Thermoregulation with Age: Role of Thermogenesis and Uncoupling Protein Expression in Brown Adipose Tissue (43691)

PHILIP J. SCARPACE,[1] MICHAEL MATHENY, STEPHEN BORST, AND NIHAL TÜMER

Geriatric Research, Education and Clinical Center, Department of Veterans Affairs Medical Center, Gainesville, Florida 32608–1197, and Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, Florida 32610

Abstract. **To investigate whether attenuation of thermogenesis in interscapular brown adipose tissue (IBAT) may account for the loss of thermoregulation with age, we examined two indices of thermogenesis after two types of cold exposure: one in which the senescent rats maintained homeothermy and the other in which the senescent rats became hypothermic. To this end, we assessed body temperature, guanosine 5′-diphosphate (GDP) binding to the IBAT mitochondrial uncoupling protein (UCP) and the induction of UCP mRNA after both 1-hr and 48-hr mild cold exposures at 8°C and after a more severe, 1-hr cold exposure at 4°C in 3- and 24-month-old F-344 rats. Thermoneutrality was determined to occur at an ambient temperature of 26°C in rats of both ages. In the 1-hr mild cold–exposed rats, there was no significant increase in GDP binding to IBAT UCP. However, after 48 hr of mild cold exposure, there was a 3-fold increase in GDP binding and a 5-fold increase in the expression of UCP mRNA despite no hypothermia in either the young or old rats. During the more severe cold exposure, the senescent rats, but not the young rats, became hypothermic. GDP binding to UCP increased 75% following cold exposure and, surprisingly was the same in young and old rats. UCP transcripts did not increase during the 1-hr cold exposure. These data, coupled with our previous findings of diminished β_3-agonist-stimulated IBAT thermogenesis, suggest that (i) IBAT thermogenesis, at least in the senescent rats, may be mediated by other than β_3-adrenergic receptors, and (ii) that altered heat dissipation or impaired thermogenesis at some site other than interscapular BAT is responsible for the observed hypothermia.** [P.S.E.B.M. 1994, Vol 205]

The capacity for thermoregulation diminishes with age in both humans and rodents. In humans, thermoregulation in response to either heat, cold, or infection is impaired in the elderly (1, 2). In rodents, thermoregulation is impaired in senescent animals after cold exposure and varies according to the severity of the cold stress and the age of the animal (3–6). Furthermore, studies in mice suggest that older animals have less chance of survival in the cold than do younger animals (7).

In the rat, nonshivering thermogenesis in brown adipose tissue (BAT) is an important contributor of the heat necessary to maintain body temperature. In rats acclimated to thermoneutrality, thermogenesis in BAT contributes approximately one-third of the increase in metabolic rate following acute cold exposure (8). However, in cold-acclimated rats, the principal site of thermogenesis following acute cold exposure is BAT, which contributes greater than two-thirds of the increase in the metabolic rate (8). BAT thermogenesis is mediated by catecholamine activation of adenylate cyclase through sympathetically innervated β-adrenergic receptors (9). This process accelerates lipolysis, and the liberated fatty acids serve as substrates for mitochondrial oxidation and provide the signal to uncouple mitochondria, producing high rates of substrate oxida-

[1] To whom requests for reprints should be addressed at GRECC (182), Department of Veterans Affairs Medical Center, Gainesville, FL 32608–1197.

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tion and an increase in heat production without the phosphorylation of adenosine 5′-diphosphate (ADP) (10). The degree of uncoupling and subsequent heat production can be assessed by the number of guanosine 5′-diphosphate (GDP) binding sites on the uncoupling protein (UCP) in BAT mitochondria. Increases in the number of binding sites are equated with increases in BAT thermogenic activity (11). In addition, prolonged (several hours) exposure to cold induces the synthesis of UCP and further increases the capacity for thermogenesis (12).

Attenuation of sympathetically activated thermogenesis in BAT may account for the loss of thermoregulation with age. For example, we and others have reported that norepinephrine-stimulated O_2 consumption, one measure of thermogenesis, is decreased in senescent rats (4, 13). Moreover, we reported that in senescent rats β-adrenergic signal transduction in BAT is impaired (14). There are 50% fewer β-adrenergic receptors and a similar decrease in the ability of β-agonists to activate adenylate cyclase in senescent compared with young rats (14). Recent evidence suggests that the β-adrenergic receptor in BAT is pharmacologically distinct from either the β_1- or β_2-adrenergic subtypes and predominantly consists of the putative β_3 subtype (15, 16). Using the specific β_3-agonist, CGP-12177A, we recently demonstrated that agonist-stimulated O_2 consumption and GDP binding to interscapular BAT (IBAT) mitochondrial UCP is reduced in senescent compared with young rats (13).

