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EFFECT OF COLD ON SERUM SUBSTRATE AND GLYCOGEN CONCENTRATION IN YOUNG AND OLD FISCHER 344 RATS

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Abstract

This investigation evaluated the hypothesis that the age-related decline in cold-induced thermogenesis observed in male (F344) rats is associated with altered substrate concentrations of glucose, lactate, and/or liver and muscle glycogen. Body mass-independent O₂ consumption, core temperature, and serum glucose and lactate concentrations were measured at rest and during 4 h of exposure to 5°C in male F344 rats ages 6, 12, and 26 months. At the end of the 4-h cold exposure, liver, soleus, and gastrocnemius tissues were removed, frozen, and analyzed for glycogen concentration and/or citrate synthase activity. Core temperature decreased during cold exposure and was consistently less in the 26-month versus the 6- and 12-month rats. There were no significant differences between the 6- and 12-month-old rats with respect to cold-induced O₂ consumption, but measures were significantly lower in the 26-month-old rats. During cold exposure, serum lactate and glucose concentrations increased in the 26-month-old animals compared to those in the 6- and 12-month-old rats, while liver glycogen concentrations decreased in all groups, and gastrocnemius glycogen contents decreased in the 12- and 26-month-old rats. Citrate synthase specific activity ($\mu\text{mol} \cdot [\text{min} \cdot \mu\text{g} \cdot \text{protein}]^{-1}$) did not differ with age. These data suggest that carbohydrate availability (as measured by serum glucose and muscle glycogen) is not a limiting factor in the attenuated cold-exposed thermogenic response of the 26-month-old male F344 rat. However, it appears that the 26-month-old rat may have a diminished capacity to fully oxidize carbohydrate during cold exposure.

Keywords

aging rats; cold-induced thermogenesis; glucose; lactate; liver and muscle glycogen

INTRODUCTION

Age-Related declines in thermogenesis of 26-month-old rats exposed to low environmental temperatures involve attenuated nonshivering thermogenesis of brown adipose tissue (McDonald *et al.*, 1989a) and result in cold-induced hypothermia. Blunted muscle thermogenesis (nonshivering and/or shivering) may also play a significant role in the development of this hypothermia (Lee and Wang, 1985). Diminished thermogenic contributions from muscle in the older rat may reflect declining muscle mass (Masoro, 1985; Yu *et al.*, 1985) and/or lower muscle oxidative capacity (Beyer *et al.*, 1984) which in turn may result from the aging process per se or from the rats' sedentary lifestyle. Our previous data indicate no decrease in lean body mass in sedentary male F344 rats as old as 24 month. Moreover, we found that, after 6 months of moderate exercise training, 24-month-old F344

male rats increased their heat production sufficiently to maintain core temperature during cold exposure (McDonald *et al.*, 1988). This moderate exercise training, however, had only minor effects on the older rats' percent lean body mass and muscle citrate synthase activity (a measure of muscle oxidative capacity [McDonald *et al.*, 1988]), making it unlikely that the improved thermogenic response of the older rats was due simply to altered quantity and/or oxidative capacity of skeletal muscle.

Another possible contribution to the decreased cold-exposed thermogenesis of the older rats is an age-related alteration in the availability and/or type of substrate (e.g., carbohydrate vs. fat) utilized by skeletal muscle during cold exposure (Lee and Wang, 1985). Data from animal and human investigations suggest that aging individuals derive a greater portion of energy from carbohydrates than from fat (Carlson and Prenow, 1961; Fitts *et al.*, 1984; Martineau and Jacobs, 1988). Preferential use of carbohydrates as an energy source during shivering could result in more rapid depletion of available carbohydrates. In addition, increased serum lactate concentrations that would accompany heightened anaerobic carbohydrate metabolism in muscle could lead to decreased serum pH, thereby adding to the stress of cold exposure. This study evaluates the hypothesis that the age-related decline in cold-induced thermogenesis observed in male F344 rats is associated with altered concentrations of serum glucose, serum lactate, and/or liver and muscle glycogen, and that a greater dependence on the glycolytic pathway may be involved in the attenuated muscle thermogenesis of older rats.

