Mitotic Activity in Mice is Suppressed by Energy Restriction–Induced Torpor[1]

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ABSTRACT We monitored core body temperatures by telemetry in energy-restricted (201 kJ/wk) and control (397 kJ/wk) C57BL/6 and SHN/C3H F1 mice to determine whether torpor may be involved in the suppression of mitotic activities resulting from energy restriction. The energy restriction regimen employed inhibited the development of cancer and greatly extended longevity in both these mouse strains. Male and female C57BL/6 mice subjected to energy restriction from 4 wk of age and tested at 3 mo of age became torporific (body temperature <31°C) at ambient air temperatures of 20–22°C, whereas control animals stayed euthermic (>35°C). Energy restriction also induced torpor in 3- and 13-mo-old SHN/C3H F1 female mice, whereas 3-, 13- and 24-mo-old control mice were euthermic. Energy restriction decreased mitotic activities to ~30% of control values in both jejunum and epidermis in 3-mo-old female C57BL/6 mice maintained at 20–22°C. However, this suppression of mitotic activities was antagonized by housing the energy-restricted mice at 30°C for 2 wk, indicating that torpor plays a substantial role in suppressing mitotic activities in energy-restricted mice. J. Nutr. 122: 1446–1453, 1992.

INDEXING KEY WORDS:

- *mice* • *energy restriction*
- *torpor* • *mitotic activity*

Energy restriction decreases tumor incidences, retards aging processes and extends maximum survival times in mice and rats (Weindruch and Walford 1988). Its mechanism, however, is not well understood. One hypothesis is that energy restriction decreases energy metabolism by lowering body temperature (Sacher 1977). In fact, mice (*Mus musculus*) become heterothermic readily after short periods (24 to 48 h) of food deprivation (Himms-Hagen 1985, Hudson and Scott 1979, Webb et al. 1982). Duffy et al. (1991) recently reported that chronically energy-restricted (ER) mice show a lower mean body temperature than control mice.

Torpor is a state characterized by a decrease in body temperature accompanied by drastic changes in physiological activities. Hibernation is the extreme stage of torpor, characterized by a dramatic fall in body temperature and energy consumption. In addition to these changes, cellular turnover rates are slowed during hibernation or torpor (Vinogradova 1988). Lok et al. (1988) found that energy restriction (75% of ad libitum feeding) decreased mitotic activities and DNA synthesis in the jejunum, colon, esophagus, epidermis and mammary glands of mice. However, it remains unknown whether energy restriction suppresses mitotic activities in mice by inducing torpor.

The major aim of the present study was to determine whether a regimen of energy restriction proven to increase the life span in mice causes a suppression of cellular turnover rates by inducing torpor. We report that the body temperature of ER mice decreased dramatically each day to reach a level as low as an ambient room temperature. The mitotic activities in intestinal and epidermal cells were suppressed when the ER mice were housed at the typical ambient air temperatures (20–22°C). However, mitotic activities of ER mice returned to the high levels observed in control mice if the torpor of ER mice was prevented by raising the ambient air temperature.

MATERIALS AND METHODS

Mice. Mice [C57BL/6 (B6)], 4 wk of age, were purchased from Nippon Clea (Tokyo, Japan). Female mice of the mammary tumor–prone SHN strain

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(Nagasawa et al. 1976) were mated with C3H/He males (Charles River Japan, Kanagawa, Japan) in our animal facility at Akita. The resulting SHN/C3H F1 (SHN/C3H) female offspring were weaned at 21 d. Mice were assigned randomly to either a control or an ER group upon arrival at our vivarium or at the time of weaning. The animals were individually housed in plastic cages with wood shavings at 20–22°C, with a relative humidity of 50% and a 14-h light (0400–1800 h):10-h dark (1800–0400 h) photocycle. Telemeters were implanted in randomly selected mice from control and ER groups. The numbers of SHN/C3H female mice implanted with telemeters were as follows: 3-mo-old controls, $n = 6$; 13-mo-old controls, $n = 6$; 24-mo-old controls, $n = 3$; 3-mo-old ER mice, $n = 6$; 13-mo-old ER mice, $n = 6$. In addition, other mice (Group Ct→ER, $n = 6$) were subjected to short-term energy restriction by switching them from the control diet at 12 mo of age to the ER diet for 1 mo. The B6 male mice implanted were 3 mo old ($n = 6$/diet group). In addition to male B6 mice, telemeters were implanted in 3-mo-old B6 females. The latter mice were divided into three groups. Control ($n = 8$) and ER1 ($n = 8$) mice were housed at room temperatures (20–22°C). The remaining group (ER2, $n = 8$) was housed at the same room temperature (20–22°C) as all other groups until 2 wk before killing, when they were transferred to an incubator maintained at 30°C, without changing the photocycle.