These data suggest that the hypothermia observed in senescent rats following cold exposure may be a result of the attenuated thermogenesis in BAT. To test this hypothesis, we examined two indices of thermogenesis in IBAT after two types of cold exposure: one in which the senescent rats maintained homeothermy and the other in which the senescent rats became hypothermic. To this end, we assessed body temperature, GDP binding to the IBAT mitochondrial UCP, and the induction of UCP mRNA after a 48-hr, mild cold exposure at 8°C and at 1-hr, more severe cold exposure at 4°C in 3- and 24-month-old rats.

Methods and Materials

Animals. Male F-344 NNia rats of 3 and 24 months of age were obtained from Harlan Sprague-Dawley (Indianapolis, IN) under contract with the National Institute on Aging. Upon arrival, rats were examined and remained in quarantine for one week. Animals were cared for in accordance with the principles of the Guide to the Care and Use of Experimental Animals. Rats were housed individually in microisolated cages and maintained on Purina Rat Chow *ad libitum* with a 12:12-hr light-dark cycle (06:00 to 18:00 hr). Experiments were begun 60–90 min after the beginning of the light cycle. Food and water were provided during the overnight periods of cold exposure and bedding material was kept at an absolute minimum. Ambient temperature was 26°C.

Body Temperature. Body temperature was measured using battery-operated biotelemetry devices consisting of a radio transmitter with a pulse rate that varies in direct proportion to temperature (Mini-Mitter, Sunriver, OR). Each transmitter was calibrated and surgically implanted into the peritoneal cavity, and the animals were allowed to recover for one week. Receivers were placed under each cage. Core body temperature was measured remotely every 3 min and averaged over 6-min or 15-min intervals.

Isolation of IBAT Mitochondria. Rats were sacrificed by cervical dislocation under 50 mg/kg pentobarbital anesthetic. The circulatory system was perfused with 20 ml of cold saline and IBAT excised and trimmed of visible white fat. Mitochondria for use in the GDP binding and cytochrome *c* oxidase assays were isolated as described previously (17). The final pellet was resuspended in 100 m*M* sucrose, 1.2 m*M* K-EDTA, 12.2 m*M* choline Cl, and 40 m*M* K-TES, pH 7.1. Mitochondria were used immediately in the GDP-binding assay or stored frozen at −70°C for the cytochrome *c* oxidase assay.

GDP Binding Assay. The density of binding sites was determined from a multipoint Scatchard analysis of [^{3}H]GDP binding (specific activity adjusted to 1.012 Ci/mmole, Dupont-NEN, Boston, MA) as previously described (17). Briefly, protein (50 μg) and 0.02–3.0 μ*M* [^{3}H]GDP were incubated both with and without 1.5 m*M* unlabeled GDP for 15 min at 37°C in a total volume of 250 μl of the buffer used for final suspension with the addition of 0.1 mg/ml fatty acid-free BSA and 2 μ*M* rotenone. The reaction was terminated by dilution with buffer, and the bound [^{3}H]GDP separated from free by filtration over glass fiber filters (Whatman GF/B, Clifton, NJ). Specific binding was determined from the difference in binding with and without 1.5 m*M* unlabeled GDP.

Cytochrome c Oxidase. Cytochrome *c* oxidase activity, a measure of mitochondrial mass, was determined by the rate of oxidation of ferrocytochrome *c* as measured by the decrease in its absorbance at 550 mμ (14).

O_2 Consumption. O_2 consumption was assessed on up to four rats simultaneously with an oxyscan analyzer (OXS-4; Omnitech Electronics, Columbus, OH) as described previously (17).

