids. Conversely, expression of genes involved in inflammation was significantly decreased in white adipose tissue from mice with long-term CR (Higami et al., 2006). Specifically, several genes encoding cytokines and acute phase plasma proteins, such as C–C chemokine ligand 2/monocyte chemoattractant protein 1, colony stimulating factor 1 receptor, and serum amyloid A3 protein, were downregulated by long-term CR (Higami et al., 2006). Thus, the health benefits of CR appear to emerge from a reduction in fat mass that is associated with enhanced functional alterations in gene expression activity. The relationships between a low T_b and CR, as well as cold exposure and fat mass have been well documented (Holloszy and Smith, 1986; Redman et al., 2008). The influence of a low T_b during CR and long-term cold exposure independent of CR on the expression of certain genes in white adipose tissue remains to be determined.

A decrease in T_b occurs in mammals undergoing CR in response to a reduction in thyroid hormones and metabolic rate for the purpose of energy conservation (Duffy et al., 1990a; Lane et al., 1996; Redman et al., 2008). As described in the proposed model (Fig. 1), evidence suggests that low T_b has an important role in mediating some of the health outcomes caused by CR (Koizumi et al., 1996; Turturro and Hart, 1991). Results obtained in mice undergoing CR demonstrate that certain CR effects are attenuated when the animals are maintained in a high temperature environment (Koizumi et al., 1996). Moreover, studies investigating the effects of reduced T_b reveal an independent influence on longevity and an overlap with CR regarding potential lifespan extending mechanisms (Conti, 2008). Specifically, mice chronically exposed to a cold environment show a reduction in the incidence of tumours (Holloszy and Smith, 1986) that has been suggested to occur because of the cold-induced reduction of fat mass (Vaanholt et al., 2009). Indeed, cold exposed mice have significantly lower levels of fat mass compared to mice living in thermoneutral environments (Vaanholt et al., 2009). To our knowledge, however, direct alterations in adipokines, ghrelin, inflammation, and disease risk in response to long-term therapeutic reductions in T_b via cold exposures have not been examined in humans. Similarly, although links have been reported with low GH and insulin, little is known regarding the connections between the genes that influence longevity, such as those altered in long-lived Ames and Snell dwarf mice or in the GH receptor/GH protein binding gene knock-out mice and their low T_b (Hauck et al., 2001).

Sympathetic nervous system axons innervate BAT and are activated following interaction with the neurotransmitter NE (Cannon and Nedergaard, 2004). In response to cold exposures NE interacts with β-adrenergic receptors, induces UCP1 expression, increases UCP1 activity, and provides substrates for BAT thermogenesis [reviewed in Mattson, 2010]. The activation of BAT is induced by cold exposure in humans (Fig. 1) and BAT activity is significantly increased in winter compared to summer, but is diminished with age and the level of activity is inversely proportional to body mass index and visceral fat mass (Saito et al., 2009). Therefore, lower levels of BAT translate into a greater susceptibility of developing obesity, insulin resistance, and cardiovascular disease, compared to individuals with higher levels of BAT [reviewed in Mattson, 2010] that may significantly influence longevity (Fig. 1). Further, overnourished young rats from a reduced nursing litter size (three pups per litter) demonstrated excess weight gain, reduced thermogenic capacity, decreased UCP1, and less cold-induced BAT activation as adults, compared to rats raised in a normal litter size (eight pups per litter) (Xiao et al., 2007). This suggests that disruptions in BAT/thermogenic activity early in life may contribute to lifelong alterations in BAT activity, UCP1 expression, and susceptibility to obesity. Indeed, cold-induced alterations in rat BAT levels and activity are highly variable at different stages throughout the lifespan (Florez-Duquet et al., 1998). It would be of interest to explore the age-associated changes in BAT activity based on early life exposures to varying environmental temperatures and how the rate of BAT decline is associated with the development of fat mass and disease. It has been suggested that the rate of ageing may be associated with environmental influences at critical periods of early development (Flouris et al., 2005, 2009; Vaiserman et al., 2002). Given that ROS production is attenuated with increased expression and acti-

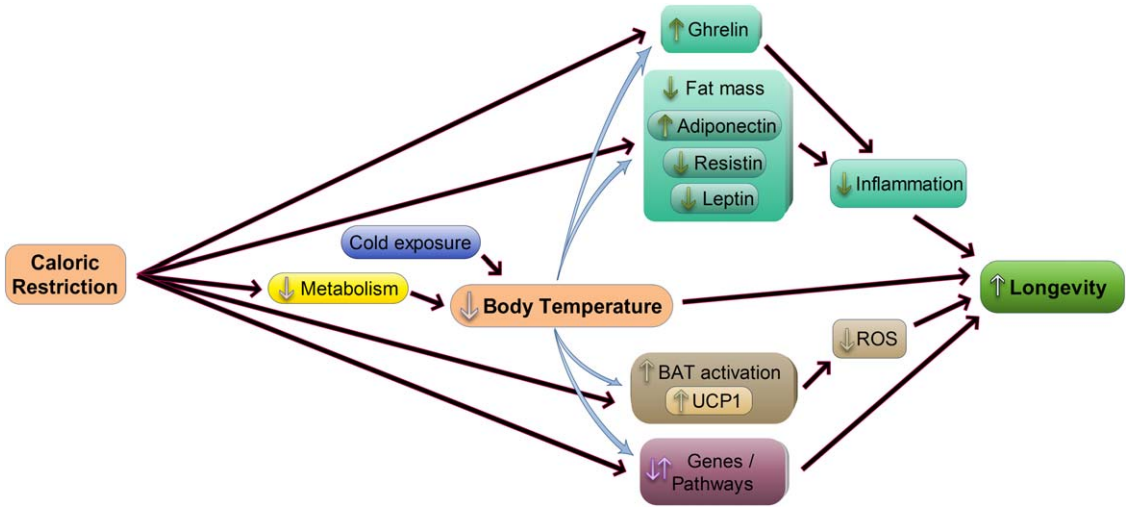

Fig. 1. Proposed model describing the relationship between caloric restriction and body temperature and their influence on longevity.