The mice were maintained under pathogen-free conditions throughout the experiment. Body weights were determined every 2 wk. All animals were handled in accordance with the animal welfare guidelines of Akita University.

Diet. The formulations of the two diets in the present study were previously described (Koizumi et al. 1987, Koizumi et al. 1990, Weindruch et al. 1986). Two diet groups (control and ER) were studied. Mice in the control group ate the control diet (a purified, pelleted, 23.2% casein diet) at a level of 397 kJ/wk. The control mice were given 27 g per week, which averaged 20% less than the amount consumed by mice given completely free access to the diet. Mice in the ER group ate 201 kJ/wk of the restricted diet (a purified, pelleted, 39.7% casein diet further enriched in vitamins and minerals). The mice were given 16 g per week, thereby consuming approximately the same amount of protein, fat, vitamins and minerals per week as did the control mice, but only 32% as much carbohydrate. These diets met the NRC requirements (National Research Council 1978) except for chromium intake, which was only ~5% of the recommended amount. Although the chromium intakes were smaller than the recommended values, neither increases in tumor incidences nor other detrimental effects were historically observed in various strains of mice (Koizumi et al. 1987, Weindruch et al. 1986, Weindruch and Walford 1982).

The control B6 and SHN/C3H mice not monitored for body temperature were fed 4 g of control diet daily on Monday through Thursday morning and 11 g on Friday morning. The ER mice of both strains not monitored for body temperature were fed 3.5 g of the ER diet per day on Monday and Wednesday and 9 g on Friday mornings between 0900 and 1000 h as previously reported (Koizumi et al. 1990, Koizumi et al. 1987). The telemetered control mice of both strains were fed ~3.9 g of the control diet daily [27 g/wk (397 kJ/wk)] between 0900 and 1000 h. The telemetered ER mice were fed ~2.3 g of the ER diet [16 g/wk (201 kJ/wk)] daily at 1800 h. Control mice consumed diets in the dark period, although they were given food in the morning. The ER mice, however, consumed diets within 3 to 4 h after they were given food. Although the two groups of mice were given diets at different times, both groups consumed diets during the dark period.

Core body temperature recording. Radio-transmitting telemeters (Model TA10TA-F20 transmitter, Prime Tech Inc., St. Paul, MN), weighing 2 g, were implanted in the peritoneal cavity of the mice. The transmitters were coated with silicone elastomer to prevent rejection. The implantations were performed under an anesthesia of 2–4% enflurene in a gas mixture containing oxygen (4 L/min) and nitrous oxide (1 L/min). The transmitters were calibrated at various known temperatures against a Japanese Bureau of Standards calibrated thermometer (Tokyo, Japan). Radiosignals were detected using receivers (Model CTR86 receiver, Prime Tech Inc.). The voltage signals from the receivers were recorded continuously using recorders (Model Linear, Shimazu, Japan) and were transferred to a computer (PC98, NEC, Tokyo, Japan) every 15 min. The computer automatically converted voltage to temperature using a voltage vs. temperature calibration curve. The telemeter is effective for at least 4 mo and all temperatures were determined within 3 mo of shipment of the equipment. We started recording the body temperature 1 wk after surgery for a period of at least 24 h. A core body temperature of <31°C has been defined as torpor by others (Hudson and Scott, 1979), and this definition was also used in the present study. We defined body temperatures of 35–39°C as euthermic and <35°C as heterothermic.

Mitotic index. Mice were dosed with colchicine (1 mg/kg body wt in distilled water) intraperitoneally in the morning (0600–0800 h) (Stevens Hooper 1961) and killed by cervical dislocation 3 h after injection. The small intestine and pieces of abdominal skin were collected from individual animals, transferred into formalin (100 mL/L distilled water) for 24–48 h and then processed for paraffin sectioning. Tissue specimens were stained with hemotoxylin and eosin.