UCP mRNA. Total cellular RNA was determined by extraction from 100 mg of minced and sonicated IBAT tissue using a modification of the method of Chomczynski and Sacchi (18). For extraction, 1 ml of RNAzol B (Biotecx, Friendswood, TX) and 100 μl of chloroform were added to the sonicated IBAT, and this preparation was mixed vigorously, incubated 15

min on ice, centrifuged, and the aqueous phase retained. The RNA was twice precipitated with an equal volume of isopropanol, washed with 70% ethanol, suspended in a 10-m*M* EDTA solution, and heated to 65°C for 10 min. After centrifugation, the supernatant was harvested. The integrity of the isolated RNA was verified using agarose gels (1%) stained with ethidium bromide. The RNA was quantified by spectrophotometric absorption at 260 mμ using multiple dilutions of each sample. The accuracy of optical density quantification was verified by comparison with ethidium Br-stained agarose gel quantification with known ribosomal RNA standards (Pharmacia Biosystems, Piscataway, NJ). For measurement of UCP mRNA levels, several concentrations of serially diluted RNA samples were immobilized on nylon membranes (Gene Screen, Dupont NEN, Boston, MA) using a slot blot apparatus (Biorad, Richmond, CA). The membranes were baked at 80°C for 2 hr. The baked membranes were prehybridized using 25 m*M* potassium phosphate, 750 m*M* NaCl, 75 m*M* Na citrate, 5X Denhardt's solution, 50 μg/ml denatured salmon sperm DNA, and 50% formamide. After incubation for 14–16 hr at 42°C, the membranes were hybridized with a ^{32}P random prime-labeled cDNA probe in the prehybridization buffer with the addition of 10% dextran sulfate (19). The cDNA clone for UCP was kindly provided by Dr. Leslie Kozak, the Jackson Laboratory, Bar Harbor, ME (19). After hybridization for 14–16 hr at 42°C, the membranes were washed and exposed to X-ray film (Kodak X-AR, Rochester, NY) for 96 hr at −70°C using intensifying screens. The developed autoradiogram was scanned using a Biorad Model 620 Video Densitometer. Optical density per μg total cellular RNA was calculated by comparison with internal laboratory standards of IBAT UCP mRNA present on each nylon membrane.

Northern analysis indicated this probe hybridizes to two mRNA species, a major band corresponding to 1.5 kilobases and a minor band corresponding to 1.9 kilobases (data not shown). The probe did not hybridize to any mRNA species from the cerebral cortex (data not shown). This is similar to what has previously been reported for the rat (12).

Results

Thermoneutrality in Young and Old Rats. The capacity for thermogenesis in BAT is dependent on the ambient temperature in which the animals are housed. Cold-acclimated rats have a greater capacity for thermogenesis than those adapted to thermoneutrality. Because thermoneutrality may be different for young and senescent rats, in the first set of experiments, the ambient temperature for thermoneutrality was determined. Basal O_2 consumption was measured in young and old rats following a one-week adaptation at ambient temperatures between 23° and 28.5°C. In both age groups the minimum O_2 consumption was observed at 26°C (Fig. 1). At this temperature the O_2 consumption was significantly less in the senescent compared with the young rats ($P < 0.01$). Rats in subsequent experiments were maintained for one week at 26°C.

Mild Cold Exposure. Two indices of thermogenesis, GDP binding and the expression of UCP, were assessed in young and senescent rats after mild cold exposure (conditions that were not severe enough to overcome the rats' ability to thermoregulate). Body temperature was assessed in control rats maintained at 26°C and cold-exposed rats maintained at 8°C for 48 hr (Fig. 2). After a brief period of adjustment, body temperature was indistinguishable between cold-exposed and control rats of both ages (Fig. 2). Body temperature of the rats in all the groups became synchronized and displayed the normal circadian rhythm with elevated nighttime and reduced daytime temperatures. All animals survived this mild cold exposure with no ill effects.

During the 48-hr cold exposure there was a small (6%–7%) decrease in body weight in both the young and senescent rats (Table I). There was a considerably larger decrease of 30% in the wet weight of the IBAT following cold exposure in rats of both ages, and this may represent the utilization of substrate (Table I). In contrast to the reduction in IBAT wet weight, mitochondrial protein per IBAT increased by 55% in the young and by 37% in the senescent rats. Furthermore, cytochrome *c* oxidase activity per IBAT increased by 250% and 230%, respectively, in the young and old rats (Table I).

To investigate the role of thermogenesis in IBAT in maintaining body temperature, GDP binding to the mitochondrial UCP and the induction of UCP mRNA was assessed in IBAT at the end of 1 hr and 48 hr in control and 8°C cold-exposed rats. The number of GDP binding sites was assessed by multipoint Scatchard analysis of [^{3}H]GDP binding to isolated mitochondria (Fig. 3). In control rats, there was no difference with age in the number of GDP binding sites (Table II). After 1 hr of mild cold exposure, there was a small nonsignificant increase in GDP binding to UCP in both the young (60.6 ± 7.7 pmol/mg protein vs 84.5 ± 14.7, $n = 8$) and old (69.4 ± 15.1 vs 90.9 ± 13.9, $n = 8$) rats. Moreover, after 48 hr of mild cold exposure, the density of GDP binding sites significantly increased in the young rats by 176% and the old rats by 227% (Table II). In addition, the total number of GDP binding sites per tissue increased by a similar extent in both young and old rats following 48 hr of cold exposure (Table II). In contrast, there was no change in the dissociation constant (341 ± 35 n*M*) of UCP for GDP with either age or cold exposure.