MATERIALS AND METHODS

Animals and animal care

Seventy-five male inbred F344 rats, ages 6, 12, and 26 months, were obtained from the National Institute on Aging's animal colony maintained by Harlan Sprague-Dawley Laboratory (Indianapolis, IN). Body weights are presented in Table 1. Upon arrival, animals were housed in laminar flow units (Duo-Flo, Lab Product, Maywood, NJ) which supply clean air by filtering incoming air with high-efficiency particle filters. Rats were housed individually in hanging wire-bottom cages (20 × 25 × 18 cm) and kept on a 12:12 h light-dark photoperiod (lights on at 0600, off at 1800) in a room at 24–26°C. They were fed ad libitum autoclaved NIH-31 Laboratory Chow (Teklad Research Diets, Indianapolis, IN) and acidified distilled water, pH 3.5.

Although necropsy and microbiological examinations were not performed routinely, animals were inspected daily for external symptoms of disease. Only rats with no external signs of disease were used in this study. In addition, serological examinations were performed on 12 rats (Microbiological Associates Inc., Bethesda, MD). Each rat tested negative for 9 rat viruses and *Mycoplasma pulmonis*. Internal tumors were observed on several of the older rats; however, no rats were excluded from the study.

Experimental protocol

Three experimental protocols were used in this investigation. In the first protocol (Initial), at 0700 food and water were removed from five rats in each of the three age groups. Three hours later, these rats were anesthetized with methoxyflurane. Liver, soleus, and gastrocnemius muscles were quickly dissected, frozen between tungsten clamps, cooled in liquid nitrogen, and stored at –7°C for subsequent analysis of glycogen concentration (liver and gastrocnemius) or citrate synthase (gastrocnemius and soleus) activity. A second group of rats (non-cold-exposed: NCE), five of each age, was anesthetized with halothane, and cannulae were placed in the left carotid artery 24 h prior to the experiment. Twelve hours later, these rats were placed into water-jacketed metabolic chambers (see below) for overnight acclimation. Animals were given ad libitum access to food and water until 0700 at which time food and water were removed

from the cages. A pilot study showed that rats undergoing surgery for implantation of carotid artery cannulae consumed food and water in the 24-h postsurgical period. At 1000, O₂ consumption was measured for 6 h at thermoneutrality (24–26°C) (McDonald *et al.*, 1987). Finally, a third group of rats (cold-exposed: CE), approximately 10 of each age, was treated identically to the NCE rats, except that O₂ consumption was measured from 1000 to 1200 at 25°C and from 1200 to 1600 at 5°C. The initial rat group served as a control for all experimental procedures including surgery, time in chamber, and cold exposure, while the NCE animals were a control only for the effects of cold exposure. Blood samples (1 ml) were collected from both the NCE and CE rats at 1130, 1400, and 1600. We have previously determined that 4.0 ml of blood can be removed from a 400-g rat without a significant drop in hematocrit (McDonald, 1990). Blood was collected in unheparinized collection tubes, separated to obtain serum, and stored at –70°C for subsequent analysis of lactate and glucose. Saline (0.9% NaCl, 1.0 ml) was used to replace blood lost at each of the three blood collection points. After drawing blood at 1600, both the NCE and CE animals were removed from the chambers and anesthetized with metophane, and the liver, soleus, and gastrocnemius muscles were dissected, freeze clamped, and stored at –70°C for subsequent analysis of glycogen concentration and citrate synthase activity. Due to the small mass of the soleus muscles, we were able to measure only citrate synthase activity.

O₂ consumption measurement

The complete procedure for measuring O₂ consumption has been described elsewhere (McDonald *et al.*, 1987). Briefly, metabolic chambers are part of an open-circuit system. Air is delivered at a rate of 3.0 liter/min to the acrylic chamber containing the animal. The volume of the system is 6 liters, which results in a 99% washout time of ~9.5 min. Room air was first passed through a desiccant (Drierite, calcium sulfate anhydrous), and then the O₂ content of air samples were recorded using an Applied Electrochemistry O₂ analyzer (model S-3A/I, Pittsburgh, PA). For each rat, O₂ consumption values were measured continuously (baseline checked every 20 min), and hourly values represent the average of three 20-min periods. Data for O₂ consumption are presented independent of body mass (i.e., ml · min^{–1} · kg body mass^{–0.67}). The use of the exponent 0.67 is based on Heusner's (1985) observations that metabolic rate within a species varies with body mass by this power function.

Measurements of core temperature

One to two weeks prior to O₂ consumption measurement, NCE and CE rats were anesthetized with halothane and a temperature-sensing radio wave transmitter was implanted in their peritoneal cavities. The transmitters were part of a computer-controlled telemetry system (Minimeter, Bend, OR) used for recording core body temperature. These temperatures were recorded at 5-min intervals throughout the entire 6-h experimental period; the hourly temperature values reported here represent the average of values recorded 20 min before and after each hour mark.

