Longevity of cold-exposed rats: a reevaluation of the "rate-of-living theory"

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HOLLOSZY, JOHN O., AND E. KAYE SMITH. *Longevity of cold-exposed rats: a reevaluation of the "rate-of-living theory."* J. Appl. Physiol. 61(5): 1656–1660, 1986.—It has been postulated that increased energy expenditure results in shortened survival. To test this "rate-of-living theory" we examined the effect of raising energy expenditure by means of cold exposure on the longevity of rats. Male 6-mo-old SPF Long-Evans rats were gradually accustomed to immersion in cool water (23°C). After 3 mo they were standing in the cool water for 4 h/day, 5 days/wk. They were maintained on this program until age 32 mo. The cold exposure resulted in a 44% increase in food intake ($P < 0.001$). Despite their greater food intake, the cold-exposed rats' body weights were significantly lower than those of control animals from age 11 to 32 mo. The average age at death of the cold-exposed rats was 968 ± 141 days compared with 923 ± 159 days for the controls. The cold exposure appeared to protect against neoplasia, particularly sarcomas; only 24% of the necropsied cold-exposed rats had malignancies compared with 57% for the controls. The results of this study provide no support for the concept that increased energy expenditure decreases longevity.

energy expenditure; food intake; neoplasia; weight gain

IT HAS BEEN POSTULATED that metabolic rate is one of the major factors that determine longevity. Rubner (32) concluded that life duration is related to energy turnover on the basis of comparative studies showing that small short-lived animals have higher metabolic rates than large animals with longer life-spans. He estimated that lifetime energy expenditure is about 200 kcal/g body wt. (Although roughly correct for some species, this value is off by a factor of >3 for humans.) Along the same lines, Pearl (30) proposed that life duration is inversely proportional to metabolic rate per unit of body mass and suggested that depletion of a vital irreplaceable substance, which occurs at a rate proportional to the rate of energy expenditure, causes aging. As formulated by Pearl (30), this "rate-of-living theory" is a direct outgrowth of vitalism and makes little sense in the context of current biological knowledge. However, the rate-of-living theory has been restated in modern form in terms of tissue damage caused by accelerated formation of superoxide radical and its derivatives (6, 9, 13, 16, 17). At low rates of O_2 utilization, damage due to production of free radicals of O_2 may be minimized by free radical scavenger mechanisms (9, 13). However, when rates of O_2 consumption are increased, these mechanisms may not be able to keep pace with the rate of free radical formation (8).

Studies of the effects of cold exposure have been cited as providing experimental support for the rate-of-living theory (cf. Ref. 11). In these studies, rats were kept at 6°C (19) or 9°C (22–24) continuously. The cold-exposed animals had a chronic increase in metabolic rate and a large decrease in life-span (19, 22–24). A factor that complicates interpretation of these studies is that the rats were not specific pathogen free, raising the possibility that the chronic cold stress may have shortened life-span by aggravating the effects of chronic infections (22). Furthermore, most stresses, including the stimuli that increase metabolic rate such as cold exposure or exercise, are normally intermittent and separated by long recovery periods during which reversal of the effects of the acute stress can occur. In contrast, continuous cold exposure represents an unremitting stress, and there is evidence that chronic stress can have deleterious effects on health and longevity regardless of whether or not it is associated with an increased metabolic rate (cf. Refs. 10, 18, 29, 35, 37).

In the present study we reevaluated the effects on longevity of increasing the metabolic rate by means of cold exposure. To minimize the nonspecific effects of chronic stress we used intermittent instead of continuous cold exposure. To avoid the complicating effects of chronic infections we used specific-pathogen-free rats. The results do not support the concept that an increase in metabolic rate shortens life-span.

MATERIALS AND METHODS

Specific-pathogen-free male Long-Evans rats aged 3 mo were obtained from Charles River, Wilmington, MA. Eight percent of the animals, selected at random, were killed and necropsied. Cultures were obtained on their respiratory tracts, tympanic bullae, and gastrointestinal contents, and serum was tested for antibodies against pathogenic viruses and mycoplasma. All the tests were negative, providing evidence that the rats were pathogen free.