In a separate group of rats, the induction of UCP

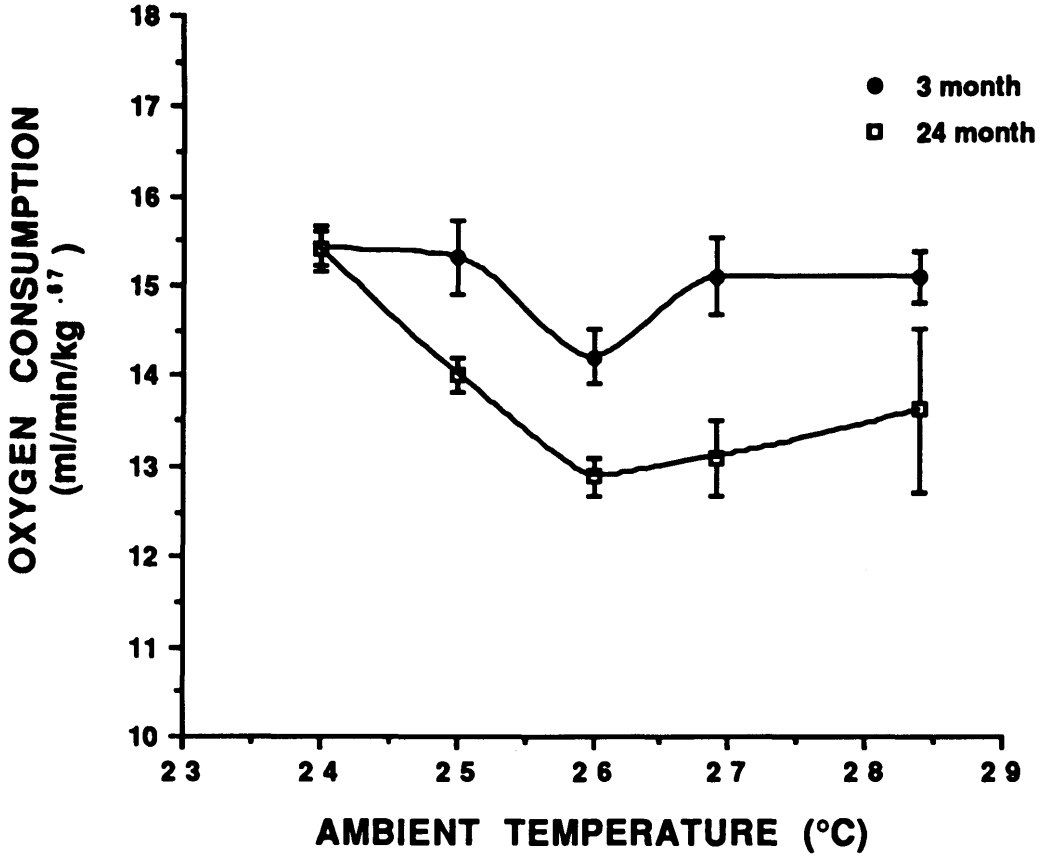

Figure 1. O_2 consumption in (●) 3-month- and (□) 24-month-old rats following acclimation for one week at the specified ambient temperature. O_2 consumption was determined for a 1-hr period. Data represents the mean ± SE of five to eight rats per age.

