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Brown Adipose Tissue Thermogenesis During Aging and Senescence

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We have found that cold- and norepinephrine-induced brown adipose tissue (BAT) nonshivering thermogenesis (NST) is significantly lower in old male Fischer 344 rats and is associated with the decreased ability of these animals to maintain homeothermy. This decline in BAT thermogenesis is not as great in females. Although the mechanism(s) underlying this gender difference in the age-related decrease in brown fat NST are not completely elucidated, they do not appear to reflect decreased sympathetic neural activity of BAT in the older males vs. females. Rather, our investigations, strongly suggest that the blunted cold-induced heat production of BAT reflects less functional BAT. The fact that the older animals have less functional BAT than do their younger counterparts may predispose them to the accumulation of excess body fat. Our studies have also found that near the end of the natural life of these rats, they enter a state of senescence that can be identified by spontaneous rapid body weight loss, resulting from decreased food intake. In this state, the rats are considerably more susceptible to cold than are comparably aged presenescent (body weight stable) rats of the same chronological age. The greater hypothermia exhibited by the senescent vs. presenescent rats during cold exposure is associated with a significant reduction in the amount of functional brown fat and in the amount of heat each brown fat cell can generate. It is the intent of this review to discuss the findings of these investigations.

KEY WORDS: Brown adipocyte proliferation, cold exposure, nonshivering thermogenesis, norepinephrine, sympathetic nervous system, uncoupling protein one (UCP1)

INTRODUCTION

Age-related declines in thermogenesis/regulatory energy expenditure in humans and in rodents have been well documented (Falk *et al.*, 1994; Lee and Wang, 1985; McDonald *et al.*, 1987; Scarpace *et al.*, 1992, 1994, 1996; Schaefer *et al.*, 1996; Tatelman and Talan, 1990). These alterations often result in an inability to maintain thermal homeostasis during cold exposure (Collins, 1987; Horvath *et al.*, 1955; Inoue *et al.*, 1992; Talan and Ingram, 1986; Wagner and Horvath, 1985) and may also contribute to the age-related increase in adiposity. Several investigations have shown that in humans oxygen consumption, a measure of energy expenditure, declines at a rate of 1 to 2% per decade after the age of 30 (McCarter, 1995). Since the amount of food intake remains relatively constant during the same time interval, this decline in energy expenditure results in increased body weight. While the mechanisms associated with the changes in energy expenditure remain to be elucidated, most investigations in humans have focused on the concomitant loss in lean body mass as a primary cause.

Another potential contributor to this attenuated energy expenditure is blunted sympathetic-activated thermogenenesis [i.e., nonshivering thermogenesis

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(NST)]. This possibility is consistent with data showing that norepinephrine clearance, expressed as a function of fat-free weight or body surface area, was significantly greater in lean older physically fit men as compared to overweight sedentary individuals of similar age (Poehlman *et al.*, 1990). Fat mass did not predict sympathetic neural (SNS) activity. These findings suggest that the well-known increase in physical activity-induced energy expenditure in older individuals may help to offset depressed thermogenesis in the aged individual. Moreover, some have proposed that blunted SNS activity following meal ingestion contributes significantly to a diminished thermic effect of feeding in older vs. younger subjects (Poehlman and Horton, 1990). While much research is still needed to determine more clearly the role of the SNS in blunted thermogenesis of the elderly, these studies provide evidence of a link between SNS activity and body weight changes that may occur during aging.

Although investigations in humans have provided valuable evidence that thermogenesis, particularly SNS-activated thermogenesis, may be attenuated in old age, these investigations, for the most part, have been limited to correlational and descriptive studies. Mechanistic studies have been carried out using laboratory animals as models for human aging. Our laboratory has used primarily the male and female Fischer 344 (F344) rat as a model to study mechanisms that may underlie the age-related attenuation of cold-induced thermogenesis. We have focused largely on the effects of chronological aging on SNS-activated nonshivering thermogenesis, most of which occurs in brown adipose tissue (BAT) and, more recently, on the changes in thermogenesis and in the regulation of energy balance that occur near the end of life. It is the intent of this review to discuss the findings of these investigations.