Analytical procedures

Serum lactate concentrations were measured using a quantitative enzymatic determination kit from Sigma Diagnostics (St Louis, MO); glucose was analyzed by the glucose oxidase method (Beckman Glucose Analyzer 2, Beckman Instruments, Inc., Fullerton, CA). Liver and muscle were analyzed for glycogen content via the method of Hassid and Abraham (1957). Muscle protein (from the 700 × g supernatant) and citrate synthase activity (assay run at 25°C) were analyzed using a Bradford protein assay kit from Bio-Rad (Richmond, CA) and the method of Serre (1969), respectively.

Statistics

We used analysis of variance (ANOVA) with a factorial design (age, temperature, and time as main effects) to evaluate the serum data, ANOVA with age and temperature as main effects to evaluate glycogen and citrate synthase data, and one-way ANOVA where appropriate. Repeated measures ANOVA was used to analyze O₂ consumption and body temperature over time. When a significant main effect was found, the Fischer least significant difference post hoc test was used to determine differences between the groups. Differences were considered significant at $p \leq 0.05$.

RESULTS

Oxygen consumption

Body mass-independent O₂ consumption of NCE rats did not differ between the age groups or during 6 h at thermoneutrality. Resting values for the NCE rats averaged 8.6 ± 0.5 for the 6-month-old rats, 10.4 ± 2.1 for the 12-month-old rats, and $8.5 \pm 1.2 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg body mass}^{-0.67}$ for the 26-month-old rats. These values did not differ from those of the NCE rats at any of the other time points or from those of the CE rats prior to their cold exposure (Fig. 1). However, during cold exposure, O₂ consumption values at 1, 2, 3, and 4 h were significantly higher in the CE than in the NCE rats (Fig. 1). Moreover, there was a significant effect of age on O₂ consumption when analyzed by repeated measures ANOVA. At no time did rates of O₂ consumption in the CE rats differ within or between the 6- and 12-month-old groups, but by 2 h of cold-exposure, the rates were significantly lower in the 26-month-old rats (Fig. 1).

Body temperature

There were no significant differences in body temperature of NCE and CE rats at thermoneutrality. For the NCE rats, body temperatures averaged: 35.9 ± 0.8 , 6 month; 36.8 ± 0.1 , 12 month; and $36.4 \pm 0.3^\circ\text{C}$, 26 month. For the CE rats, average body temperatures were 35.8 ± 1.0 , 6 month; 36.2 ± 1.2 , 12 month; and $36.4 \pm 0.3^\circ\text{C}$, 26 month. Although core body temperatures of all CE rats significantly declined, repeated measures analysis revealed that, during the 4 h of cold exposure, the temperatures of the 26-month-old rats were consistently below those of the 6- and 12-month-old animals ($p < 0.01$; Fig. 2).

Serum glucose and lactate

Serum glucose and lactate concentrations did not significantly differ among NCE and CE rats at thermoneutrality; there were also no effects of cold on these concentrations in the 6- and 12-month-old rats (Table 2). However, the concentrations of both serum glucose and lactate were significantly higher in 26-month-old CE rats compared to 6- and 12-month-old CE animals, with a significant interaction of age and temperature.

Liver and gastrocnemius glycogen concentration

Although liver weights were higher in the 26-month-old compared to the 6- and 12-month-old initial and CE rats (Table 1), liver glycogen (per gram liver or per whole liver) was significantly lower in the NCE and CE rats regardless of age (Table 3).

Gastrocnemius muscle glycogen levels (per gram muscle or per whole muscle) in the 6-month-old rats was not altered by acute cold exposure. In contrast, gastrocnemius glycogen levels in the 12- and 26-month-old rats decreased in response to acute cold exposure whether data were expressed per gram muscle or per whole muscle. Gastrocnemius weights were significantly lower in the 26- compared to the 6- and 12-month-old groups (Table 1).

Soleus and gastrocnemius citrate synthase activity

Citrate synthase in soleus and gastrocnemius muscles (per mg protein) did not significantly differ with age in either the NCE or CE groups (Table 4).