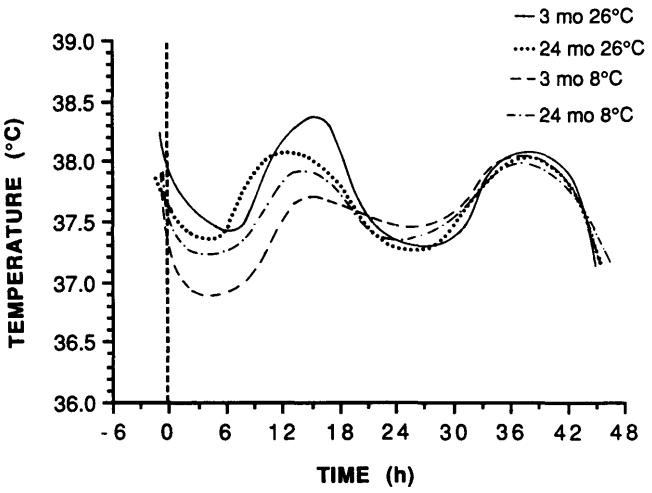

Figure 2. Body temperature measured every 3 min in 3-month- and 24-month-old rats maintained at 26°C or 8°C. The zero time corresponds to 09:00 hours. Curves were hand drawn through data points. After a period of adjustment, the normal circadian rhythm was observed. Data represents the mean of five to six rats per group. The average standard error was 0.13, and the error bars were left off for the sake of clarity.

was assessed by measuring the amount of UCP mRNA following a mild cold exposure at 8°C for 48 hr. There was no significant difference in the amount of UCP mRNA in young control rats compared with old control rats. However, cold exposure increased the expression of UCP transcripts by 5-fold in both the young and old rats (Table II).

One-Hour Severe Cold Exposure. The results of the mild cold exposure indicated that, under conditions when both the young and senescent rats were able to maintain body temperature, the increases in mitochondrial GDP binding after 1 hr and 48 hr were equal in rats of both ages. In the next series of experiments, rats were exposed for 1 hr to a colder temperature, 4°C, at which the senescent rats, but not the young rats, became hypothermic (Fig. 4). In a preliminary experiment, we observed that, if the senescent rats were maintained for a longer period at 4°C, they were never able to thermoregulate and two of the rats died by 24 hr. For these reasons, cold exposure at 4°C was limited to 1 hr, a time length at which hypothermia was observed in the senescent rats, but there was no evidence of other ill effects (Fig. 4). Despite the observed hypothermia in the senescent rats, the amount of O_2 consumption was not different between young (33.2 ± 0.5 ml/min/kg$^{.67}$) and old (34.3 ± 0.4) rats (n = 6/age).

Under these conditions, GDP binding to mitochondrial UCP and the induction of UCP transcripts were examined. Neither IBAT weight nor cytochrome

Table I. Effects of a 48-hr Cold Exposure and Age on Body and IBAT Parameters in F-344 Rats[a]

Parameter	Age			
	3 Months		24 Months	
	26°C	8°C	26°C	8°C
Body wt (g)	301 ± 6	283 ± 6[b]	462 ± 14	427 ± 12[b]
IBAT wt (mg)	300 ± 26	198 ± 22[c]	289 ± 13	205 ± 19[d]
Mitochondrial protein (mg/IBAT)	1.51 ± 0.21	2.34 ± 0.31	1.50 ± 0.20	2.05 ± 0.36
Cytochrome *c* oxidase activity (nmol/IBAT)	351 ± 86	1218 ± 138[c]	398 ± 43	1035 ± 232[c]

[a] Body weight represents the weight prior to (26°C) and after 48-hr cold exposure (8°C). All other data represent separate control and cold-exposed rats. Data represents the mean ± SE of five to seven rats per group. Mitochondrial protein and cytochrome *c* oxidase activity were determined in the isolated mitochondrial fraction.
[b] $P < 0.001$ (3 months) or $P < 0.005$ (24 months) for difference between before and after cold exposure by paired *t*-test.
[c] $P < 0.02$ (BAT wt), $P < 0.001$ (cytochrome *c*, 3 months) or $P < 0.01$ (cytochrome *c*, 24 months) for difference with cold exposure by Student's *t*-test.
[d] $P < 0.005$ for difference from 24-month-old control by Student's *t*-test.