COLD- AND NOREPINEPHRINE-INDUCED THERMOGENESIS IN OLD MALE RATS

Exposing rats and mice to cold elicits a significant SNS response that is associated with increased BAT nonshivering thermogenesis (Himms-Hagen, 1985; Himms-Hagen *et al.*, 1990). This thermogenesis involves interaction of norepinephrine released from sympathetic nerves with plasma membrane adrenergic receptors. The sequence of events that is initiated by this interaction culminate not only in increased fatty acid oxidation and thus increased heat production [by activating uncoupling protein 1 (UCP1)], but also in induction of the synthesis of proteins that increase the thermogenic capacity of BAT. Similar effects can be elicited by administration of norepinephrine. Foster and his colleagues have shown that in rats raised at room temperature, BAT can account for a significant portion of the increased heat production (oxygen consumption) induced by several hours of cold exposure (25%) or by norepinephrine administration (42%) (Foster and Frydman, 1978, 1979). Thus, if SNS-mediated thermogenesis is blunted in older animals, their metabolic response to exogenous norepinephrine and/or cold exposure should be attenuated. To test this hypotheses, we measured oxygen consumption in older (24-month) and younger (4-month) male F344 rats at rest and during exposure to 2 h of 6°C or infused (3.5 mg/min/kg) with norepinephrine for 60 min (McDonald *et al.*, 1988). Resting oxygen consumption did not differ significantly between the two age groups (Fig. 1). This finding, one that would be confirmed in subsequent investigations, was surprising given the general consensus that resting oxygen consumption in humans decreases with advanced age (McCarter, 1995; NIH, 1989). It most likely reflects the fact that our older rats had not lost significant amounts of lean body mass. Although both young and old rats increased body mass-independent (ml/min/kg body mass$^{0.67}$) oxygen consumption in response to cold and to norepinephrine, this increase was significantly less in the old than in the young rats. This finding suggested strongly that NST in BAT was attenuated in the older rats. Support-

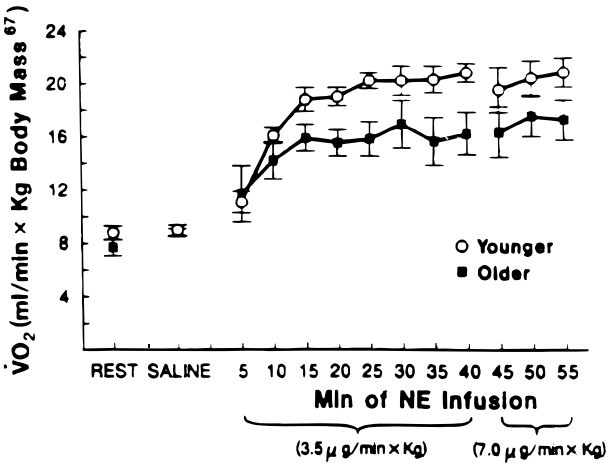

Fig. 1. Mass-independent oxygen consumption of younger (4 months) and older (24 months) rats at rest, during saline infusion, and during jugular infusion of norepinephrine (from McDonald *et al.*, 1988, with permission).

ing this interpretation were our measurements of purine-nucleotide (GDP) binding to BAT mitochondria isolated from these rats. This binding, which is an *in vitro* index of the thermogenic state of brown adipocytes (Himms-Hagen, 1985), was 50–60% lower in mitochondria from the old vs. young animals. This was true whether the data were expressed per total amount of brown fat (this amount being significantly lower in the old vs. young rats), per brown fat mitochondrial protein (the levels of which were also significantly lower in the old rats), or per kilogram body mass$^{0.67}$. Moreover, we found a significant correlation ($r = .66$) between maximal GDP binding and norepinephrine-induced oxygen consumption.

To further evaluate the contribution of brown fat thermogenesis to the response of the older rats and to determine if skeletal muscle thermogenesis (either shivering or nonshivering) was also blunted with age, we measured blood flow via radioactively labeled microspheres in brown fat and skeletal muscle of conscious young and old rats at rest and during cold exposure (McDonald *et al.*, 1989). Foster and colleagues have demonstrated that blood flow to BAT, and presumably other tissues as well, is directly related to its oxygen consumption (Foster and Frydman, 1978). We found that during 4 h of cold exposure, blood flow to BAT was significantly increased (5.5- to 11-fold) while that to muscles (gastocnemius, soleus, and psoas) was not. Thus, in these cold-exposed rats, both young and old, BAT, rather than skeletal muscle, was the major contributor to the animal's cold-induced thermogenesis.

This conclusion was confirmed in a subsequent study in which we measured the cellular incorporation of [^{14}C]-2-deoxyglucose into brown fat and skeletal muscle of conscious 6-, 12-, and 26-month-old rats to assess glucose utilization and thus, *in vivo* oxidative metabolism. (McDonald *et al.*, 1994). Cold-induced BAT thermogenesis involves the oxidation of fatty acids, accompanied by increased glucose uptake into the adipocytes. Cold exposure did not significantly increase glucose utilization by skeletal muscle although it dramatically stimulated glucose utilization in BAT, again indicating that the primary thermogenic tissue in both the young and old cold-exposed rats was BAT, rather than skeletal muscle. Notably, in both the blood flow and glucose utilization studies, we saw no decrement in BAT from the old rats when the values were expressed per unit of BAT mass or BAT protein. However, when the total amount of BAT was taken into consideration, BAT glucose utilization was blunted in the older male rats. Thus, the blunted BAT thermogenic response of the older males appears to reflect the presence of less functional BAT.