DISCUSSION

Our data suggest that the availability of substrate to shivering muscle is not the limiting factor in the attenuated ability of the old male F344 rats to maintain homiothermy during cold exposure. Instead, it appears that old rats metabolize relatively more carbohydrate via glycolytic than oxidative pathways during acute cold exposure, based on our observation that the availability of glucose to muscle is greater for old than for young rats.

Although muscle glycogen concentrations of the 12- and 26-month-old rats decreased to similar levels and serum glucose increased in the oldest rats, only the latter exhibited attenuated cold-exposed thermogenesis and significant hypothermia. This indicates that carbohydrate availability was not the limiting factor in the older rats' ability to maintain homiothermy. The increased serum lactate concentrations observed in 26-month-old compared to young rats suggests that old rats preferentially metabolized glucose via non-oxidative glycolytic pathways in response to 4 h of acute cold exposure. The increased lactate in the old rats is consistent with incomplete glucose oxidation and could result in attenuated flux through the TCA cycle. This in turn, may contribute to decreased muscle thermogenesis since less energy is released as heat during glucose oxidation to lactate than during glucose oxidation to carbon dioxide and water. Other investigators have shown lower activity of aerobic enzymes in 28- and 38-month-old male Wistar rats (Bass *et al.*, 1975) and decreased activity of mitochondrial enzymes in soleus muscle of 22- and 24-month-old Wistar rats (Sanchez *et al.*, 1983; Simard *et al.*, 1985; Kiltgaard *et al.*, 1989). Although we did not find lower citrate synthase specific activity in the 26-month-old gastrocnemius or soleus muscles, we did observe smaller muscle masses in these rats. This latter change could have a negative impact on total oxidative capacity of muscle and thus may contribute to the attenuated thermogenesis of old rats in response to cold exposure. An alternative explanation for the increased serum lactate concentration in old rats could be a decrease in the conversion of lactate to glucose in the liver (via the Cori cycle); however, current literature does not speak to this possibility.

The slight increase in glycogen content of the gastrocnemius muscle isolated from CE 6-month-old rats is consistent with reports on young rats exhibiting a glycogen-sparing effect during acute cold exposure (Smith and Davidson, 1982; Bukowiecki, 1988; Shibata *et al.*, 1989). These findings have generally been interpreted to mean that the rate of glucose uptake increases during cold exposure. Indeed, Vallerand *et al.* (1987) report that cold exposure potentiates glucose uptake in muscle with or without the presence of insulin. The observation that 12- and 26-month-old rats showed declining muscle glycogen levels during cold exposure implies that glucose uptake of thermogenic tissues in cold-exposed rats diminishes within this age span. It appears that old rats exposed to cold metabolize intracellular carbohydrate stores (rather than circulating reserves) to a greater degree than do young rats.

The age-related decline in cold-exposed mass-independent thermogenesis observed in our 26-month-old F344 rats is of interest because we have previously observed no such declines in 6-, 12-, and 24-month-old male F344 rats even after 6 h of exposure to 6°C (McDonald *et al.*, 1989b). These differences in the cold-induced responses of 24- versus 26-month-old rats may reflect the fact that the life expectancy of the F344 rat decreases rapidly after the age of 24 mo (Yu *et al.*, 1985). That is, blunting of physiological functions may be greater in animals that are older than the median life span. Thus, our data are consistent with the concept that physiological aging is not linear with time but instead is more closely associated with rapid functional declines expressed late in life.

The observed decrease in body temperature in all three age groups in response to acute cold exposure could be confounded by the experimental manipulations (i.e., surgery, anesthesia, etc.) (Avakian and Horvath, 1981; Dulloo *et al.*, 1988). Although the effects of halothane should be minimal 24 h after surgery (Miller *et al.*, 1980; Hoffman *et al.*, 1982), postsurgical stress could contribute to the attenuated thermogenic response observed. Our previous studies indicate that even non-surgically treated male F344 rats (23–27 month) were unable to adequately maintain their body temperature during 6 h of acute cold exposure (McDonald *et al.*, 1989b). The stress of surgery, or stress in general, may have exacerbated this aging response via modulating factors such as catecholamines. Norepinephrine has been shown to have vasoconstrictive activity which decreases blood flow to muscle and could ultimately affect muscle thermogenesis (Smith and Davidson, 1982; Bukowiecki, 1988; Shibata *et al.*, 1989). However, all age groups were treated identically and the aged 26-month-old rat still appeared more sensitive to the stress of the acute cold exposure. Therefore, surgical stress or the effects of anesthesia cannot entirely explain the attenuated thermogenic response of the aged 26-month-old animal.