The rats were housed in a temperature- and light-controlled room with its own ventilation system, with 15 air exchanges/h (100% intake and 100% exhaust), in a facility in which no other rats were housed. The animal room was lighted between 7 A.M. and 7 P.M. and maintained at 20°C. To avoid introducing infections into the

rat colony, the people who entered the room to care for the animals did not work with other rats or in areas where they were exposed to other rats.

At the age of 6 mo, rats were assigned to either a control group (54 rats) that was not subjected to any experimental treatment or a cold-exposure group (24 rats). Cold exposure consisted of immersion of the rats up to the upper border of their scapulae in water kept at 23°C. Cold exposure was initially for 10 min/day and was gradually increased over a 3-mo period until the rats were standing in the cool water for 4 h/day, 5 days/wk. They were maintained on this program until age 32 mo, at which time the cold exposure was discontinued. Rectal temperature was measured before and immediately after the cold exposure at least once every 3 mo during the study and never decreased during the cold exposure; i.e., the rats were able to increase heat production sufficiently to maintain body temperature. The rats were dried with towels before being returned to their cages. They showed no signs of distress before, during, or after the periods of cold exposure.

The rats were fed a diet of Purina rat chow and water ad libitum. Food intake was measured daily for 1 wk/mo for 10 rats/group; the food intake measurements were rotated, i.e., the first 10 rats during the first month, the next 10 rats the second month, and so on. Food intakes of rats that reduced their intakes because of terminal illness are not included in the averages (this was done by not including the food intakes for the last 2 mo of life for all the rats in the averages).

To obtain a rough estimate of caloric expenditure during the cold exposure four 12-mo-old rats that had adapted to the cold exposure for 6 mo were immersed to the upper border of their scapulae in a measured amount of water adjusted to 23°C in a steel barrel (i.e., the usual procedure for cold exposure of the rats) in a room in which temperature was kept at 23°C. The increase in water temperature over a 60-min period was measured and used to calculate energy expenditure. This procedure was repeated four times on different days, and the values were averaged.

A detailed necropsy including gross and histological examination was performed on all the rats except those in which autolysis was too far advanced by the time their death was discovered. Eighteen of the controls and nine of the cold exposed rats were killed (CO_2 chamber) late during their terminal illness when they appeared to be in discomfort. The animals that were killed were necropsied within 30 min. Animals that were found dead were refrigerated and necropsied within 24 h. Cultures were obtained, and serum was tested for antibodies against pathogenic viruses and mycoplasma on the rats that were killed during their terminal illness; these rats were found to be specific pathogen free. The statistical significance of the difference in survival between the groups was evaluated with the generalized Wilcoxon (Breslow) test (7). The significance of differences in the cause of death and the incidence of disease processes was evaluated using the χ^2 test. The food intake data were analyzed using two-way analysis of variance with testing of subhypotheses with the use of appropriate contrasts. Statistical analyses were performed using the General Linear Models procedure of the Statistical Analysis System (3).

RESULTS

Food intake and body weight. At all ages, the cold-exposure group ate significantly more food than the controls ($P < 0.001$); this increase in food intake averaged ~11 g/day, or 44% (Table 1). There was a significant decrease ($P < 0.01$) in food intake with age in both the cold-exposed and the control groups (Table 1). This decrease was marked after age 27 mo.

Despite the greater food intake of the cold-exposed rats, their body weights were significantly lower than those of the control rats between the ages of 11 and 32 mo ($P < 0.01$; Fig. 1). After age 32 mo the two groups' weights became similar as the result of weight loss by the surviving control rats and of weight gain by the surviving experimental animals after discontinuation of the cold exposure at age 32 mo. The control groups' weight loss began at about age 26 mo, with a decrease in average weight of the group of about 100 g by age 32 mo. This decline was due to loss of weight of the surviving animals, not to a longer survival of smaller rats.

