Impaired Febrile Response with Age: Role of Thermogenesis in Brown Adipose Tissue (43442)

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Abstract. **We demonstrated previously that in *Escherichia coli*-infected rats, the heat necessary for the febrile response is a result of thermogenesis in brown adipose tissue (BAT). To investigate whether senescent rats have an impaired febrile response to infection and whether such an impairment is a result of attenuated sympathetically activated thermogenesis in BAT, we assessed body temperature and the increase in mitochondrial guanosine 5′-diphosphate (GDP) binding sites in interscapular BAT in response to *E. coli* administration in young and senescent male F-344 rats. There was a significant delay of 2 hr in the onset of fever in the older animals. In addition, in senescent rats, the peak fever (1.0 ± 0.1 Δ°C vs 2.2 ± 0.1) and the cumulative fever (383 ± 43 Δ°C·min vs 775 ± 69) were significantly less than in the young rats ($P < 0.005$). Baseline levels of GDP binding were the same in young and old rats. In young rats, during the rising phase of the fever, *E. coli* infection resulted in a 50% increase in the density of GDP binding sites in BAT mitochondria. In contrast, there was no increase in GDP binding in the older rats following infection. The failure to increase GDP binding may be a result of a reduced ability to unmask reserve GDP binding sites. Alternatively, there may be fewer total GDP binding sites (masked and unmasked) in senescent rats and these sites may already be unmasked. Collectively, these data suggest that the impaired febrile response with age is due to reduced thermogenesis in BAT.**

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Fever is a highly complex physiological response to infection that appears to serve as an important, nonspecific, host defense mechanism (1) as well as an important indicator in the early diagnosis of severe infection (2). Elevated body temperature during bacteremia is directly correlated with survival, since mortality rates are lower in patients who develop a high fever during bacteremia compared with those who develop a low grade fever or are afebrile (3).

Elderly people, in particular, have an impaired febrile response to infections, but the mechanism underlying this clinical observation remains obscure (2). The febrile response to acute bacteremia involves interaction between bacterial products and macrophages and is mediated by endogenously released cytokines (4). Perhaps the best-studied endogenous pyrogen is interleukin 1 (4). Its pyrogenic effects are mediated centrally and involve activation of the sympathetic nervous system and, ultimately, stimulation of thermogenesis in the periphery (5, 6). The ability to produce interleukin 1, however, appears to be unchanged with age in both humans and rats (7, 8), which suggests that the reduced febrile response with age may be a consequence of impaired peripheral thermogenic mechanisms.

Evidence is mounting that in rats the major peripheral mechanism responsible for the increase in heat production following infection is thermogenesis in brown adipose tissue (BAT). Muscle has often been considered the likely source of heat production; however, recent studies indicated that blood flow and oxygen consumption were reduced in the hindlimb of interleukin 1β-treated rats (5). In contrast, blood flow and oxygen use were increased in BAT, which suggests that thermogenesis in BAT is an important source of

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heat production for the febrile response to interleukins in rats (5). It is not clear whether thermogenesis in BAT is important in humans, although BAT is present in humans of all ages (9).

Thermogenesis in BAT is stimulated by catecholamine activation of adenylate cyclase through sympathetically innervated β-adrenergic receptors. 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 oxidation and an increase in heat production independent of the phosphorylation of ADP (10). One of the most reliable indices of an increase in BAT thermogenesis is an increase in the density of available guanosine 5′-diphosphate (GDP) binding sites in isolated BAT mitochondria (11, 12). We reported recently that following *Escherichia coli* infection in rats, GDP binding increases, with the greatest increase occurring during the rising phase of the fever (6). Similarly, others have reported increased GDP binding in response to interleukin 1β or endotoxin administration in rats (5, 13–15).

Additionally, we reported previously that both β-adrenergic signal transduction and thermogenesis in BAT is decreased with age (16, 17). Specifically, the increase in GDP binding in response to β-agonist stimulation was absent in older rats. These findings led to the hypotheses that the febrile response to infection may be impaired with age and this impairment may be due to a decreased capacity for thermogenesis in BAT. In the present study, we tested these hypotheses by assessing the febrile response to *E. coli* peritonitis in young and senescent rats and correlating this with BAT mitochondrial GDP binding.