GENDER DIFFERENCES IN THE EFFECTS OF AGE ON COLD-INDUCED THERMOREGULATORY RESPONSES

Gender differences in thermoregulatory responses have been reported in humans. For example, Wagner and Horvath (1985) found that aged human females maintained higher rectal temperatures during cold exposure than did similarly aged males. To evaluate such differences in our model system, we measured oxygen consumption, colonic temperature, body composition, and the binding of GDP to isolated BAT mitochondria of male and female F344 rats, aged 5, 23, and 27 months of age (McDonald *et al.*, 1989). After 6 h of cold exposure (6°C), the colonic temperatures of the 23- and 27-month-old males were significantly lower than those prior to cold exposure (averaging 1.8 and 2.9°C, respectively). In contrast, only the 27-month-old females exhibited significant hypothermia (an average decrease of 0.7°C). Moreover, the degree of hypothermia was considerably less in the females than in the males. Heat production (as measured by oxygen consumption), expressed in terms of lean body mass, was also significantly lower in the 27-month-old males than in the females.

That these gender differences reflected differences in BAT, rather than skeletal muscle, thermogenesis was supported by several observations. Among these was the finding that the old females did not have more lean body mass (grams or percent) than did the older males. In fact, the absolute amount of lean body mass (g) was significantly greater in the males (the percentage lean body mass also tended to be higher in the males, although this difference did not reach significance). In contrast, the magnitude of BAT mass, protein content, and mitochondrial GDP binding were all significantly greater in the 27-month-old female rats than in the similarly aged males (McDonald *et al.*, 1989). Moreover, cellular incorporation of [^{14}C]-2-deoxyglucose indicated that total glucose utilization by BAT was significantly higher (~70%) in cold-exposed 26-month-old female vs. male rats (McDonald *et al.*, 1994).

Because the thermogenic capacity of brown fat as well as its thermogenic response to cold are heavily dependent on sympathetic activation of the tissue, our

results suggested the possibility that the effects of age and gender on BAT mass, protein content, mitochondrial GDP binding, and glucose utilization thermogenesis might reflect diminished sympathetic signaling in the old vs. young males and in the old males vs. females. In fact, age-related alterations in the sympathetic activity to a variety of tissues have been observed in previous investigations of rodents and humans. However, age has generally been associated with increased, rather than decreased, activity of the SNS (Mazzeo and Grantham, 1989; Morrow *et al.*, 1987; Pfeifer *et al.*, 1983; Rowe and Troen, 1980; Supiano *et al.*, 1990; Ziegler *et al.*, 1976). Kawate *et al.* (1993) have reported that neural activity to interscapular BAT of aged vs. younger C57BL/6J mice was significantly higher at room temperature and during cold exposure. Nonetheless, we considered it important to evaluate sympathetic signaling to brown fat in the F344 rats. For this, we used the method of competitive inhibition of tyrosine hydroxylase by α-methyl-*p*-tyrosine (Brodie *et al.*, 1966) to estimate norepinephrine turnover in 6-, 12-, and 26-month-old male and female rats (McDonald *et al.*, 1993). As in previous studies, BAT mass (relative to body mass) and BAT protein concentration were significantly higher in the old females than in the old males, consistent with greater BAT thermogenic potential in the former. There were no significant differences in norepinephrine turnover in any of the rat groups maintained at room temperature (22–24°C). However, this changed during cold exposure (6°C). That is, although BAT norepinephrine turnover increased in all rats, there were both age and gender differences. Specifically, turnover (nmol norepinephrine/hr) was significantly lower in the 26-month-old females (0.86 ± 0.47) than in the 6- (1.73 ± 0.64) or 12- (1.58 ± 0.64) month-old rats; while in the males, norepinephrine turnover was highest in the 26-month-old rats [1.89 ± 0.47 vs. 0.98 ± 0.31 (6 month) and 0.69 ± 0.27 (12 month)]. Thus, these data negated the hypothesis that the lower thermogenic capacity/responses of the old males vs. old females reflects blunted sympathetic stimulation to BAT. Alternatively, they suggested that the gender differences in BAT thermogenic responses reflected the presence of less functional BAT (and thus lower BAT thermogenic capacity) in the old males vs. old females and/or altered receptor/signal transduction of the sympathetic signals at the brown adipocyte. Both of these possibilities are considered below.