Increased energy utilization. To obtain a rough estimate of the increase in caloric expenditure during the cold exposure four 12-mo-old rats, average weight 407 ± 14 g, that had adapted to the cold exposure for 6 mo were immersed to the upper border of their scapulae in a measured amount of water adjusted to room temperature (23°C) in a steel barrel (i.e., the usual procedure used for

TABLE 1. *Food intake*

Age Period, mo	Food Intake, g	
	Control	Cold exposure
8–12	27.1±3.6	38.1±3.7
13–17	26.8±2.9	37.9±3.0
18–22	24.7±4.0	37.7±2.9
23–27	24.0±3.6	35.1±3.8
28–32	20.8±2.1	29.6±4.5

Values are means ± SD. Food intake for cold exposure vs. control group, $P < 0.01$ for all age periods.

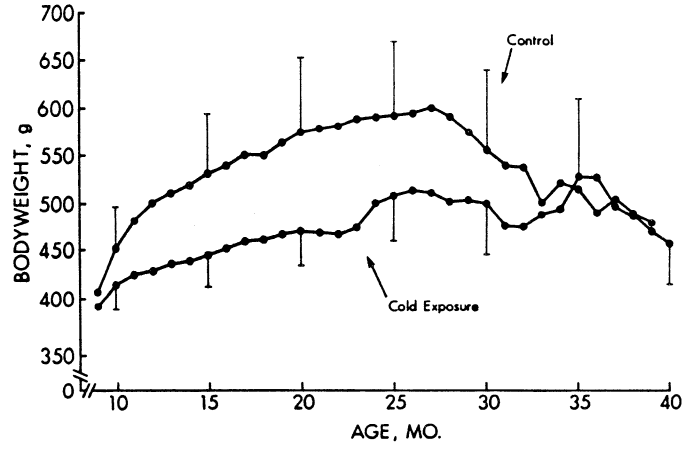

FIG. 1. Average body weights of cold-exposed rats and controls. Cold exposure was begun at age 6 mo and gradually increased over 3 mo until rats were standing in 23°C water for 4 h/day.

cold exposure of the rats). The increase in water temperature over a 60-min period was used to calculate energy expenditure. The average water volume was 23.2 ± 1.4 liters, and the increase in water temperature in 60 min averaged 0.94 ± 0.05°C in four trials.

This represents an average heat loss to the water of 5.4 kcal/h for each rat. This is, of course, an underestimate as *1*) the animals' wet head, shoulders and, usually also, front legs (which rested against the side of the barrel) were above the water; and *2*) there is additional heat loss in the expired air. The average metabolic rate of freely eating rats measured over 24-h periods averages ~113 kcal·kg body wt^{-1}·24 h^{-1} (27). This represents a metabolic rate of 1.92 kcal/h for a 407-g rat. Thus metabolic rate was increased about threefold above that of rats at normal room temperature during the 4-h periods of cold exposure.

Longevity. The average age at death of the cold-exposed rats was 968 ± 141 days (mean ± SD), whereas that of the control rats was 923 ± 159 days. These values are not significantly different. As shown in Fig. 2, there were no major differences in the shapes of the mortality curves of the two groups.

Necropsy findings. The cold exposure appeared to protect against development of certain malignancies. As shown in Table 2, neoplasia was the cause of death in only 19% of the necropsied cold-exposed rats compared with 50% in the control group. The largest difference was in the development of sarcomas; none of the necropsied cold-exposure rats had sarcomas compared with an incidence of 17.4% in the controls (Table 3). The cold exposure may also have protected against development of carcinomas, with a 50% lower incidence in the cold-exposed than in the control rats; however, with the small number of animals involved, the difference in the incidence of carcinoma was not statistically significant.