In summary, attenuated cold-induced thermogenesis in aging rats (as measured by O₂ consumption) could be due to several factors, including decreased substrate availability to the muscle, decreased substrate uptake into the muscle, or decreased utilization of available substrate. Because we observed increased glucose and lactate in the serum of the cold-exposed older rats, availability of circulating substrate does not appear to be a major factor in the attenuated cold-induced muscle thermogenesis. It is possible, however, that incomplete oxidation of carbohydrate (as indicated by increased serum lactate) contributes to muscle's attenuated thermogenic response. We suggest that cold-exposed aged male F344 rats metabolize carbohydrate via glycolytic rather than oxidative pathways to a greater degree than do younger rats.

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FIG. 1.

Mass-independent O_2 consumption ($\dot{V}O_2$) of younger (6 and 12 month) and older (26 month) rats during acute cold exposure. Resting O_2 consumption values did not significantly differ and averaged: 6 month, 8.68 ± 0.5 ; 12 month, 8.77 ± 0.5 ; and 26 month, $8.91 \pm 0.4 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg body mass}^{-0.67}$. *Values are significantly different from those of 6- and 12-month animals ($p \leq 0.05$). There was a significant effect of age on O_2 consumption when analyzed by repeated measures analysis of variance ($p \leq 0.05$).

FIG. 2.

Core body temperatures of younger (6 and 12 month) and older (26 month) rats during acute cold exposure. Resting values averaged: 6 month, 35.8 ± 1.04 ; 12 month, 36.2 ± 1.16 ; and 26 month, $36.36 \pm 0.34^\circ\text{C}$. By repeated measures analysis of variance, there was a significant effect of age on body temperature ($p < 0.01$).

TABLE 1

Weights of body, liver, soleus, and gastrocnemius in male F344 rats

Age (months)	6	12	26
Body weight (g)			
Initial	342.6 ± 17.1 ^a (5)	392.3 ± 12.0 ^b (5)	390.9 ± 19.3 ^b (5)
No Cold (NCE)	365.6 ± 13.2 (6)	359.5 ± 11.9 (6)	347.0 ± 15.0 (5)
Cold (CE)	350.4 ± 8.6 ^a (12)	387.6 ± 5.0 ^b (14)	383.0 ± 8.4 ^b (12)
Liver weight (g)			
Initial	10.4 ± 0.4 ^{a#} (4)	9.7 ± 0.8 ^a (4)	13.0 ± 1.1 ^{b#} (4)
No Cold (NCE)	8.9 ± 0.9 [@] (5)	7.5 ± 0.9 (3)	8.9 ± 1.6 [@] (3)
Cold (CE)	8.5 ± 0.3 [@] (9)	9.1 ± 0.4 (9)	10.5 ± 0.6 [@] (8)
Soleus weight (g)			
Initial	0.13 ± 0.01 (5)	0.14 ± 0.01 (5)	0.11 ± 0.01 (5)
No Cold (NCE)	0.14 ± 0.01 (5)	0.14 ± 0.01 (4)	0.11 ± 0.01 (4)
Cold (CE)	0.15 ± 0.01 ^a (11)	0.14 ± 0.01 ^a (11)	0.12 ± 0.01 ^b (8)
Gastrocnemius weight (g)			
Initial	1.78 ± 0.13 ^{a#@} (4)	1.79 ± 0.03 ^a (4)	1.48 ± 0.09 ^{b#} (5)
No Cold (NCE)	1.86 ± 0.08 ^{a#} (5)	1.62 ± 0.05 ^b (4)	1.27 ± 0.07 ^{c@} (4)
Cold (CE)	1.66 ± 0.04 ^{a@} (11)	1.76 ± 0.03 ^a (11)	1.39 ± 0.03 ^{b#@} (8)

Values are means ± SE. Within a row, values not sharing common letter superscripts are significantly different ($p < 0.05$). For each variable, values in a column not sharing common symbol superscripts are significantly different ($p < 0.05$). Numbers in parenthesis = the number of rats/group; NCE = non-cold-exposed; CE = cold exposed. Initial group had no surgical manipulations.