THERMOGENIC CAPACITY OF BROWN ADIPOSE TISSUE: AGE AND GENDER EFFECTS

As previously discussed, our initial studies of gender differences provided evidence consistent with less functional BAT in the old males vs. females. This evidence included less BAT mass, BAT protein, BAT mitochondrial protein, total GDP binding to BAT mitochondria, and cold-induced glucose utilization by BAT. Further support for age and gender differences in the amount of functional BAT in the older rats derived from our measurements of UCP1 in BAT, generally considered to be an index of thermogenic capacity. After 4 h of cold exposure, 26-month-old females had 65.5% more UCP1 than did their male counterparts (250.7 ± 44.1 vs. 151.5 ± 48.4 μg per IBAT depot). This difference increased to 111% when the values of UCP1 were expressed independent of body mass (Gabaldon *et al.*, 1995; Fig. 2). Notably, the age and gender differences in GDP binding and UCP1 were abolished when these variables were expressed in terms of milligram mitochondrial or BAT protein rather than in terms of the total amount in the tissue. This supports the hypothesis that it is the amount of functional BAT that is the primary contributor to the age-related reduction in BAT thermogenic capacity.

Although norepinephrine is a major regulator of brown fat growth and UCP1 transcription, our measurements of norepinephrine turnover in BAT (as discussed above) indicated that the lower UCP1 values in older males could not be explained by blunted sympathetic activity. This led us to consider thyroid hormone as a potential mediator of the gender differences because the active form of this hormone [3,5,3′-triiodothyronine (T_3)] can modulate the sympathetic (β-adrenergic) stimulation of UCP1 transcription (Rehnmark *et al.*, 1990; Silva and Larsen, 1986). While we found no significant age or gender differences in total serum thyroxine in rats that had been exposed to cold (6°C) for 4 h, we did observe an effect of age (but not gender) on free serum thyroxine (Gabaldon *et al.*, 1995). Concentrations of the latter averaged 37.5–56.5% lower in the 26- vs. the 6- and 12-month-old rats. Moreover, the activity of type II thyroxine 5′-deiodinase in interscapular BAT was approximately threefold higher in the old females than in the old males (Gabaldon *et al.*, 1995). Since this enzyme converts thyroxine to T_3 BAT in the older males would presumably have less intracellular T_3 than would BAT in the

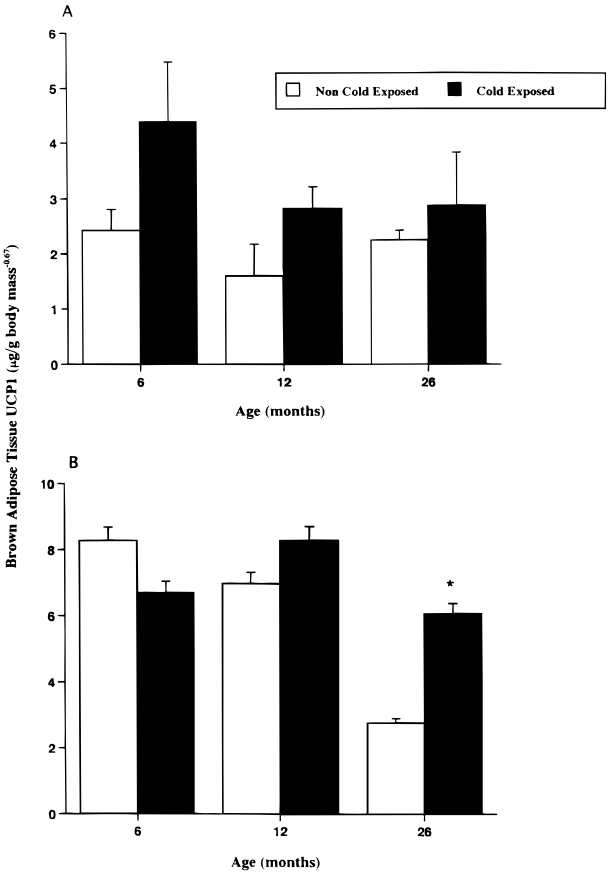

Fig. 2. Mass-independent BAT UCP1 concentration of male (A) and female (B) F344 rats. *, Significantly different from non-cold-exposed group of same age (adapted from Gabaldon *et al.*, 1995, with permission).