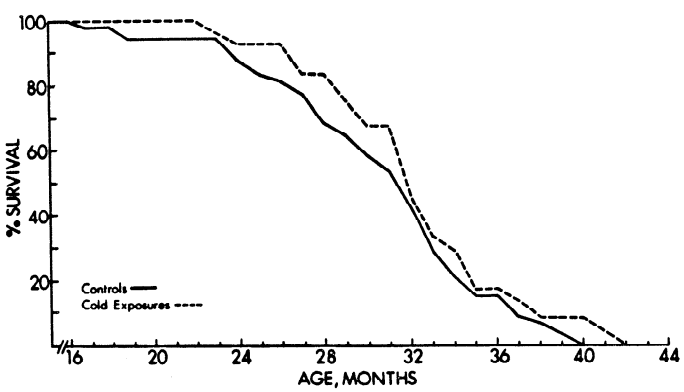

FIG. 2. Survival curves for 2 groups.

TABLE 2. *Apparent cause of death*

Group	Number Necropsied	Neoplasia		Renal Disease		Periarteritis Nodosa	
		n	%	*n*	%	*n*	%
Controls	46	23	50.0	20	43.5	3	6.5
Cold exposure	21	4	19.0*	13	62.0	4	19.0

* Cold exposure vs. controls, $P < 0.02$.

TABLE 3. *Prevalence of various pathological processes*

	Total		Sarcoma, %	Carcinoma, %	Lymphoma-leukemia, %
	n	%			
			Incidence of malignancies		
Controls	26/46	56.6	17.4	28.3	10.9
Cold exposure	5/21	23.8*	0*	14.3	9.5
	Total		Severe Nephrosis, %	Moderate Nephrosis, %	Urolithiasis, %
	n	%			
			Incidence of renal disease		
Controls	42/46	91.3	58.7	21.7	10.9
Cold exposure	21/21	100	66.7	19.0	19.0
	Total		Myocardial Fibrosis, %		Periarteritis, %
	n	%	Severe	Mild	
			Incidence of cardiovascular pathologies		
Controls	30/46	65.2	2.2	52.1	10.9
Cold exposure	21/21	100†	23.8*	61.9	23.8

* Control vs. cold exposure, $P < 0.01$; † Control vs. cold exposure, $P < 0.05$.

The chronic nephropathy that most older rats develop (1, 25) was the major cause of death in the cold-exposed rats; however, the incidence of severe nephrosis at the time of death was similar in the cold-exposed and control rats (Table 3).

Periarteritis nodosa, which is a common vascular lesion seen with aging in the rat (1), was the cause of death in 6.5% of the control rats and in 19% of the cold-exposed animals (Table 2, $P = 0.12$). There was a large difference in the development of severe myocardial fibrosis between the groups, with a 10-fold higher incidence in the cold-exposed animals (Table 3).

None of the rats had evidence of chronic infections at necropsy.

DISCUSSION

The cold-exposed rats in this study did not have a shortened life-span despite an increase in energy expenditure sufficient to result in a slowing of growth, a lower maximum weight, and an increase in food intake of ~44%. This finding provides evidence against the rate-of-living theory. The present results differ from those of previous studies in which cold exposure resulted in a marked shortening of life-span (19, 22–24). A major difference in experimental design that may help to explain these disparate results relates to the cold stress used.

It has been assumed that the marked reduction in life-span of the rats subjected to continuous cold exposure in these earlier studies was due to the increase in metabolic rate and thus provides support for the rate-of-living theory (cf. Ref. 11). However, continuous cold exposure is an unremitting stress, and chronic stress due to a variety of causes, such as fear, anxiety, noise, or overcrowding has deleterious effects on health and longevity (18, 29, 31, 35, 37). The harmful effects of stress are probably mediated by chronic elevation of the "stress hormones," i.e., catecholamines, adrenocorticotropin,

corticosteroids (2), regardless of whether or not there is an increase in energy expenditure. Therefore, to try to minimize the nonspecific effects of chronic stress and maximize the effects of increased energy expenditure, we used a much more intense cold stress but applied it for relatively brief periods separated by long recovery intervals.