TABLE 2
Serum concentrations of glucose and lactate in male F344 rats

Age (months)	6	12	26
<i>Glucose</i> (mmol · L ⁻¹)			
No Cold (NCE)			
Resting	7.9 ± 0.5 (4)	7.1 ± 0.9 (4)	5.5 ± 0.4 (4)
2 h	7.3 ± 0.5 (4)	7.3 ± 1.2 (3)	6.3 ± 0.5 (4)
4 h	7.6 ± 0.3 (4)	7.0 ± 1.0 (4)	6.8 ± 1.1 (4)
Cold (CE)			
Resting	7.8 ± 0.3 (6)	8.3 ± 0.4 (10)	7.3 ± 0.5 [#] (7)
2 h	8.1 ± 1.0 ^a (5)	8.3 ± 0.7 ^a (8)	14.2 ± 2.1 ^{b@} (7)
4 h	9.5 ± 1.5 ^a (6)	9.8 ± 1.2 ^a (9)	15.1 ± 2.8 ^{b@} (6)
<i>Lactate</i> (mmol · L ⁻¹)			
No Cold (NCE)			
Resting	0.62 ± 0.07 (5)	0.73 ± 0.10 (4)	0.69 ± 0.13 (4)
2 h	0.59 ± 0.09 (4)	0.71 ± 0.12 (3)	0.69 ± 0.11 (4)
4 h	0.84 ± 0.30 (4)	0.70 ± 0.12 (4)	0.73 ± 0.12 (4)
Cold (CE)			
Resting	0.66 ± 0.06 (10)	0.70 ± 0.08 (10)	0.72 ± 0.11 [#] (7)
2 h	1.48 ± 0.60 ^a (9)	0.96 ± 0.14 ^a (9)	3.38 ± 0.64 ^{b@} (7)
4 h	1.68 ± 0.69 ^a (8)	1.21 ± 0.34 ^a (9)	4.21 ± 1.24 ^{b@} (6)

Values are means ± SE. Within a row, values not sharing common letter superscripts are significantly different ($p \leq 0.05$). Within a column and experimental trial (i.e., cold or no cold), values not sharing common symbol superscripts are significantly different ($p \leq 0.05$). Both serum glucose and lactate showed significant interactions of age and temperature: $F(17, 59) = 3.69$, $p = 0.0291$ and $F(17, 59) = 4.61$, $p = 0.0001$, respectively. Numbers in parenthesis = the number of rats in a group; NCE = non-cold-exposed; CE = cold exposed.

TABLE 3
Glycogen concentration/content of liver and *gastrocnemius* in male F344 rats

Age (months)	6	12	26
Liver			
Initial			
$\mu\text{mol} \cdot \text{gram}^{-1}$	$323.1 \pm 26.3^{\text{a}\#}$	$463.5 \pm 43.2^{\text{b}\#}$	$203.9 \pm 54.4^{\text{c}\#}$
$\mu\text{mol} \cdot \text{Liver}^{-1}$	$3309.8 \pm 130.8^{\text{a}\#}$	$4356.1 \pm 180.0^{\text{b}\#}$	$2608.0 \pm 776.8^{\text{a}\#}$
	(5)	(5)	(5)
No Cold (NCE)			
$\mu\text{mol} \cdot \text{gram}^{-1}$	$100.3 \pm 23.0^{\text{a}}$	$125.8 \pm 17.2^{\text{a}}$	$65.7 \pm 16.3^{\text{a}}$
$\mu\text{mol} \cdot \text{Liver}^{-1}$	$836.1 \pm 176.4^{\text{a}}$	$936.92 \pm 181.9^{\text{a}}$	$551.8 \pm 131.2^{\text{a}}$
	(5)	(4)	(3)
Cold (CE)			
$\mu\text{mol} \cdot \text{gram}^{-1}$	$91.6 \pm 20.8^{\text{a}}$	$94.1 \pm 13.2^{\text{a}}$	$36.4 \pm 3.2^{\text{a}}$
$\mu\text{mol} \cdot \text{Liver}^{-1}$	$808.1 \pm 201.3^{\text{a}}$	$874.7 \pm 141.3^{\text{a}}$	$376.1 \pm 32.3^{\text{a}}$
	(9)	(10)	(8)
Gastrocnemius			
Initial			
$\mu\text{mol} \cdot \text{gram}^{-1}$	$15.9 \pm 6.2^{\text{a}}$	$77.9 \pm 9.9^{\text{b}\#}$	$23.0 \pm 2.9^{\text{a}\#}$
$\mu\text{mol} \cdot \text{muscle}^{-1}$	$25.5 \pm 7.2^{\text{a}\#}$	$138.7 \pm 16.1^{\text{b}\#}$	$33.8 \pm 4.7^{\text{a}\#}$
	(4)	(4)	(5)
No Cold (NCE)			
$\mu\text{mol} \cdot \text{gram}^{-1}$	$27.4 \pm 5.1^{\text{a}}$	$70.6 \pm 5.5^{\text{b}\#}$	$76.3 \pm 9.8^{\text{b}\#}$
$\mu\text{mol} \cdot \text{muscle}^{-1}$	$49.7 \pm 8.1^{\text{a}\#}$	$113.7 \pm 8.8^{\text{b}\#}$	$97.1 \pm 14.9^{\text{b}\#}$
	(5)	(4)	(4)
Cold (CE)			
$\mu\text{mol} \cdot \text{gram}^{-1}$	35.5 ± 5.2	$32.8 \pm 2.8^{\text{a}}$	$41.0 \pm 8.9^{\text{a}}$
$\mu\text{mol} \cdot \text{muscle}^{-1}$	$59.1 \pm 8.7^{\text{a}}$	$57.4 \pm 5.6^{\text{a}}$	$56.7 \pm 12.1^{\text{a}}$
	(9)	(10)	(8)