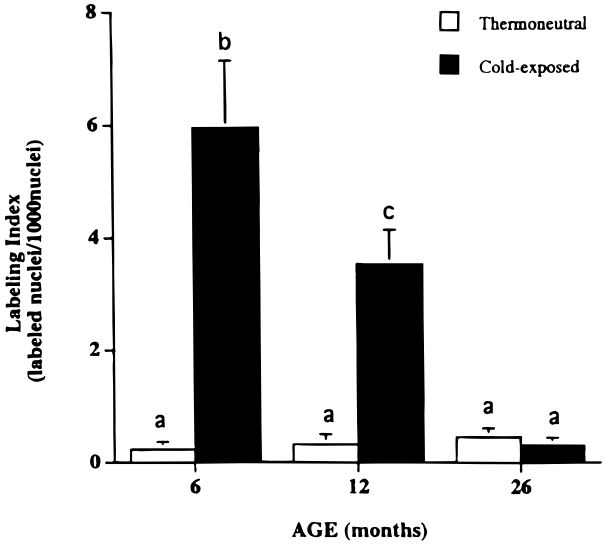

Fig. 3. Cell proliferation in interscapular brown adipose tissue of male Fischer 344 rats after 5 days at either thermoneutrality (25°C) or cold (6°C). Values are means ± SE. Values sharing a common superscript letter are not significantly different (from Florez-Duquet *et al.*, 1998, with permission)

older females and this difference could contribute to the lower levels of UCP1 in the males.

Of considerable interest was our finding that the 26-month-old rats that were maintained at 26°C (thermoneutrality) rather than being cold exposed did not exhibit gender differences with respect to UCP1 or BAT thyroxine 5′-deiodinase activity. This suggested that the pathways that determine steady-state UCP1 levels in "unstimulated" BAT were intact but that during cold exposure, when stimulation of UCP1 would be expected to occur in the brown adipocytes, the older males were less able to respond than were the females.

While we have not directly tested this hypothesis, we have found that old males exposed to 5 days of cold (10°C) do not exhibit significant brown preadipocyte proliferation/maturation whereas younger rats (6- and 12-month-old) do (Fig. 3). This study involved measurement of the *in vivo* incorporation of 5-bromo-2′-deoxyurindine (BrdU) into brown preadipocytes and mature adipocytes and thus was a measure of the degree of brown preadipocyte proliferation and maturation (Florez-Duquet *et al.*, 1998). In contrast to the unresponsiveness of the old males, cold exposure stimulated brown preadipocyte proliferation (and maturation into mature brown adipocytes) in both 6- and 26-month-old females (Wagner *et al*, in preparation).

This gender difference in the effects of age on brown fat growth in response to cold exposure could involve several mechanisms. Among these is the possibility that in contrast to the females and younger males, the older males: (a) do not have sufficient preadipocytes to proliferate; (b) the number of preadipocytes in older males is sufficient, but the cells have lost their ability to respond to the cold-induced "growth" signals; and (c) the signals for growth are absent in the older males. We are currently evaluating all of these possibilities.

SIGNAL TRANSDUCTION OF SYMPATHETIC STIMULATION: AGE AND GENDER EFFECTS

In addition to having less thermogenically active BAT, older males may also have brown adipocytes

that are less responsive to sympathetic signaling than comparably aged females or younger males. This could be due to changes in receptor number, receptor characteristics, and/or signal transduction. Scarpace and his colleagues have, in fact, suggested that the density of brown fat β-adrenergic receptors declines with age (Scarpace *et al.*, 1988), with β_1-adrenergic receptor density decreasing to a greater extent than β_2-adrenergic receptors. This age-related decrease in receptor density was concomitant with a reduction in adenyly cyclase activity, further suggesting that there is a blunting of SNS-mediated signal transduction with age. These findings in brown fat (Scarpace *et al.*, 1988) were consistent with several investigations reporting age-related declines in β-adrenergic responsiveness in other tissues, such as myocardium, cerebral microvessels, brain, and kidney (Mooradian and Scarpace, 1991; Scarpace *et al.*, 1992; Shu and Scarpace, 1994; Sugawa and May, 1993; Wilson and Dillingham, 1992).

In addition to lower β_1 and β_2 receptor densities in BAT from older rats, Scarpace and his colleagues found evidence of an age-related attenuation of β_3-adrenergic-mediated thermogenesis in BAT (Scarpace and Matheny, 1991). That is, in response to administration of CGP-12177, a compound that is both a β_3 agonist and a β_1 antagonist, increases in oxygen consumption and body temperature were 60% less in old vs. young F344 rats maintained at thermoneutrality. In addition, binding of GDP to BAT mitochondria and the levels of UCP1 mRNA in BAT from old rats were significantly lower in the old vs. young rats.