This approach has the added advantage of being more biologically relevant, because stimuli that increase metabolic rate in everyday life, such as exercise or cold exposure, are normally of limited duration and followed by periods of recovery. The finding that our rats did not have a decrease in longevity despite an approximately threefold increase in energy expenditure during the 4-h periods of immersion in cool water, suggests that the shortened survival of cold-exposed rats in previous studies (19, 22–24) was due to nonspecific effects of chronic stress rather than to an increased rate-of-living.

An unexpected finding of considerable interest is that the animals subjected to 4 h of daily immersion in cool water had a significantly reduced incidence of malignancies, particularly of sarcomas and perhaps also of carcinomas (Table 3). It is well documented that severe chronic food restriction results in a marked decrease in the incidence of neoplasia in rats and mice (5, 14, 21, 28, 38). Thus the finding that our cold-exposed rats had a decreased incidence of neoplasms is particularly surprising in view of their very high food intake. The magnitude of the reduction in the incidence of malignancies in the intermittently cold-exposed rats seems sufficiently impressive to warrant studies designed to elucidate the mechanisms involved.

The finding that the cold-exposed rats had a significant increase in the incidence of severe patchy myocardial fibrosis provides evidence that our effort to minimize the nonspecific pathological effects of chronic stress by making the cold exposure intermittent was not completely successful. High levels of catecholamines can cause myocardial necrosis (cf. Ref. 10). It seems possible that this accentuation in the cold-exposed animals of the myocardial fibrosis frequently seen in old rats (1) is the end result of repeated cold-induced increases in catecholamine secretion (20). However, in no case did the myocardial fibrosis appear to be the cause of death.

The original basis for the concept that metabolic rate determines the rate of aging was the finding in comparative studies of different species that there is a correlation between metabolic rate and life-span (32). There is a relationship between longevity and metabolic rate, and it has been reported that the regression of log life-span on log metabolic rate (or log body weight) accounts for ~60% of life-span variance (33). This correlation does not provide information regarding cause and effect. However, three lines of experimental evidence, from studies of the effects of food restriction, exercise, and cold exposure, have been used to support the rate-of-living theory.

It was generally believed that a reduction in food intake causes metabolic rate per unit of body weight to decrease, and it has been suggested that the extension of life-span that occurs in rodents in response to caloric restriction (5, 28) is due to a decrease in metabolic rate (cf. Refs. 12, 34). However, recent studies have shown that life-prolonging food restriction does not result in a decrease in metabolic rate per gram of lean body mass in rats (27); furthermore, food-restricted rats actually consume more food per gram body weight per day and per lifetime than rats eating ad libitum (26, 39).

In early studies on the effects of exercise, rats given free access to running wheels did not live as long as sedentary controls (4, 36). These studies have been cited as support of the rate-of-living theory (cf. Ref. 11). However, recent studies have not confirmed these early reports (15, 21). On the contrary, in the recent studies exercising rats lived significantly longer than freely eating (15, 21) or pair-fed (21) sedentary animals.

Another line of experimental evidence that has been used to support the rate-of-living theory came from the studies showing that chronic cold exposure shortens the life-span of rats (19, 22–24). However, our finding that rats subjected to intermittent immersion in cool water, which resulted in a large increase in energy expenditure, lived as long as the controls, does not support the interpretation that the shortened survival of chronically cold-exposed rats is due to elevation of metabolic rate. Instead, we think it likely that the shortening of life-span in continuously cold-exposed rats is due to the deleterious, nonspecific effects of chronic stress (10, 18, 29, 35, 37).

We conclude from the results of the present study of the effects of intermittent cold exposure and of recent studies of the effects of food restriction (26, 27, 39) and of exercise (15, 21) that there is no experimental support for the rate-of-living theory of aging. On the contrary, intermittent increases in energy expenditure may actually have health benefits (Refs. 15, 21, and Table 3) that deserve further investigation.

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