vation of UCP1 [reviewed in Rial and Zardoya, 2009], and that the uncoupling or thermogenic activity of BAT can be increased with intermittent cold exposures (Freeman et al., 1989), it is tenable to suggest that the consistent absence of cold exposures starting at early life could contribute to the susceptibility of increased fat mass and ROS-induced damage. This provides an interesting avenue for future research and is consistent with the novel contention that high ambient temperatures are contributing, at least in part, to global obesity trends (Hansen et al., 2010). Although data is limited, evidence suggests that CR and low T_b may be linked to longevity (Conti, 2008). Global increases in ambient temperatures highlight the need to identify the effects of low T_b for the purpose of developing future therapeutic interventions, particularly among older populations.

6. Concluding remarks

To our knowledge, the earliest evidence supporting the connection between reduced T_b and longevity emerged 18 years before the first published CR study by McCay and colleagues in 1935 [reviewed in South et al., 1972] (McCay et al., 1935). The important discovery that a low calorie diet could decrease T_b and increase lifespan, initiated the movement of exploring longevity through CR with the consideration of low T_b as secondary. After decades of research, the contention that low T_b can increase lifespan still has merit, which is supported by recent studies that show an increase in lifespan with low T_b independent of CR (Conti et al., 2006). The problem in homeotherms, however, is developing practical processes by which low T_b can be achieved without CR. Currently, two techniques have been utilized that include genetic manipulations in animal models and cold exposure (Conti et al., 2006; Holloszy and Smith, 1986; Vaanholt et al., 2009). Experimental manipulation of the control of thin mice has shown that low T_b can increase lifespan and that the mechanisms responsible likely overlap with those that alter longevity by means of CR (Conti et al., 2006). Cold exposure studies have been carried out using experimental protocols that include a wide range of temperatures during either continuous or intermittent exposures (Heroux and Campbell, 1960; Holloszy and Smith, 1986; Johnson et al., 1963; Vaanholt et al., 2009). The literature suggests that continuous cold exposure could be potentially harmful whereas intermittent cold exposures result in health benefits but do not extend lifespan (Heroux and Campbell, 1960; Holloszy and Smith, 1986). Furthermore, the germane literature that reports cold-induced changes in biological outcomes in humans is limited to investigations of acute changes that occur from a single exposure to a cold environment (Imbeault et al., 2009). To our knowledge, "cold therapy" in humans has not been applied to investigate mechanisms that overlap with CR-mediated longevity or relate to diseases or disorders that influence longevity such as diabetes and obesity. Thus, cold exposure experiments with refined long-term protocols that induce intermittent decreases in T_b warrant further study.

Seasonal programming during early life has been linked to longevity such that season of birth (autumn and winter) is significantly associated with increased longevity compared to those born in summer (Flouris et al., 2005, 2009). Thus, the possibility exists, as others have suggested, that the rate of ageing could be pre-programmed based on early life environmental influences (Vaiserman et al., 2002). The intriguing finding that BAT is considerably malleable during early life, as shown by responses to intermittent cold exposures (Florez-Duquet et al., 1998), suggests a potential mechanism for pre-programmed rate of ageing. Given that BAT levels are associated with obesity (van Marken Lichtenbelt et al., 2009), and that ROS production can be reduced by increased UCP1 content, which is increased by cold exposure, [reviewed in Mattson, 2010] (Florez-Duquet et al., 1998; van Marken Lichtenbelt et al., 2009), early life environmental temperature may stimulate BAT and this could influence later life. It has recently been shown that the age-associated decline in BAT activity is attenuated with CR (Valle et al., 2008). Moreover, the small amount of BAT existing in adult humans is activated by cold exposure and the level of activation is inversely proportional to age and obesity (van Marken Lichtenbelt et al., 2009). Future studies should investigate the factors that contribute to the rate of BAT decline that may include, at least in part, environmental and/or T_b. Furthermore, investigation of intermittent cold-induced BAT activity for the purpose of determining its involvement with the development and/or progression of age-associated diseases such as diabetes should be included in future research.

The aim of this review was to explore the connections between CR, low T_b, and longevity. We conclude, based on the presented evidence, that part of the benefits induced by CR are mediated by T_b, and that a low T_b may exert independent biological changes that promote longevity. The mechanisms that explain CR- and temperature-mediated longevity remain unclear, yet are likely to overlap. This information has been summarized in a model to put forth a theory based on relevant longevity research. Little is understood about the inner workings of the connections between T_b and

longevity, which should be explored in future work. Importance of developments in this area is growing in the context of increasing ambient temperatures that characterize recent and current climate change.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.arr.2010.10.001.

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