Materials and Methods

Animals. Male F-344 NNia rats of 6 and 24 months of age were obtained from Harlan Industries (Indianapolis, IN) under contract with the National Institute of Aging. Upon arrival, the rats were examined and remained in quarantine for 1 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 and maintained on Purina Rat Chow *ad libitum* with a 12:12-hr light:dark cycle (0600 to 1800 hr). All experiments were started at 0730 hr. Food was removed the morning of the experiment. Ambient temperature was 25°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 1 week. Receivers were placed under each cage. Core body temperature was measured remotely every 3 min and averaged over a 15-min interval. This method allowed repeated noninvasive measurement of temperature without the stress and increase in body temperature normally associated with handling the rats.

Inoculation. Nonhemolytic *E. coli* (ATCC 25922; American Type Culture Collection, Rockville, MD) were grown in Tripticase soy broth with 5% sheep blood at 37°C for 18 hr prior to use. The concentration of organisms was determined by spectrophotometric comparison to McFarland Nephelometer standards. *E. coli* (5×10^8 colony-forming units) suspended in 1 ml of pyrogen-free saline was injected intraperitoneally at 0730 hr on the day of the experiment. Control rats received 1 ml of pyrogen-free saline.

Isolation of BAT Mitochondria. At the time of sacrifice, the circulatory system was perfused with 20 ml of cold saline and interscapular BAT was excised and trimmed of visible white fat. Mitochondria for use in the GDP binding and cytochrome *c* oxidase assays were isolated as described previously (6). Briefly, BAT was suspended in 250 m*M* sucrose, 2 m*M* K-EDTA, 5 m*M* potassium *N*-Tris(Hydroxymethyl) methyl-2-aminoethanesulfonic acid (K-TES) (pH 7.2), and a protease inhibitor cocktail consisting of 1 μ*M* leupeptin, 100 μ*M* benzamidine, and 100 μ*M* phenylmethylsufonyl fluoride. BAT was finely minced with a tissue chopper and homogenized with six strokes of a motor-driven pestle. The homogenate was passed through four layers of cheesecloth and centrifuged at 8715*g* for 10 min at 4°C. The pellet was suspended in the above buffer with 2% fatty acid-free bovine serum albumin and centrifuged two additional times at 750*g* for 10 min at 4°C. The supernatants, which had been retained, were combined and centrifuged at 8715*g* for 10 min, and the pellet was resuspended in 250 m*M* sucrose and recentrifuged three additional times. 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 an 8-point Scatchard analysis of [^{3}H]GDP binding (sp act adjusted to 1.012 Ci/mmol; Dupont-NEN, Boston, MA), as described previously (6). Briefly, protein (50 μg) and 0.02–3.0 μ*M* [^{3}H]GDP were incubated both with and without 1.5 m*M* 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 bovine serum albumin and 2 μ*M* rotenone. The reaction was terminated by dilution with buffer and the bound [^{3}H]GDP was separated from free [^{3}H]GDP 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 the absorbance at 550 mμ (17).

Results

The effects of an injection of 5×10^8 colony-forming units of live *E. coli* into the peritoneal cavity on body temperature and the induction of fever were assessed in 6- and 24-month-old male rats that served as their own controls. Body temperature was assessed for 24 hr preceding the experiment. Daytime body temperatures were relatively constant, which was similar to our previously reported data on the circadian rhythm of body temperature in rats (6). Baseline temperatures were calculated from body temperatures for 1 hr prior to *E. coli* injection and were unchanged with age (Table I). A fever was evident within 30 min after infection in the young animals, but in the senescent rats the onset of fever was delayed by greater than 2 hr (Fig. 1, Table I). The magnitude of the fever was quantitatively less in the older compared with the younger rats throughout the time course of the experiment (Fig. 1). The peak fever at 6 hr after the injection was 2-fold greater in the 6-month-old compared with the 24-month-old rats. Similarly, the cumulative fever (area under the curve) was also 2-fold greater in young animals (Table I). In separate vehicle-injected rats, there was no evidence of an increase in body temperature (data not shown). All the animals recovered from the infection, and there was no evidence of a fever the following day. In addition, the rats remained quiescent during the course of the study, and there was no evidence of shivering.

Table I. Body Temperature and Fever Parameters after *Escherichia coli* Infection in Young and Old Rats[a]

Parameters	Age	
	6 months	24 months
Baseline (°C)	36.9 ± 0.2	37.5 ± 0.4
Peak fever (Δ°C)	2.2 ± 0.2	1.0 ± 0.1[b]
Area (Δ°C·min)	775 ± 69	383 ± 43[b]
Time to onset of fever (hr)	0.4 ± 0.1	2.3 ± 0.1[b]

[a] Data represents the mean ± SE of five rats of each age.
[b] $P < 0.001$ (peak fever and time to onset of fever) or $P < 0.005$ (area) for difference from young rats by Student's *t* test.