Statistics. Comparisons of mean body weights, mean core body temperatures, mean ranges and mitotic indices, and cell numbers, which are presented

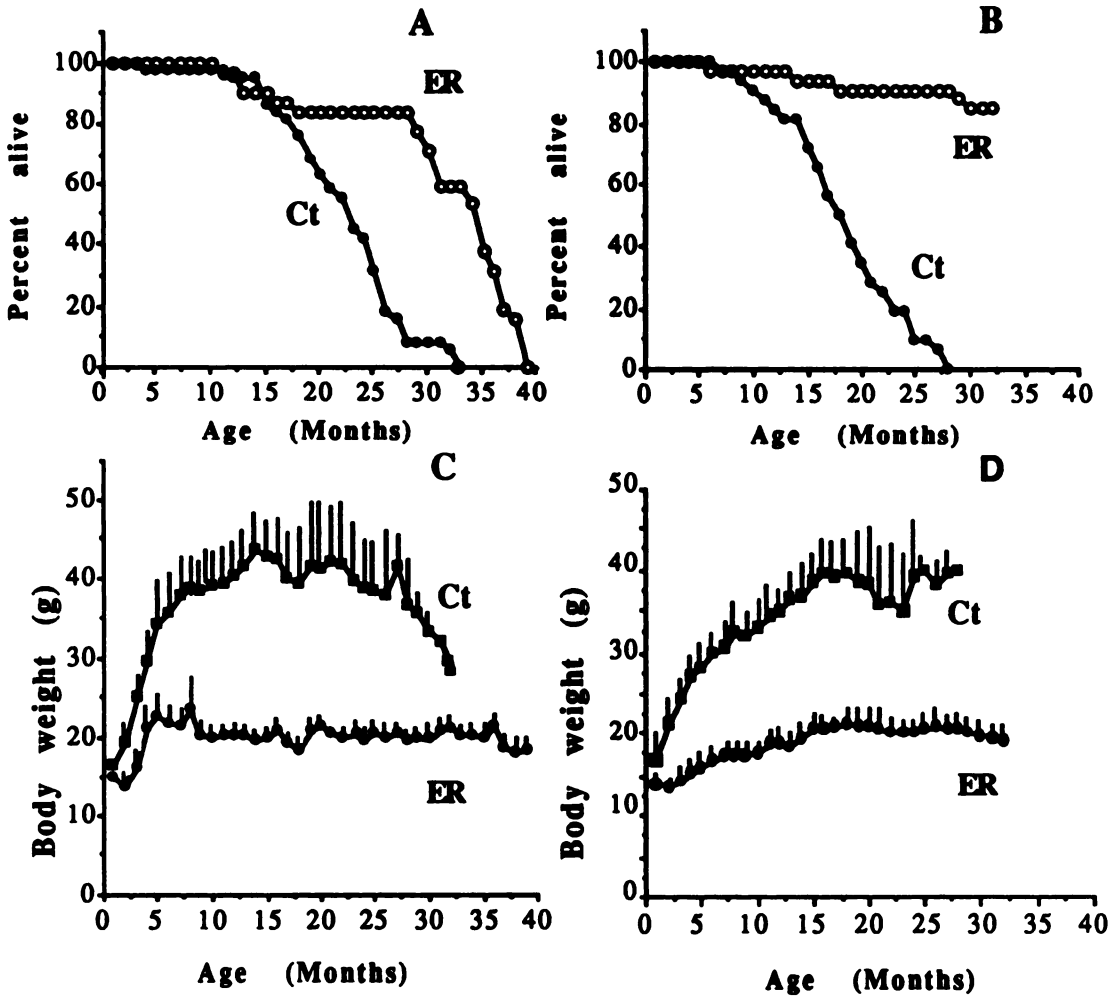

FIGURE 1 Survival curves and body weight gain of control (Ct) and energy-restricted (ER) mice. *A*: Survival curve for B6 male mice (n = 38 for control group, n = 32 for ER group). *B*: Survival curve for SHN/C3H female mice (n = 32 for control group, n = 33 for ER group). *C*: Body weight gain of B6 male mice. Bars indicate 1 SD. *D*: Body weight gain of SHN/C3H female mice. Bars indicate 1 SD.

as means ± SD, were conducted using ANOVA followed by Duncan's multiple range test (Gad and Weil 1989). A value of $P < 0.05$ was considered significant. Differences in tumor incidences or mortality were analyzed by Fisher's exact test (Gad and Weil 1989). Survival rates and ages of lymphoma deaths in ER and control mice were compared using Cox-Mantel test (Gad and Weil 1989).

