Increased Life Span Transgenic Mice with a Reduced Core Body Temperature Have an
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discriminate between the many possible high-resolution models by relying on the dichroism of the EXAFS spectra.

The structural changes of the Mn$_4$Ca complex on advancing through the S$_i$ state intermediates can be placed in the context of the polarized EXAFS data to assist in deriving a mechanism for photosynthetic water oxidation. The FTIR data, in conjunction with model II (Fig. 4), suggest that Mn$_A$, which may be ligated by Asp$^{70}$, does not change oxidation state and remains Mn(III) or Mn(IV) throughout the Kok cycle. The C-terminal Ala$^{144}$ may be a ligand to Mn$_B$, which is proposed to undergo a Mn$^{2+}$→MnD transition (26–28). Recent FTIR data suggest that His$^{332}$ monitors structural changes of the Mn$_4$Ca cluster, but no evidence for a Mn-centered oxidation was reported (29). Because Mn$_A$ is closer to His$^{332}$, Mn$_B$ may remain Mn(III) or Mn(IV) throughout the cycle. Consequently, Mn$_B$ is a likely candidate for Mn oxidation during the S$_1$→S$_2$ transition.

The dichroism in the polarized EXAFS data from single crystals provides a powerful filter for choosing among many of the proposed structural models. Also, as shown in this study, the combination of XRD and polarized EXAFS on single crystals has several advantages for unraveling structures of x-ray damage-prone, redox-active metal sites in proteins. XRD structures at medium resolution are sufficient to determine the overall shape and placement of the metal site within the ligand sphere, and refinement by means of polarized EXAFS can provide accurate metal-to-metal and metal-to-ligand vectors. In addition, different intermediate states of the active site (including different metal oxidation states), which may be difficult to study with XRD at high resolution, can be examined. The structural model from polarized EXAFS from the S$_1$ state presented here, and from the other S states, will provide a reliable foundation for the investigation of the mechanism of photosynthetic water oxidation and for the design of biomimetic catalysts for water splitting.

References and Notes

24. Materials and methods are available as supporting material on Science Online.
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Supporting Online Material

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Material and Methods

SOM Text
Figs. S1 to S7
Tables S1 and S2
References

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Transgenic Mice with a Reduced Core Body Temperature Have an Increased Life Span

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Reduction of core body temperature has been proposed to contribute to the increased life span and the antiaging effects conferred by calorie restriction (CR). Validation of this hypothesis has been difficult in homeotherms, primarily due to a lack of experimental models. We report that transgenic mice engineered to overexpress the uncoupling protein 2 in hypocretin neurons (Hcrt-UCP2) have elevated hypothalamic temperature. The effects of local temperature elevation on the central thermostat resulted in a 0.3° to 0.5°C reduction of the core body temperature. Fed ad libitum, Hcrt-UCP2 transgenic mice had the same caloric intake as their wild-type littermates but had increased energy efficiency and a greater median life span (12% increase in males; 20% increase in females). Thus, modest, sustained reduction of core body temperature prolonged life span independent of altered diet or CR.

Temperature homeostasis in mammals is regulated centrally by neurons located in the preoptic area (POA) of the hypothalamus, a region that includes the medial and lateral part of the preoptic nucleus, the anterior hypothalamus, and the nearby regions of the septum. This region is believed to contain the central thermostat, which keeps core body temperature (CBT) within a very narrow range even when the animal is exposed to a wide range of ambient temperatures. Lesion and thermal stimulation studies have demonstrated that the POA senses changes in local and peripheral temperatures and coordinates thermoregulatory responses [for review, see (1)].

With the aim of generating animals with a reduced CBT, we hypothesized that local heat production within or proximate to the POA, by mimicking an increase in CBT, might ac-
Hypocretins (hypocretin 1 and 2), also known as orexins, are neuro-peptides derived from a common precursor and activated by the intermembrane space to the matrix, thereby dissipating the proton gradient energy in the form of heat (2). Hypocretins (hypocretin 1 and 2) are exclusively expressed in hypocretin neurons (Hcrt-UCP2 mice). UCP2 is an inner mitochondrial membrane protein that uncouples oxidative phosphorylation from respiration by leaking hydrogen ions from the intermembrane space to the matrix, thereby dissipating the proton gradient energy in the form of heat (2). Hypocretins (hypocretin 1 and 2), also known as orexins, are neuro-peptides derived from a common precursor that participate in the regulation of the sleep/wake cycle, energy balance, food intake, and endocrine and autonomic functions (3–5).

Hypocretins are exclusively expressed in approximately 3000 neurons in the lateral hypothalamus (LH) at a distance of 0.8 mm from the POA (6) and provide an anatomically restricted site for heat generation in the hypothalamus.