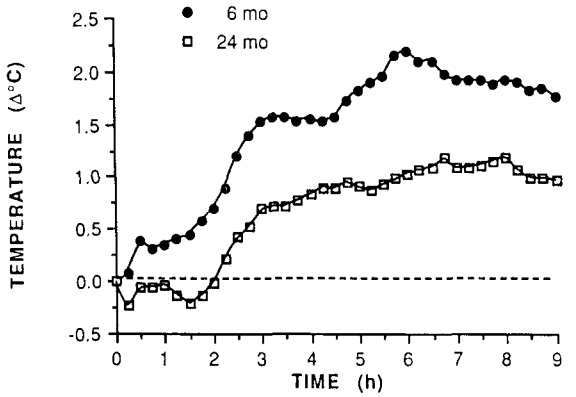

Figure 1. The increase over baseline in body temperature following infection with 5×10^8 colony-forming units of *E. coli* administered at Time 0 to 6-month (●)- and 24-month (□)-old rats. Baseline temperatures are given in Table I. Data represents the mean of five rats in each group. Standard errors ranged from 0.05 to 0.21 and were left off for the sake of clarity.

To investigate the role of BAT thermogenesis in the impaired febrile response in the senescent rats, GDP binding to the uncoupling protein of interscapular BAT mitochondria was assessed following *E. coli* infection in young and old rats. From our previous study in young rats, we determined that during the initial phase of the fever, thermogenesis (increased oxygen consumption and increased GDP binding) was at a maximum, and that at a later time during the stable phase of the fever, the increase in GDP binding was less (6). Therefore, paired rats in each age group were either vehicle injected or infected with 5×10^8 colony-forming units of *E. coli* and sacrificed at 1.75 hr and 3.0 hr after the injection, representing periods during the rising phase of the fever for the young and old animals, respectively.

Interscapular BAT weight, as well as two measures of mitochondrial mass, mitochondrial protein, and cytochrome *c* oxidase activity, were unchanged with either age or infection (Table II). Body weight, as expected, increased with age, but was the same in control and infected groups (Table II).

The number of GDP binding sites was assessed by multipoint Scatchard analysis of [^{3}H]GDP binding to isolated mitochondria (Fig. 2). In the young rats, at 1.75 hr after the infection, there was a 50% increase in the density of available GDP binding sites without a change in the dissociation constant (Fig. 2, Table III). This increase was evident whether expressed as binding sites per milligram of mitochondrial protein or as binding sites per cytochrome *c* oxidase activity (Table III). There was no significant difference between the density of GDP binding sites in the old control rats compared with the young control rats (Table III). In contrast to the young rats, in the senescent rats at 1.75 hr after infection, there was no increase in the density of GDP binding sites whether expressed as binding sites per milligram of mitochondria protein or binding sites per cytochrome *c* oxidase activity (Table III).

However, at 1.75 hr after infection, the young animals are in the rising phase of the fever, whereas the old animals are afebrile (Fig. 1). For this reason in a separate experiment, we investigated GDP binding at 3 hr after infection, a time when the fever was stable in the young animals and rising in the old animals (Fig. 1). In the young rats, there was a 42% increase in the

Table II. Effects of Age and *Escherichia coli* Infection on Body and Interscapular BAT Parameters[a]

Parameter	Age			
	6 months		24 months	
	Control	Infected	Control	Infected
Body wt (g)	321 ± 9	306 ± 10	413 ± 5	414 ± 8
BAT wt (mg)	256 ± 22	267 ± 9	234 ± 31	302 ± 34
Mitochondrial protein (mg)	1.61 ± 0.22	1.59 ± 0.18	1.65 ± 0.25	1.82 ± 0.32
Cytochrome *c* oxidase activity (nmol/mg protein)	619 ± 62	638 ± 48	682 ± 91	535 ± 53

[a] Cytochrome *c* oxidase activity is expressed as activity per mg of mitochondrial protein. Data represents the mean ± SE of five to eight rats.