RESULTS

Survival curves, mortalities, growth curves and tumor incidences. Survival curves and body weight gains are summarized in **Figure 1**. Energy restriction increased the survival rate of B6 mice significantly (Fig. 1 and **Table 1**). It also reduced net incidences of lymphoma significantly (Table 1). Furthermore, energy restriction delayed the onset of lymphoma significantly (Cox-Mantel test, $z = 5.25$, $P < 0.001$). The earliest lymphoma death in control mice occurred at 12 mo of age, and 50% of lymphoma deaths had occurred by 25 mo of age. In contrast, in ER mice, the earliest lymphoma death occurred at 30 mo of age and 50% of lymphoma deaths occurred by 35.5 mo. Energy restriction also extended longevity and reduced mammary tumor incidences significantly in SHN/C3H female mice (Fig. 1, Table 1). By 31 mo, all 32 control mice had died, whereas 28 of 33 ER mice survived (Fig. 1). Cumulative mortalities and mammary tumor incidences by 32 mo were significantly reduced by energy restriction (Table 1). The increased life span in ER mice of these two strains seemed to be mainly the result of a lower incidence of neoplastic diseases.

The mean body weights of adult B6 and SHN/C3H mice were proportional to energy intakes (Fig. 1). The ratio of the mean body weight of the ER mice to that of the control mice was 0.50 (20 g vs. 40 g) for B6 mice and 0.54 (19 g vs. 35 g) for SHN/C3H F1 mice, roughly equal to the ratio of the energy intakes.

TABLE 1

Tumor incidences, longevities and maximum life spans of control and energy-restricted (ER) C57BL/6 male mice and SHN/C3H female mice[1]

Group	Neoplastic diseases: Lymphoma	Lung tumor	Mammary tumor	Hepatoma	Ovarian tumor	Non-neoplastic diseases	Age of 50% survival[2]	Age of the last death[2]	Comparison of survival rate[3]
			number of mice				*y*	*y*	
B6 male									
Control (n = 38)	32*	1	0	0	0	5	1.89	2.71	z = 6.17
ER (n = 32)	15	0	0	0	0	17	2.80	3.25	P < 0.001
SHN/C3H female[4]									
Control (n = 32)	1	0	22*	2	2	4	1.50	2.33	z = 7.51
ER (n = 33)	1	0	0	0	0	4	>2.67	>2.67	P < 0.001

[1]Non-neoplastic diseases include unknown causes of death, hydronephrosis and bleeding; *indicates that control mice had higher incidences than ER mice (P < 0.05) by Fisher's exact test.
[2]Age of 50% survival indicates the age to which 50% of mice survived. Age of the last death indicates the age when the last mice died.
[3]Survival rates of control and ER mice were compared using Cox-Mantel test (see Materials and Methods).
[4]SHN/C3H mice were observed until 32 months of age.

Core body temperature in control and ER mice. A representative circadian body temperature profile expressed in clock time is shown in **Figure 2*A*** for control SHN/C3H F1 mice. The core body temperatures were fairly constant in control mice during the light and dark phases. The highest body temperature was recorded during the active phase (dark period) around midnight and the lowest in the inactive phase (light period). Irrespective of age, control mice maintained daily body temperatures between 39 and 35°C and remained euthermic (**Table 2**). A typical body temperature change of a 13-mo-old ER mouse is shown in Figure 2*B*. Similar torpor profiles were found in all ER and Ct→ER mice examined (Table 2) in both strains. Torpor started at midnight and ended in the morning. The lowest body temperature recorded was 23.0°C (ambient temperature = 21°C). The highest body temperature for ER mice was recorded at ~1800 h, indicating that body temperature started to rise before feeding time.