Values are mean $\pm$ SE. Within a row, values not sharing common letter superscripts are significantly different ($p \leq 0.05$). For each variable, values in a column not sharing common symbol superscripts are significantly different ($p \leq 0.05$). Numbers in parenthesis = the number of rats/group: NCE = non-cold-exposed; CE = cold exposed. Initial group had no surgical manipulations.

TABLE 4
Citrate synthase (cs) activity of the gastrocnemius and soleus muscle in male F344 rats

Age (months)	6	12	26
<i>Soleus</i>			
<i>No Cold</i> (NCE)	(4)	(3)	(4)
Mass (g)	0.13 ± 0.01	0.13 ± 0.02	0.11 ± 0.01
CS Activity			
$\mu\text{mol} \cdot (\text{min} \cdot \text{g muscle})^{-1}$	10.3 ± 0.7	9.8 ± 2.4	8.6 ± 1.0
$\mu\text{mol} \cdot (\text{min} \cdot \mu\text{g protein})^{-1}$	281.0 ± 28.0	279.0 ± 9.0	226.0 ± 13.0
<i>Cold</i> (CE)	(8)	(10)	(8)
Mass(g)	0.15 ± 0.01 ^a	0.15 ± 0.01 ^a	0.11 ± 0.01 ^b
CS Activity			
$\mu\text{mol} \cdot (\text{min} \cdot \text{g muscle})^{-1}$	8.8 ± 1.1	8.3 ± 0.9	9.2 ± 0.7
$\mu\text{mol} \cdot (\text{min} \cdot \mu\text{g protein})^{-1}$	233.9 ± 9.6	257.2 ± 10.3	238.0 ± 13.0
<i>Gastrocnemius</i>			
<i>No Cold</i> (NCE)	(4)	(3)	(3)
Mass (g)	1.89 ± 0.05 ^a	1.66 ± 0.03 ^b	1.30 ± 0.11 ^c
CS Activity			
$\mu\text{mol} \cdot (\text{min} \cdot \text{g muscle})^{-1}$	12.7 ± 1.6 ^{ab}	14.6 ± 0.9 ^b	10.2 ± 1.3 ^a
$\mu\text{mol} \cdot (\text{min} \cdot \mu\text{g protein})^{-1}$	127.0 ± 17.0	124.0 ± 7.0	108.0 ± 14.0
<i>Cold</i> (CE)	(9)	(9)	(7)
Mass(g)	1.73 ± 0.03 ^a	1.72 ± 0.04 ^a	1.34 ± 0.03 ^b
CS Activity			
$\mu\text{mol} \cdot (\text{min} \cdot \text{g muscle})^{-1}$	13.8 ± 0.5 ^{ab}	14.8 ± 0.8 ^b	12.4 ± 0.7 ^a
$\mu\text{mol} \cdot (\text{min} \cdot \mu\text{g protein})^{-1}$	128.3 ± 7.7	140.2 ± 7.5	129.0 ± 6.0

Values are means ± SE. Within a row, values not sharing common letter superscripts are significantly different ($p \leq 0.05$). Numbers in parenthesis = number of rats in groups; NCE = non-cold-exposed; CE = cold exposed. Muscle weights differ from Table 1 because not all animals were included in CS assay.