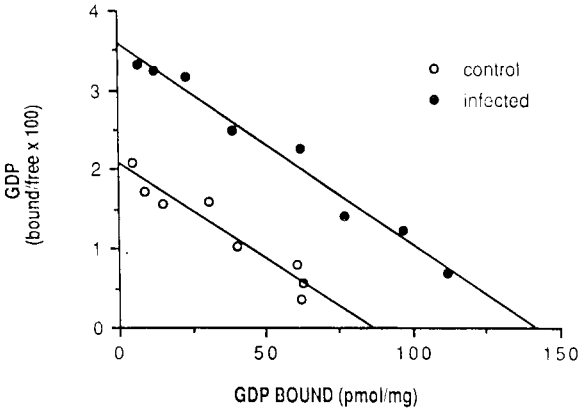

Figure 2. Scatchard analysis of [^{3}H]GDP binding to BAT mitochondria from 6-month-old rats 1.75 hr after saline (○) or *E. coli* (●) administration. Lines were determined by regression analysis with correlation coefficients of 0.99 and 0.96, binding site densities of 141 and 86 pmol/mg protein (indicated by the abscissa intercepts), and dissociation constants for [^{3}H]GDP of 375 and 327 n*M* (indicated by the negative reciprocal of the slope of the line), respectively, for control and *E. coli*-infected rats.

density of GDP binding sites at 3 hr after infection; however, this increase was not statistically significant (Table IV). When the GDP binding was expressed as binding sites per cytochrome *c* oxidase activity, there was a similar nonsignificant increase (Table IV). In the old animals, there was no significant difference in GDP binding at 3 hr after infection whether expressed as binding sites per milligram of protein or binding sites per cytochrome *c* oxidase activity (Table IV). In control rats, similar to the results at the 1.75 hr time point, there was no difference between GDP binding in young and old control rats (Table IV). The dissociation constant for GDP binding was unchanged with age or *E. coli* infection (Table III and IV).

Discussion

Fever has long been recognized as a host defense mechanism against infection. It has an evolutionary history that dates back hundreds of millions of years to lower animals (1). Although there is no direct evidence of fever's importance as a host defense mechanism in humans, its role in the early diagnosis of severe infection is undisputed (2). There is a positive correlation between the presence of a fever and decreased morbidity and mortality rates during a variety of infections (3). Elderly people in particular have especially high morbidity and mortality rates with infection, and this is likely due to a variety of factors, including the frequent absence of a fever with a consequent delay in the diagnosis of severe infection (2). In lower animals, there is direct evidence that fever is an important host defense mechanism. This was best demonstrated in the classic study by Kluger *et al.* (18), in which lizards infected with bacteria had higher survival rates when maintained at externally controlled higher temperatures.

In the present report, we have provided evidence that the febrile response to *E. coli* has both a delayed onset and is reduced in magnitude in senescent male rats. Both the peak fever and the cumulative fever were

Table III. Effects of Age and *Escherichia coli* Infection on GDP Binding at 1.75 hr after Infection[a]

Parameter	Age			
	6 months		24 months	
	Control	Infected	Control	Infected
GDP binding sites (pmol/mg protein)	98 ± 9	149 ± 13[b]	122 ± 11	112 ± 11
GDP binding sites (pmol/μmol cyc *c*)	169 ± 21	239 ± 23[b]	192 ± 20	226 ± 17
K_d (n*M*)	350 ± 43	472 ± 91	447 ± 69	392 ± 62

[a] GDP binding is expressed as binding sites per mg mitochondrial protein (Row 1) or as binding sites per μmol cytochrome *c* oxidase activity (Row 2). Data represent the mean ± SE of five to eight rats.
[b] $P < 0.01$ (Row 1) or $P < 0.05$ (Row 2) for difference from 6-month-old control by Student's *t* test.

Table IV. Effects of Age and *Escherichia coli* Infection on GDP Binding at 3 hr after Infection[a]

Parameter	Age			
	6 months		24 months	
	Control	Infected	Control	Infected
GDP binding sites (pmol/mg protein)	71 ± 11	101 ± 20	72 ± 8	91 ± 10
GDP binding sites (pmol/μmol cyc *c*)	149 ± 38	238 ± 53	178 ± 14	208 ± 23
K_d (*nM*)	462 ± 99	489 ± 62	465 ± 86	393 ± 34

[a] GDP binding is expressed as binding sites per mg mitochondrial protein (Row 1) or as binding sites per μmol cytochrome *c* oxidase activity (Row 2). Data represent the mean ± SE of five to seven rats.

reduced in the older rats. These data in male rats are similar to what we have observed in female rates, i.e., there was a longer time to the onset of the fever and the cumulative fever was less in the senescent female rats (19).

The febrile effects of endotoxin are thought to be mediated by a number of endogenous pyrogens, including interleukin 1, tumor necrosis factor, interferons (especially α-interferon), and macrophage inflammatory protein-1 (4, 20). These cytokines interact with the thermoregulatory center of hypothalamus and reset the hypothalamic thermostat to a higher level, presumably through an increase in prostaglandin production (4). However, non-prostaglandin-mediated pyrogenic mechanisms also exist (20).