Effects of torpor and environmental temperature on mitotic indices in the jejunum and epidermis. We determined the effects of torpor on the mitotic indices using the colchicine technique (Stevens Hooper 1961). Three groups of 3-mo-old female B6 mice, control (n = 8), ER1 (n = 8) and ER2 (n = 8), were examined. The mean body temperatures of control and ER1 mice are summarized in Table 2. Telemetry revealed that the lowest core body temperature recorded during 24 h in the eight ER2 mice was 34.2°C. The mean body temperature of the ER2 mice (n = 8) was 36.5 ± 0.12°C and the mean daily range was 3.17 ± 0.63°C. Therefore, ER2 mice did not enter torpor when housed at 30°C. Mitotic indices were suppressed both in the small intestine (27% of the mean control values) and epidermis (30% of the mean control values) in ER1 mice, but to a lesser extent or not at all in ER2 mice (**Fig. 3** and **Table 3**). In the jejunum, villus height and width were less in ER1 mice compared with values for control mice. These differences, however, were not apparent when the restricted mice were housed at 30°C (ER2 group).

DISCUSSION

It is a widely held axiom that reduced energy intake leads to a decrease in the rate of metabolism. Because most of the metabolic energy arises from oxidative metabolism, the reduction primarily involves the oxidative pathway. Reduced oxidative metabolism should have survival benefits, because the rate of generation of reactive oxygen molecules would be expected to decrease. On the basis of this view, Sacher and Hart (1978) suggested that the life-prolonging action of energy restriction results from a reduced metabolic rate. However, results from other studies (Masoro et al. 1982, McCarter and McGee 1989) demonstrated that energy restriction reduces only transiently the rate of metabolism per unit mass in rats so that this value was similar for ER and control rats over most of the life span. The mean body weight in adulthood, as shown in the present study, was proportional to the amount of energy consumed, indicating that both ER and control mice consumed similar amounts of energy per gram of body weight. Nevertheless, one may argue that during periods of torpor, mice can conserve more energy than at other times. However, much energy is consumed in warming bodies to a euthermic level (Nedergreerd and