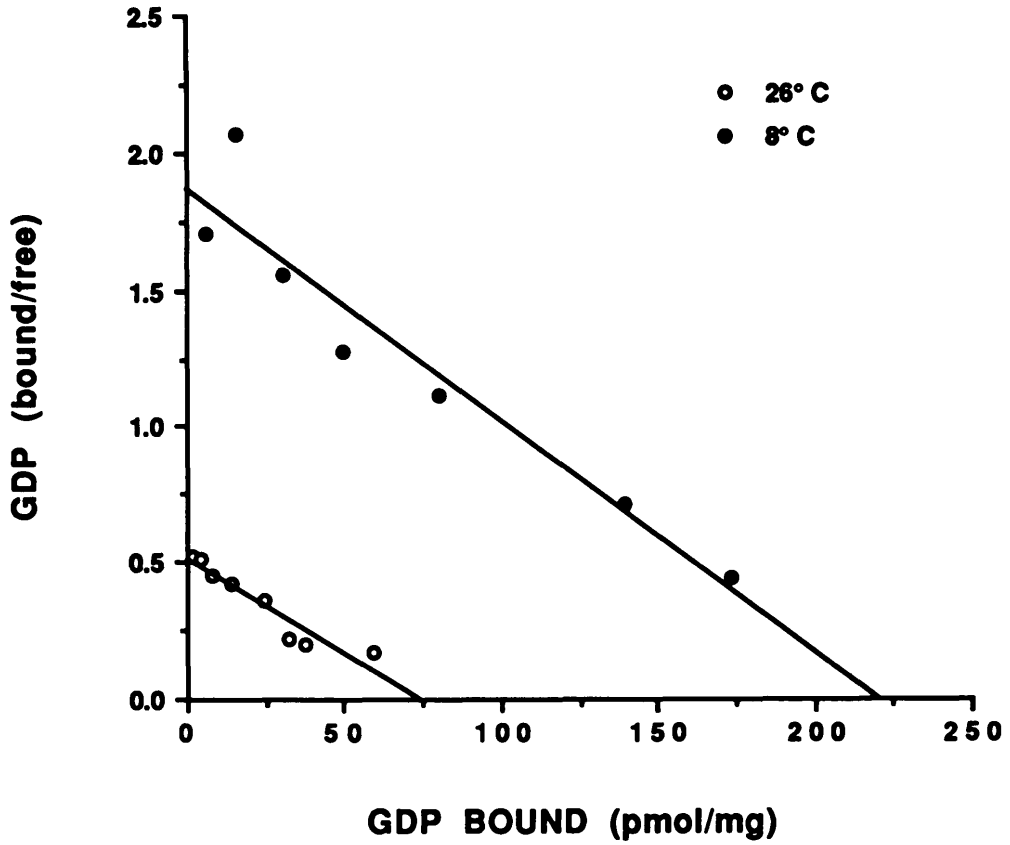

Figure 3. Scatchard analysis of [^{3}H]GDP binding to IBAT mitochondria from 24-month-old rats maintained at 26°C (○) or exposed at 8°C for 48 hr (●). Lines were determined by regression analysis with correlation coefficients of 0.95 and 0.96, binding site densities of 75 and 220 pmol per mg protein (indicated by the abscissa intercepts), and dissociation constants for [^{3}H]GDP of 582 and 471 n*M* (indicated by the negative reciprocal of the slope of the line), respectively, for control and cold-exposed rats.

c oxidase activity was altered by the 1-hr cold exposure (data not shown). As expected, the density of GDP binding sites was increased in both young and senescent rats following a 1-hr cold exposure (Table III). Surprisingly, the extent of the increase (75%) was the same in rats of both ages. Similarly, the total number of GDP binding sites per tissue increased by approximately the same extent in young and old rats (Table III). The magnitude of the increase in GDP binding after the 1-hr severe cold exposure was greater than after the 1-hr mild cold exposure, but less than after the 48-hr mild cold exposure.

The 48-hr cold exposure was associated with an increase in UCP transcripts, and presumably, the greater increase in GDP binding reflects binding to both existing UCP and newly synthesized UCP. To determine if newly induced UCP plays a role in thermogenesis after a 1-hr cold exposure, the induction of

Table II. GDP Binding and Induction of UCP Following a 48-hr Cold Exposure at 8°C in Young and Old Rats[a]

Parameter	Age			
	3 Months		24 Months	
	26°C	8°C	26°C	8°C
GDP binding sites (pmol/mg protein)	67 ± 9	186 ± 33[b]	70 ± 14	229 ± 39[b]
GDP binding sites (pmol/tissue)	107 ± 25	426 ± 81[b]	105 ± 23	508 ± 138[b]
UCP mRNA (units/μgRNA)	1.4 ± 0.4	6.4 ± 0.7[b]	0.9 ± 0.2	4.7 ± 0.8[b]

[a] GDP binding is expressed as binding sites per mitochondria protein or as binding sites per IBAT. UCP mRNA is expressed as arbitrary units per μg RNA. Data represents the mean ± SE of five to seven rats per group.
[b] $P < 0.02$ (line 1), $P < 0.01$ (line 2), or $P < 0.001$ (line 3) for difference with cold exposure from corresponding age-matched control by Student's *t*-test.