While these data supported the view that BAT β-receptor density and adenyl cyclase activity in membrane fractions from BAT were diminished with age, we were interested in determining if there were thermogenic consequences of such decreases. Thus, we measured the oxygen consumption of isolated brown adipocytes from older and younger, male and female, F344 rats in response to norepinephrine and to CL-316,243, a highly selective β_3-adrenergic agonist (Gabaldon *et al.*, 1998). Control (i.e., unstimulated) oxygen consumption of the brown adipocytes did not differ significantly among the age and gender groups, supporting our previous *in vivo* finding that measures of brown fat thermogenesis in rats at thermoneutrality do not show age differences. Norepinephrine elicited increased respiration in a dose-dependent manner in all age and gender groups. Notably, there were no age or gender differences in the maximum (V_{max}) thermogenic response of the cells to norepinephrine. The EC_{50} values (i.e., the concentration of norepinephrine eliciting a value of oxygen consumption that is 50% of V_{max} and an index of the sensitivity of the cells to norepinephrine) were also unaffected by age. Moreover, although the values for the cells from the 6- and 12-month-old males were significantly less than those of the comparably aged females (indicating greater sensitivity to norepinephrine), there were no significant differences in EC_{50} between the brown adipocytes from the 26-month-old rats. When the isolated adipocytes were exposed to a single maximal concentration of the β_3-adrenergic agonist CL-316,243, oxygen consumption increased significantly in all groups to a level comparable to that induced by maximal concentrations of norepinephrine. There were no significant differences due to age, although the responses of the males tended to be moderately higher than those of the females. These findings demonstrate that even if there are age-related decreases in the β-adrenergic receptor densities of the brown adipocytes, they do not "translate" into functional thermogenic changes. This may be due to the fact that even with age-related decreases, the number of β-adrenergic receptors is in excess. Whatever the reason, our experiments indicate that there is no age-related deficit in the norepinephrine responsiveness of the isolated brown adipocytes that can explain the age-related decrease in BAT thermogenesis *in vivo*. Rather, our data provide further support for the hypothesis that the blunted *in vivo* thermogenic response of BAT in old males primarily reflects altered amounts of functional BAT.

BROWN PREADIPOCYTE PROLIFERATION: AGE AND GENDER EFFECTS

The failure of the old F344 males to retain sufficient amounts of functional BAT to maintain homeothermy suggests that brown preadipocyte proliferation does not keep pace with the turnover of mature brown adipocytes. In fact, declining tissue mass and altered cell proliferation appear to be hallmarks of aging in a variety of systems (Cristofalo and Pignolo, 1995; Martin *et al.*, 1993). This may reflect diminished preadipocyte proliferation due to age- and gender-related alterations in extracellular signaling and/or changes in the preadipocytes themselves. We have begun to explore the first of these possibilities by measuring factors known to stimulate brown preadipocyte proliferation/maturation. Our finding of no age-related decrements in circulating catecholamines (Gabaldon *et al.*,

1995) or in BAT norepinephrine turnover (McDonald, 1993 #16; see above) argues against attenuated sympathetic signaling being the explanation for the lack of adequate preadipocyte proliferation/maturation. Similarly, we found no differences in serum IGF-1 in the old vs. young males maintained at thermoneutrality (unpublished results). In contrast, there were decreases in both serum insulin and free thyroxine in the old vs. young males (but not in the females). Currently, we are examining the effects of these and of autocrine growth factors on brown preadipocyte proliferation.

CHRONOLOGICAL AGING, BIOLOGICAL SENESCENCE, AND SNS-MEDIATED THERMOGENESIS

In the course of testing our hypotheses concerning altered SNS-mediated thermogenesis of brown fat, we noticed significant heterogeneity in the ability of the old rats to maintain homeothermy during cold exposure, with the suggestion of a correlation between body weight loss and core temperature. That is, it appeared that old rats undergoing spontaneous rapid weight loss (rats that we now refer to as *senescent*) were considerably more susceptible to cold exposure than were body weight-stable rats of the same chronological age. To examine this relationship more closely, we exposed presenescent (i.e., body weight stable) rats to 6°C for 4 h every 14 days, beginning at 24 months of age and until the onset of rapid spontaneous weight loss (McDonald *et al.*, 1996). The age of onset of the spontaneous rapid weight loss ranged from 24.5 to 29 months of age, clearly indicating that chronological age is a poor predictor of senescence. All rats displaying spontaneous rapid weight loss developed severe hypothermia during the acute cold exposure, a hypothermia that generally was not present prior to the weight loss. Moreover, the interscapular BAT of rats that exhibited this weight loss (which averaged about 10% at the time of cold exposure) had 22% less mass, 36% less protein, and 48.5% less UCP1 than did the presenescent rats of the same chronological age. These differences in BAT and in the rats' thermoregulatory responses were not due to the weight loss of the senescent rats. This was clear from data obtained from 26-month-old presenescent rats (weight stable) that were food restricted such that they lost, on average, 10% of their body weight. When these food-restricted rats were cold exposed under the same conditions as were the senescent rats, they did not develop hypothermia. Thus, the loss of body weight of the senescent rats is a marker indicating that the rats have entered a different physiological state; it is not the cause of this state.