There are few age-related studies on endogenous pyrogens. Interleukin 1 production is unchanged with age (7, 8). However, the fever response of old mice is depressed compared with young mice following the injection of either interleukin 1 or tumor necrosis factor (21, 22). In addition, the febrile response of senescent rats to endotoxin or *Salmonella typhimurium* is decreased with age (23). These data, along with our observations of a decreased febrile response to *E. coli* infection, suggest either an impaired central processing of cytokines or altered heat production-dissipation in the periphery. An important peripheral mechanism responsible for heat production following infection is thermogenesis in BAT (6). Endotoxin alters regional blood flow, increasing perfusion of kidney, liver, and BAT, and decreasing flow to the back and leg muscles (5). There is increased oxygen consumption and an increased number of available GDP-binding sites in BAT mitochondria (5, 6). These data suggest that sympathetic stimulation through the β-adrenergic receptor leads to an increase in thermogenesis in BAT, culminating in a rise in body temperature. The present data support this hypothesis by demonstrating an increase in BAT GDP binding following *E. coli* infection in the young rats. Moreover, our finding of an impaired ability to increase in GDP binding in the older rats after *E. coli* infections suggests that the delayed and reduced febrile response in older rats may be due to a failure to stimulate thermogenesis in BAT.

These data are consistent with our previous reports suggesting inadequate β-adrenergic stimulation of BAT thermogenesis in older rats (16, 17). Thermogenesis in BAT is stimulated by the sympathetic nervous system. Activation involves norepinephrine binding to the β-adrenergic receptor and stimulation of adenylate cyclase (10). Signal transduction, including the number of β-adrenergic receptors and the efficacy of adenylate cyclase activation, is decreased in BAT with age (17). Functionally, there is a decrease in the ability of β-adrenergic agonists to stimulate oxygen consumption (16, 24). More importantly, the ability of agonists to stimulate an increase in BAT mitochondrial GDP binding is absent in the senescent rats (16). An increase in GDP binding to mitochondria in BAT is the most reliable index of *in vivo* activation of BAT thermogenesis (11, 12). The high capacity for mitochondrial heat production is a result of loosely coupled respiration. Mitochondria in BAT contain a unique uncoupling protein that is an H+/OH− translocater. This protein, when activated permits protons (or OH−) to pass through the mitochondrial inner membrane, allowing substrate oxidation without ATP synthesis (25). The degree of uncoupling and subsequent heat production is a function of the number of active translocaters, which can be assessed by the amount of "unmasked" or available GDP binding sites (26). Increased demands for heat, such as following acute cold exposure or infection, increases the number of GDP-binding sites (6, 27).

In the present study, we found that in the young rats, the increase in GDP binding was greatest during the rising phase of the fever, when the demand for heat production would be expected to be greatest. In contrast, at a later time, while the fever was stable, the increase in GDP binding was less than during the rising phase of the fever and was not significant. In the older rats, the situation was somewhat different. At the earliest time point, the older animals had not yet become febrile and there was no increase in GDP binding. Furthermore, at the later time point, when the fever was rising in the older rat, there was only a small increase in GDP binding and it was not significant. This finding is further supported when we included

additional data. Three older animals and one young animal were excluded from the data in Table IV since the cytochrome *c* oxidase values were not obtained, though the GDP binding values were assessed. When these values are included, the control density of GDP binding sites was 66 ± 7 pmol/mg protein (n = 9), the value from infected rats was 87 ± 9 pmol/mg protein (n = 7), and the difference remained not significant.

The failure to increase the number of GDP binding sites in the older rats may be due to an impaired ability to unmask reserve GDP binding sites. The observed diminished β-adrenergic signal transduction with age could be contributing to this impairment (17). Alternatively, the GDP binding sites may already be unmasked in the older rats. The density of unstimulated GDP binding sites was the same in senescent control compared with young control rats. However, it is possible that the total amount of GDP binding sites (masked and unmasked) is less in senescent rats. Thus, a greater proportion, if not all, of the GDP binding sites may already be unmasked in the baseline state of older rats. When challenged with infection, there should be no additional sites to be unmasked, and, thus, no increase in GDP binding would be observed.

E. coli infection induces a fever in young rats that is associated with an increase in BAT mitochondrial GDP binding. In senescent rats, there is a delay in the onset of the fever, a decrease in the magnitude of fever, and no increase in GDP binding. This failure to increase GDP binding may be a result of a reduced ability to unmask reserve GDP binding sites or it may be that the GDP binding sites are already unmasked. Collectively, these data suggest that the impaired febrile response with age may be due to reduced thermogenesis in BAT.

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