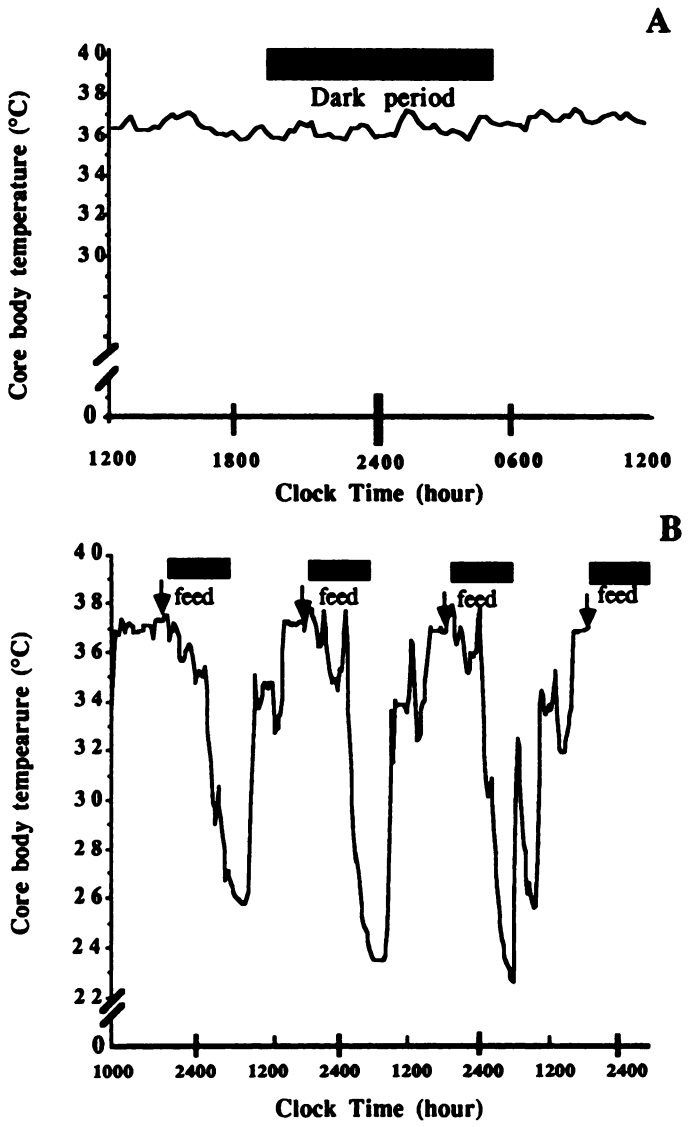

FIGURE 2 A representative circadian profile of the core body temperature of a 13-mo-old SHN/C3H mouse fed the control (*A*) or energy-restricted (*B*) diet.

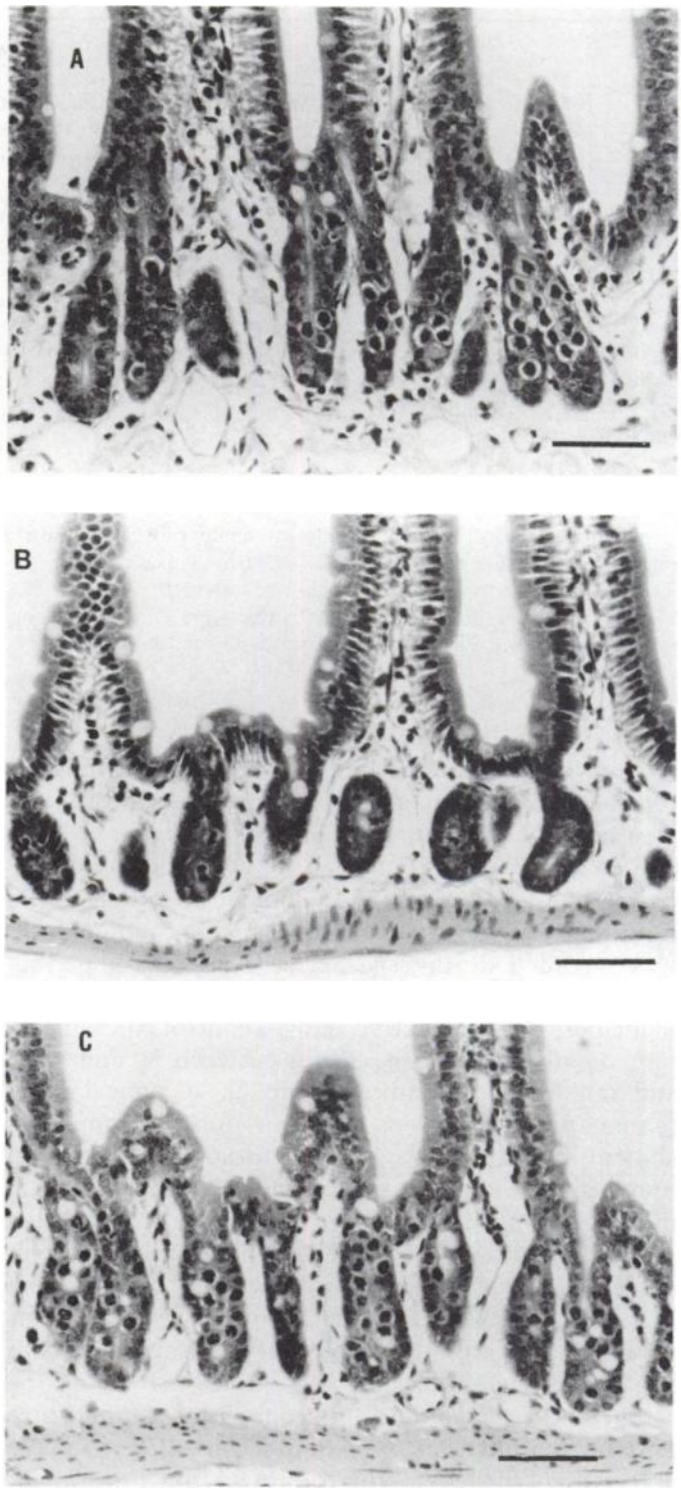

FIGURE 3 Decreased small intestine mitotic activity in mice subjected to energy restriction (ER1) and its recovery by housing energy-restricted (ER2) mice at 30°C. *A*: Jejunum from a control mouse. *B*: Jejunum from an ER1 mouse. *C*: Jejunum from an ER2 mouse. Hematoxylin and eosin staining. Scale bar = 50 μm.