The results from the above study indicated that the senescence-related reduction in BAT thermogenic response and thermogenic capacity can occur rapidly and is not a simple function of chronological age or the median life-span of the animals. While these reductions clearly involved less UCP1, it was also possible that other characteristics of brown adipocytes were altered after the entry into senescence. For this reason, we isolated brown adipocytes and evaluated the functional integrity of the β-adrenergic receptor/pathway, the availability of adequate substrate for mitochondrial oxidation, and, thus, thermogenesis, and the amount of UCP1 per cell (Gabaldon *et al.*, 1998). For assessment of the functional integrity of the thermogenic pathway, we measured the norepinephrine- and CL-316,243-stimulated oxygen consumption of brown adipocytes isolated from male and female, older presenescent and senescent F344 rats. Norepinephrine (NE) stimulated oxygen consumption in a dose-dependent manner in all groups (Fig. 4). There was no significant difference in the EC_{50} of the cells from presenescent vs. senescent rats (male or female). However, V_{max} was significantly lower in the senescent cells (48% less in the males, 49% in the females). Similarly, oxygen consumption in response to a maximal concentration of the β_3-adrenergic receptor agonist CL-316,243 was significantly lower in cells isolated from senescent vs. presenescent rats (56% lower in the males, 60% lower in the females). No such decrease in response to adrenergic stimulation was seen in cells isolated from food-restricted, 26-month-old presenescent rats undergoing comparable weight loss as the senescent rats. Thus the brown adipocytes from the senescent rats had a markedly reduced thermogenic potential that was not associated with the loss of body weight per se.

That the attenuated thermogenic response of the senescent cells did not result from deficits in the β-adrenergic pathway or the provision of substrate was shown by the fact that there were no significant differences in the cAMP generation stimulated by a range of norepinephrine concentrations. The values of V_{max} and EC_{50} were comparable in cells from presenescent and senescent rats. A maximal concentration of forskolin, which stimulates adenylyl cyclase independent of the β receptor, also elicited similar responses from both groups of cells. Similar results were obtained for lipolysis. That is, the responses of the cells from the

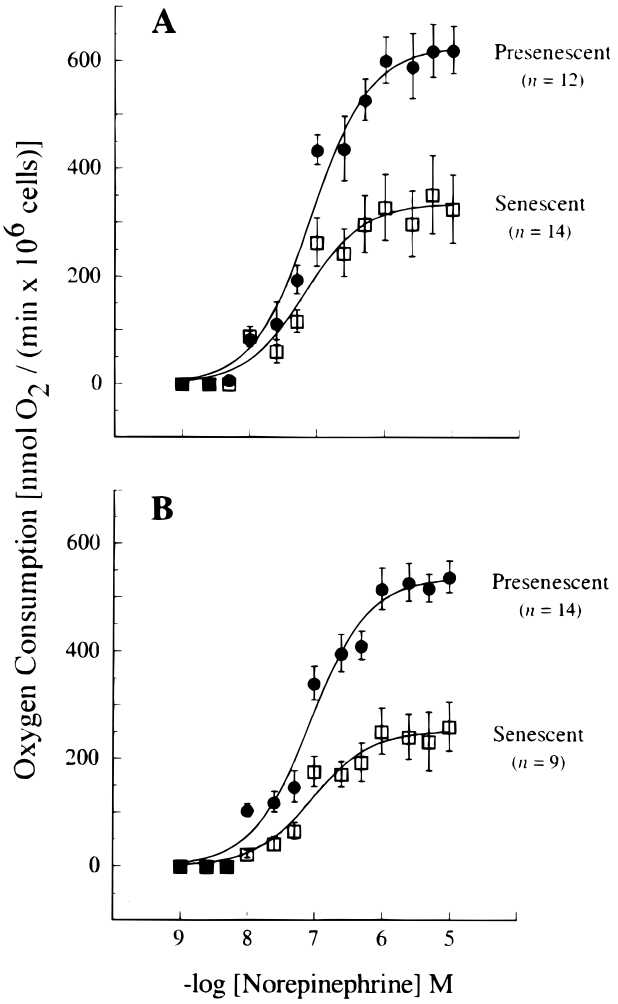

Fig. 4. NE-induced oxygen consumption of isolated brown adipocytes form male(A) and female (B) F344 rats incubated in Krebs–Ringer bicarbonated buffer at 37°C (from Gabaldon *et al.*, 1998, with permission).

senescent rats did not differ significantly from those of the presenescent animals. Thus, the attenuated thermogenic response of brown adipocytes isolated from senescent rats cannot be ascribed to altered adrenegic receptor/pathway characteristics or insufficient substrate (fatty acids) for oxidation and for activation of UCP1.