A colony of Hcrt-UCP2 mice and wild-type littermates was established from one founder generated on and backcrossed for seven generations on a C57/BL6 background (fig. S1A).

To determine whether UCP2 overexpression in hypocretin neurons resulted in heat production that could affect the POA, we measured local temperature in the LH and in the POA. Local temperature was significantly higher in both the LH and the POA of Hcrt-UCP2 mice compared with wild-type mice (Fig. 1). Temperature elevation averaged 0.65°C in the LH and 0.32°C in the POA. The smaller increase in temperature elevation in the POA compared with the LH was likely due to heat dissipation from the LH. The difference between LH and POA temperature was constant during the 24 hours of recording.

The effect of elevated hypothalamic temperature on CBT was studied using radiotelemetry in male and female mice (Fig. 2). Hcrt-UCP2 mice maintained a normal circadian variation of CBT during the light and the dark cycles. In males, no difference in the CBT values between Hcrt-UCP2 and wild-type mice was observed during the light phase or during the transition between phases. However, Hcrt-UCP2 mice consistently exhibited a significantly lower CBT during the dark phase throughout several days of recording (Fig. 2). In females, the reduction of CBT averaged 0.34°C and was more pronounced in the second part of the dark phase with a peak difference of 0.6°C (Fig. 2, A and B). A similar pattern was seen in male transgenics, with no difference observed during the light phase and the transition from light to dark, but an average CBT reduction of 0.3°C and a peak difference of 0.56°C observed during the dark phase (Fig. 2, D and E). In contrast to males, female Hcrt-UCP2 mice also showed a significant reduction of CBT in the first half of the transition from dark to light. Motor activity was similar between Hcrt-UCP2 and wild-type mice, being only marginally higher in Hcrt-UCP2 male mice at the end of the light-dark transition, a time when no difference in CBT was observed. In females, motor activity of Hcrt-UCP2 mice was marginally lower in the last part of the dark phase, when CBT also was lowest (Fig. 2, C and F). After injection with Escherichia coli lipopolysaccharides (LPS), Hcrt-UCP2 mice developed a fever response similar in amplitude and duration to that of the wild-type mice, which indicates that the thermogenic capacity of Hcrt-UCP2 mice was not impaired (fig. S3). The CBT profile was identical between transgenic and wild-type mice during the stress peak and the light phase, but Hcrt-UCP2 mice...
maintained a temperature slightly higher than wild-type mice during the first half and the end of the dark phase. Overall, the data indicate that the reduction of basal CBT observed in Hcrt-UCP2 mice did not result from reduced locomotor activity or impaired thermogenic ability, but is consistent with an effect on the central thermostat.

We found that UCP2 overexpression reduced the number of hypocretin immunoreactive neurons by 22% and 30% in male and female Hcrt-UCP2 mice, respectively (fig. S4). It might be argued that intracellular temperature elevation, an excessive reduction in ATP synthesis, or altered intracellular Ca2+ concentrations resulting from UCP2 overexpression interferes with the normal metabolic activity of hypocretin neurons. Intracerebroventricular injection of pharmacologically high doses of hypocretin 1 reportedly elevates spontaneous physical activity and CBT (7–10). However, the possibility that a decreased number of hypocretin neurons contributed to the reduced CBT was ruled out in orexin/ataxin-3 mice (II) that showed 90% reduction of hypocretin neurons but no significantly lowered CBT (fig. S5). No differences in sleep parameters that could account for the reduction of CBT were found (SOM text).

The effects of UCP2 overexpression in hypocretin neurons on water and food consumption were also measured. Hcrt-UCP2 mice did not differ from wild-type mice in their intake of chow (measured every 3 hours or biweekly) (Fig. 3A) or water (M ± SEM: 3.7 ± 0.3 versus 3.6 ± 0.2 ml for wild-type and Hcrt-UCP2, measured biweekly). Whereas body weights of female Hcrt-UCP2 and wild-type mice did not significantly differ, male transgenic mice began to weigh significantly more than wild-type mice beginning at 20 weeks of age. By 35 weeks of age, the male transgenics weighed 10% more than the wild-type males (Fig. 3B). When subjected to 27 hours of food deprivation, Hcrt-UCP2 transgenic mice lost significantly less weight than would be predicted from their expected metabolic body mass demands as compared with wild-type littermates (genotype effect: F(1,29) = 20.64, P < 0.0001). The decrease in putative relative energy expenditure, which was similar in both male and female transgenics (Fig. 3C), is an index of increased metabolic efficiency most likely reflecting the reduced energy required to maintain a lower CBT (12).