Cannon 1990). Thus, whether torpor can reduce overall energy expenditure remains questionable. We are reluctant to support the hypothesis that the primary action of energy restriction resides in decreased energy metabolism through torpor, although the idea cannot be discarded completely at this time. We are aware of evidence that long-term life-prolonging energy restriction does not induce torpor in rats, although it does decrease the mean body temperature (Duffy et al. 1989). Therefore, torpor may exert specific actions in mice.

We demonstrated that a life-prolonging energy restriction that induces daily torpor also decreases mitotic indices. However, ER mice recovered mitotic activities in the jejunum and epidermis simultaneously if torpor was prevented by housing the mice at 30°C. Local responses initiated by contact of mucosa with food (Carey and Cooke 1991) partly regulate the growth in gut mucosa but do not seem to regulate epidermal cell turnover rates. In addition, energy intake was the same for the ER1 and ER2 groups, leading us to conclude that life-prolonging energy restriction inhibited cellular turnover rates by

TABLE 2

Mean body weight, mean core body temperature and torpor profiles in control and energy-restricted (ER) C57BL/6 mice and SHN/C3H female mice[1]

Diet	Age (mo)	No. of mice	Mean body weight	Core body temperature (°C) Mean	Core body temperature (°C) Range (high-low)[2]	Hours/day with body temperature at 39–35°C	35–31°C	31–27°C	27–23°C
			g						
B6 male									
Control	3	6	21.9 ± 1.3^{bc}	37.7 ± 0.70^{a}	1.64 ± 0.15^{c}	24	0	0	0
ER	3	6	17.7 ± 1.1^{c}	33.2 ± 0.97^{b}	13.0 ± 1.44^{a}	10.8 ± 2.41	6.54 ± 1.96	3.41 ± 1.72	3.22 ± 2.72
B6 female									
Control	3	8	19.7 ± 2.3^{bc}	37.1 ± 0.53^{a}	2.74 ± 0.46^{c}	24	0	0	0
ER1	3	8	17.6 ± 1.6^{c}	32.7 ± 1.24^{b}	13.8 ± 1.19^{a}	11.7 ± 2.47	5.20 ± 0.66	1.24 ± 0.46	5.75 ± 2.37
SHN/C3H female									
Control	3	6	24.3 ± 2.4^{b}	37.4 ± 0.35^{a}	1.92 ± 0.72^{c}	24	0	0	0
Control	13	6	34.9 ± 3.8^{a}	37.0 ± 0.46^{a}	1.41 ± 0.27^{c}	24	0	0	0
Control	24	3	39.2 ± 3.1^{a}	37.2 ± 0.40^{a}	2.35 ± 0.38^{c}	24	0	0	0
ER	3	6	17.6 ± 1.3^{c}	34.1 ± 0.15^{b}	10.2 ± 1.35^{b}	9.46 ± 0.91	11.0 ± 1.36	3.46 ± 0.66	0
ER	13	6	19.8 ± 2.3^{bc}	34.8 ± 0.54^{ab}	12.1 ± 1.77^{ab}	14.6 ± 2.32	6.81 ± 1.56	1.53 ± 0.85	1.04 ± 0.58
Ct→ER[3]	13	6	22.4 ± 3.0^{bc}	33.5 ± 1.50^{b}	12.2 ± 2.16^{ab}	12.7 ± 3.83	5.16 ± 2.24	3.05 ± 2.08	3.14 ± 2.79

[1]Values are means ± SD. Statistically significant differences between means were evaluated by Duncan's multiple range test, which was applied when one-way ANOVA indicated significant differences ($P < 0.05$). Means in a column not sharing the same superscript are significantly different.

[2]Because variances increased as means increased, values were transformed by logarithm. After transformation, variances were homogeneous. Transformed values were compared.

[3]The mean of body weight of short-term ER, i.e., Ct→ER, mice was 34.0 ± 4.1 g before starting ER and decreased to 22.4 ± 3.0 g after 1 mo of ER.

inducing torpor and that the cell turnover rates depend on environmental temperatures in ER mice.