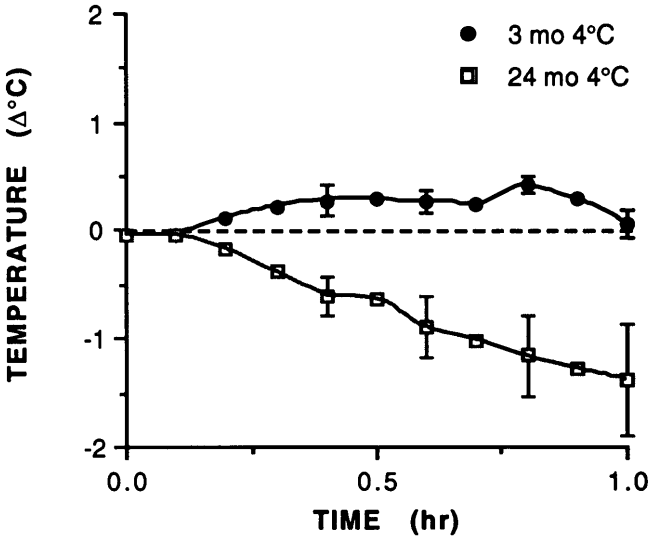

Figure 4. The change in body temperature from baseline during 1 hr of intense cold (4°C) in 3-month- and 24-month-old rats. The senescent rats were significantly more hypothermic compared with the young rats. At 1 hr, the temperature changes were +0.1 ± 0.1°C (3 months) and −1.4 ± 0.5 (24 months) ($P < 0.025$, Student's *t*-test), and the cumulative change in temperature over 1 hr was +11 ± 5°C • hour (3 months) and −45 ± 16 (24 months) ($P < 0.01$, Student's *t*-test). Data represents the mean ± SE of five rats per group. Representative SE bars shown.

UCP mRNA was assessed. In contrast to the 48-hr cold exposure, the 1-hr cold exposure was not associated with an increase in UCP transcripts. UCP mRNA decreased in the young rats and was unchanged in the senescent rats after a 1-hr cold exposure (Table III).

Discussion

The ability to regulate body temperature in response to a variety of environmental challenges diminishes with age (1, 2, 4, 20). There are several factors that may be contributing to this impaired thermoregulation, including a dysfunctional hypothalamic thermoregulatory pathway, altered heat distribution and heat dissipating mechanisms, and a reduced end organ ability to generate heat (13, 21). Previous studies suggest the latter possibility. Senescent Sprague-Dawley and senescent male F-344 rats become hypothermic following acute (less than 6 hr) cold exposure at 4°–6°C (3, 4). This hypothermia was associated with a smaller increase in O_2 consumption in the older compared with the younger rats, suggesting reduced thermogenesis (4). In addition, in IBAT, β-adrenergic signal transduction and β-adrenergic-stimulated mitochondrial GDP binding is diminished with senescence, suggesting reduced end organ thermogenic responsiveness (13, 14).

In the present report, we found that with a short exposure to the cold at 4°C, senescent F-344 rats, but not the young rats, became hypothermic, and that, with a prolonged (24-hr) exposure at this temperature, two of the senescent rats died. In contrast, when exposed to a mild cold of 8°C, rats of both ages were able to maintain homeothermy, and no ill effects were noted for as long as two days. These observations are similar to previous reports comparing the thermoregulatory ability of various strains and sexes of rats. Separate studies indicated that senescent Osborne-Mendel and senescent female F-344 rats were able to maintain their body temperature following a 5°C cold exposure (4, 5). However, another study indicated that when a colder temperature of 0°C was used, both senescent male and female Long-Evans rats became hypothermic (6). Clearly, the age of the animal, the severity of the cold stress, and the thermoregulatory capacity are all important factors in the maintenance of body temperature following cold exposure.

An important site of heat production in rodents in response to cold exposure is BAT thermogenesis (9). An acute cold stimulus increases thermogenesis in BAT, whereas acclimation to the cold increases the capacity for thermogenesis. The latter process involves hypertrophy of the tissue, including an increased number of mitochondria and increased expression of UCP (9). Thus, a cold-acclimated rat has a greater thermogenic potential than a warm (thermoneutral)-acclimated rat. In a warm-acclimated rat, when acutely exposed to cold, BAT thermogenesis contributes from 32%–37% of the increase in metabolic rate (8), whereas in a cold-acclimated rat, the contribution of BAT thermogenesis can be as great as

Table III. GDP Binding and Induction of UCP Following a 1-hr Severe Cold Exposure at 4°C in Young and Old Rats[a]