Reduction of CBT has antiaging effects and prolongs life in poikilotherms (13). In homeotherms, reduction of CBT results from calorie restriction (CR), a controlled dietary regimen that prolongs life span in rodents (14, 15) and that has been reported to delay the onset of a variety of diseases in model organisms (16–21). However, whether re-

![Fig. 2. Core body temperature and motor activity profile. CBT and motor activity were recorded simultaneously on mice implanted intraperitoneally with radiotelemetric transmitters (Data Science International, St. Paul, MN). Mice were allowed to recover for 2 weeks after surgery before recording. The recording was carried out over a period of 10 days. Panels show 5-day temperature profiles (A and D), the average temperature (B and E), and locomotor activity profiles (C and F) from 10 days of 24-hour recording in both male and female mice. The air temperature of the room was maintained at 25 ± 0.5°C [n = 15 mice per group for males, n = 5 mice per group for females, *P < 0.05 analysis of variance (ANOVA) with repeated measures followed by Fisher’s least significant difference (LSD) tests comparing 10-min intervals]. tg, transgenic; wt, wild type.](image1)

![Fig. 3. Food intake, growth curve, and energy expenditure. (A) Mean (±SEM) daily chow (11% kcal fat) intake of mice as estimated from measuring food every 3 hours over a 24-hour period or biweekly over a 2-week period (as indicated) in adult age-matched (5 to 6 months old) and weight-matched Hcrt-UCP2 mice (n = 9 males and 9 females). Mice from the two genotypes did not differ in their food intake. (B) Growth curve of male and female Hcrt-UCP2 and wild-type littermates determined from biweekly measurements of body weight (n = 9 mice per group; *P < 0.05 ANOVA). (C) Mean (±SEM) weight loss after 27 hours of food deprivation in Hcrt-UCP2 and wild-type control mice, normalized for the expected metabolic demands of their predeprivation body mass as estimated by a power function (g weight loss/g baseline weight0.568) (29). Male and female transgenic mice lost 13 and 16% less weight, respectively, during food deprivation than would have been expected from their metabolic body mass as determined by weight loss of wild-type controls (n = 6 and 9, *P < 0.005, Fisher’s protected LSD test). tg, transgenic; wt, wild-type.](image2)
duced CBT in itself prolongs life span in homeotherms has not been demonstrated. To investigate this question, we compared the survivorship of Hcrt-UCP2 mice with wild-type littermates fed ad libitum on an 11% fat (kcal) diet. Despite eating normally (Fig. 3A), the Hcrt-UCP2 genotype showed a 25% reduction in mortality rate across adulthood. As a consequence, life expectancy (median life span from birth) was 89 days (~12%) greater in transgenic as compared with wild-type males and 112 days (~20%) greater in transgenic females (Fig. 4, A and C). Survival was assessed from a total of 57 females and 89 males; six Hcrt-UCP2 males remained alive at the time of this report and were treated as censored observations. Differential mortality was evaluated by Cox proportional-hazard regression with genotype, parent, and sex as main effects and sex-by-genotype as an interaction variable (tables S1 and S2). Each main effect was significant, but there was no effect of sex upon genotype, indicating that UCP2 overexpression in hypocretin neurons has an equal impact on the mortality of both genders.

Inspection of the complementary log-mortality plots between genotypes (Fig. 4, B and D) suggests that the ratio of their hazard rates is approximately constant with time. This assessment was verified by testing the significance of age as a time-dependent covariate (table S3). The mortality rates for the two genotypes were proportional as required for Cox analysis. These data demonstrate that the reduction of CBT within a normal physiological range in Hcrt-UCP2 mice caused a parallel, proportional shift in the mortality rate trajectory, thus reducing the aging-related frailty of the mice (22). This demographic shift resembles the effects of CR upon mortality in mice and poikilotherms and differs from the effects of temperature reduction in poikilotherms, which changes the slope of mortality plots (23–28).

Although the mechanisms underlying the prolonged life span of Hcrt-UCP2 mice have yet to be elucidated, the aggregate data in several ways suggest that the mechanisms may be similar to those mediating the effects of CR. Although the reduction of CBT in Hcrt-UCP2 mice is small, metabolic requirements to maintain a lower CBT are reduced as demonstrated by the increased energy efficiency (Fig. 3C). This may lead to lower oxidative and free radical damage that, over the lifetime, ultimately prolongs life span.

We have shown that a modest and prolonged reduction of body core temperature can contribute to increased median life span in the absence of CR. The Hcrt-UCP2 “cool mice” represent a model for studying mechanisms underlying thermoregulation and metabolic regulation in mammals. They may also be useful for studying the effects of CBT on aging and longevity that are independent of the effects induced by CR.

References and Notes