In contrast, UCP1 levels per cell were significantly lower (65–72%) in senescent vs. presenescent rats. Although this reduction could account for the attenuated maximal norepinephrine- and CL-316,234-induced oxygen consumption, it is premature to exclude other potential alterations, such as reduced mitochondria per cell and/or altered concentrations/activities of oxidative enzymes. Nonetheless, decreased concentrations of UCP1 appear to be a major cause of the blunted thermogenic response of cells from the senescent rats; we are currently evaluating factors that regulate expression of UCP1.

SENESCENCE IN THE F344 RAT AS A MODEL FOR GERIATRIC FAILURE TO THRIVE

Geriatric failure to thrive is characterized by undernutrition and weight loss, loss of physical and cognitive function, and depression (Palmer, 1990). Although the etiology of this syndrome is unknown, it has been proposed that its symptoms are simply manifestations of a prelude to "natural death." That is, elderly persons, in the absence of disease, will eventually undergo a process of progressive body weight loss resulting from a reduction in the desire to eat that culminates in death (McCue, 1996; Sarkisian and Lechs 1996). Indeed, the primary measurement defining failure to thrive is weight loss resulting from anorexia (Lonergan, 1991). Our initial data evaluating the mechanism of this syndrome in the F344 rat suggest a possible contribution by the central nervous system, including the SNS.

The weight loss of senescent rats results from decreased food intake rather than increased energy expenditure (Blanton *et al.*, 1998; McDonald *et al.*, 1996). Moreover, when we compared feeding patterns of rats during their presenescent and early senescent periods (average body weight loss of 5%), we found that the lower daily food intake of the senescent rats (which averaged ~83% of that when they were presenescent) resulted from a reduction in the amount of food consumed at each meal. The observation that the amount of time spent at the food cup and the amount of food eaten per meal were significantly less after the rats had entered senescence suggests that the senescent rats became satiated sooner than did the presenescent rats. This finding is consistent with human investigations (Roberts *et al.*, 1994; Rolls *et al.*, 1995). We also noted that providing the rats with a palatable diet containing high-fat and high-sucrose or a flavored diet preferred by the rats did not significantly alter feeding behavior after entry into senescence. The fact that changes in the nutrient composition of the diet did not halt the progression to anorexia in these rats implies that the effect of nutrients on the control of food intake may be minimal in this particular model.

Among the mechanisms that could underlie the disrupted control of energy balance in the senescent animals, altered hypothalamic regulatory pathways are of major interest. Our finding that the senescent-related changes in food intake and body weight coincided with a disrupted circadian rhythm of body temperature, a variable whose regulation is centered in the suprachiasmatic nucleus of the hypothalamus, supports the view that hypothalamic function is altered in senescence (McDonald *et al.*, 1999). Our most recent studies indicate senescent-related changes in the leptin-neuropeptide Y (NPY) pathway, a major pathway regulating both food intake and SNS stimulation of energy expenditure (unpublished results). This is consistent with previous investigations describing age-related changes in hypothalamic NPY levels (Gruenewald *et al.*, 1996) and food intake regulation (Huguet *et al.*, 1993). Thus, data from our laboratory, as well as that from other investigators, provide strong evidence that the state referred to as failure to thrive and/or anorexia of aging involves alterations in central mechanisms that regulate food intake.

SUMMARY

Old presenescent (body weight stable) F344 rats exhibit increased susceptibility to cold exposure which involves attenuated heat production (as well as increased heat loss), resulting in decreased ability to maintain homeothermy. Their blunted cold-induced heat production, which occurs primarily in BAT, reflects the presence of less functional BAT rather than an altered thermogenic pathway in the brown adipocytes themselves. Males are more affected than are females, a gender difference that appears to involve a differential effect of age on the proliferation of brown preadipocytes. The fact that the older animals have less functional BAT than do their younger counterparts may predispose them to obesity. Near the end of the natural life of the rats, they enter a state of senescence that can be identified by spontaneous body weight loss, resulting from decreased food intake. These changes in energy balance are indexes of an altered physiological state, rather than its cause. In this state, the rats are considerably more susceptible to cold than are comparably aged presenescent (body weight stable) rats. The greater hypothermia exhibited by the senescent vs. presenescent rats during cold exposure is associated with a significant reduction in the amount of functional brown fat and in the amount of heat each brown fat cell can generate. These changes, as well as the altered feeding behavior, suggest hypothalamic dysfunction that may involve alterations in the NPY regulatory pathway.

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