Parameter	Age			
	3 Months		24 Months	
	26°C	4°C	26°C	4°C
GDP binding sites (pmol/mg protein)	71 ± 8	123 ± 13[b]	92 ± 14	160 ± 17[b]
GDP binding sites (pmol/tissue)	128 ± 25	277 ± 37[b]	182 ± 39	335 ± 43[b]
UCP mRNA (units/μgRNA)	1.4 ± 0.4	0.4 ± 0.1[c]	0.9 ± 0.2	1.0 ± 0.5

[a] Data represents the mean ± SE of five to seven rats per group.
[b] $P < 0.01$ (3 months) or $P < 0.05$ (24 months) for difference with cold exposure from corresponding age-matched control by Student's *t*-test.
[c] $P < 0.05$ for difference with cold exposure from corresponding age-matched control by Student's *t*-test.

70% (8). One possible explanation for the decrease in the thermoregulatory ability of senescent rats could be that thermoneutrality occurs at different ambient temperatures for young and old rats. A previous study indicated the zone of thermoneutrality for young and senescent rats to be 24°–30°C (4). Within this zone the temperature for thermoneutrality may be different for the two ages of rats. If it were higher in the young rats and both ages were housed at the same temperature, then the mild cold-acclimation experienced by the young rats could account for their greater capacity for thermogenesis. In the present study we demonstrated that this is not the case. Thermoneutrality was observed at an ambient temperature of 26°C for rats of both ages.

In the present study, GDP binding in IBAT was assessed under two different cold-exposure conditions, one in which the senescent rats maintained body temperature and another in which they experienced hypothermia. In both cases, the young rats were able to maintain homeothermy. Examination of the GDP binding supported these observations. However, even under conditions where the senescent rats experienced hypothermia (1 hr at 4°), the amount of O_2 consumption was the same in the older (hypothermic) and younger (homeothermic) cold-exposed rats. This suggests that thermogenesis was not deficient in the senescent rats. First, the increase in GDP binding in both young and old rats was greater after a 48-hr cold exposure compared with a 1-hr cold exposure at either 4°C or 8°C. The 48-hr cold exposure was associated with a concomitant increase in the expression of UCP transcripts, whereas the 1-hr cold exposure was not. This suggests the greater increase in GDP binding in the 48-hr cold-exposed rats was a combination of both binding to newly synthesized UCP and unmasking of GDP binding sites on existing UCP. In contrast, in acute cold-exposed rats, the data suggest that only unmasking of GDP binding sites on existing UCP contributed to the increase in binding. Second, the increase in GDP binding was the same in the young and old rats despite the hypothermia in the senescent rats after the 1-hr severe cold exposure. In addition, the induction of UCP mRNA was equal in young and old rats after the 48-hr cold exposure. These data suggest that GDP binding and the gene expression of UCP in IBAT are unchanged with age following cold exposure.

The findings described above are surprising in light of our previous reports. β-adrenergic signal transduction is decreased in BAT with age and β-adrenergic-induced GDP binding in IBAT mitochondria is virtually absent in senescent rats (13, 14). These data, coupled with the present findings, suggest that the cold-induced thermogenic response is not principally mediated by β_3-adrenergic receptors in IBAT, at least not in senescent rats. This contention is based on the contrast between the β_3-agonist-induced GDP binding (decreased with age) and the cold-induced GDP binding (preserved with age). Furthermore, the data suggest that a mechanism other than failure of thermogenesis in *interscapular* BAT is responsible for the hypothermic response observed in the older rats. This latter contention is based on the report that in warm-acclimated rats (as are the rats in this study) IBAT thermogenesis contributes one-third of the total increase in metabolic rate with cold stimulus (8) and on our observation that thermogenesis in IBAT was preserved with age despite hypothermia in the senescent rats. This suggests either that heat dissipation may be altered with age or that the capacity for thermogenesis at other sites, even possibly other BAT sites, is decreased with age.

In summary, the increase in GDP binding in IBAT following mild and severe cold exposure was unchanged with age despite a hypothermia in the senescent rats following severe cold exposure. In addition, the induction of UCP mRNA by cold exposure was unchanged with age. These data, coupled with our previous findings of diminished β_3-agonist-stimulated BAT thermogenesis, suggest (i) that IBAT thermogenesis, at least in the senescent rats, may be mediated by other than β_3-adrenergic receptors, and (ii) that altered heat dissipation or impaired thermogenesis at some site other than interscapular IBAT is responsible for the observed hypothermia.

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