Studies in rodents clearly show that energy restriction prevents or delays the onset of major diseases and alters their incidences (Weindruch and Walford 1988). Tumors are the major cause of death in most mouse strains. Therefore, energy restriction is able to extend longevity mainly by delaying the onset of tumors or decreasing tumor incidences (Weindruch and Walford 1988). Hamsters in hibernation and torpor have been shown to develop fewer chemically induced tumors, and they experienced increased longevity compared with those in an awake state (Althoff et al. 1985, Lyman et al. 1981). These effects of torpor or hibernation are, at least in part, associated with a low cell turnover rate. This hypothesis is supported by Malkinson (1991), who showed that tumor incidences in mice show a good correlation with basal cell turnover rates in the organs with high cell turnover rates. For example, A/J mice, which have a high incidence of pulmonary tumors, also have a high basal cell turnover rate in the lung (Malkinson 1991).

We previously reported that long-term energy restriction delays puberty and prevents animals from reaching maturity (Koizumi et al. 1990). It also decreased the prolactin production and decreased the number of mammatrophs (Hamada et al. 1990, Koizumi et al. 1989, Koizumi et al. 1990, Koizumi et al. 1991). These effects of energy restriction on the neuroendocrine system suggest hypothalamic involvement. Hypothalamic involvement is also suggested by hibernation studies. The hypothalamic nuclei seem to be involved in the entrance to, maintenance of, and arousal from hibernation through changing the dynamics of opioids in the central nervous system and peripheral organs (Nurnberger et al. 1991, Wang et al. 1987). The interaction of the central nervous system with gastrointestinal stimuli may be critical for understanding the effects of energy restriction on the neuroendocrine system as well as thermogenesis.

Energy restriction–induced torpor has not attracted much attention, although torpor may explain diverse effects of energy restriction in mice. Low body temperature may have decreased cell turnover rate by slowing the cell cycle and may have resulted in the preservation of stem cells in old animals. There is also indirect evidence that some biochemical and physiological changes induced by energy restriction and by torpor or hibernation may have similarities. For example, antioxidant mechanisms are activated by energy restriction (Koizumi et al. 1987, Lee and Yu

TABLE 3

Suppression of mitotic activities in C57BL/6 female mice by energy restriction and recovery of mitotic activities by housing energy-restricted (ER) mice at 30°C[1]

Group	Housing temperature	Body weight	Intestine[2] Villus width	Villus height	Crypt size	Mitotic index[3]	Epidermis[4] Mitotic index
		g	*μm*	*cells*	*cells/crypt*	%	%
Control	20–22°C	19.7 ± 2.27[a]	78.8 ± 6.20[a]	84.6 ± 8.63[a]	33.2 ± 2.12[a]	25.3 ± 1.90[a]	1.28 ± 0.21[a]
ER1	20–22°C	17.6 ± 1.64[ab]	56.0 ± 3.03[b]	76.5 ± 3.66[b]	27.5 ± 2.02[b]	6.86 ± 2.19[c]	0.39 ± 0.14[b]
ER2	30°C	17.4 ± 0.83[b]	75.8 ± 3.93[a]	82.6 ± 3.09[a]	30.8 ± 3.47[ab]	20.1 ± 3.18[b]	1.21 ± 0.33[a]

[1]Values are means ± SD for eight mice per group. For statistical test, see Table 1.
[2]Jejunum, 1 cm long, was excised from each mouse and examined individually; 30–50 crypts/specimen were counted.
[3]Mitotic index was defined by the percentage of mitotic cells in the total cell population in crypts or dermis.
[4]Skin pieces (1 × 1 cm) were collected from individual mice. More than 2500 cells/skin sample were counted.

1990) and by hibernation (Buzadzic et al. 1990). Another similarity between hibernation and energy restriction is evidence that regression of reproductive function is a precondition for hibernation (Darrow et al. 1988) and is found in ER mice (Koizumi et al. 1989, Koizumi et al. 1990, Koizumi et al. 1991). We are thus tempted to hypothesize that long-term energy restriction in mice shows striking similarities in physiological changes to hibernation. Our hypothesis, however, awaits further research that provides information on the long-term effects of housing temperatures on ER mice.

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