Published in final edited form as:

Am J Physiol. 1994 March ; 266(3 Pt 2): R749–R755.

Adrenergic stimulated skeletal muscle glycogenolysis in perfused hindlimbs of young and old male Fischer 344 rats

Lisa M. Larkin, Barbara A. Horwitz, Kristen C. Eiffert, and Roger B. McDonald

Departments of Nutrition and Animal Physiology, University of California, Davis, California 95616

Abstract

Epinephrine (Epi)- and forskolin (FSK)-stimulated glycogenolysis of skeletal muscle was evaluated in perfused hindlimb isolated from male Fischer 344 (F344) rats, ages 6, 12, and 26 mo. Muscle glycogen stores were reduced by sciatic nerve stimulation and replenished by infusing 10 mM glucose, 500 μ U insulin, and 5 μ Ci [14 C]glucose via a left carotid artery cannula. Then the hindlimb was perfused with a modified Krebs-Henseleit buffer (pH 7.4). At *minute 20* of the perfusion, Epi [O.O (perfusate), 0.25, 0.50, or 0.75 μ M] or 40 μ M FSK were infused for 10 min. Radioactivity (14 C) in the effluent perfusate was collected every 60 s during a 20-min preinfusion, a 10-min Epi infusion, and a 20-min postinfusion period and was used to determine the rate of muscle glycogen utilization. Total 14 C release increased with Epi and 40 μ M FSK. However, the pattern of release did not differ significantly with age. In general, the fraction of the perfusate released as 14 CO₂ increased in the presence of FSK and Epi but did not significantly differ with age. [14 C]lactate released in response to Epi increased in the 6-mo-old group, remained unchanged in the 12-mo-old group, and decreased in the 26-mo-old group compared with 0.0 Epi (perfusate) values. It appears that stimulation of skeletal muscle glycogenolysis via adrenergic receptor or postreceptor/adenosine 3',5'-cyclic monophosphate-mediated mechanisms is unaffected by age. However, the utilization of carbohydrate by isolated hindlimb muscle is altered in the aging rat, resulting in a more oxidative metabolism.

Keywords

aging; epinephrine; forskolin; glycogen

Previous investigations suggested that the ability to maintain homeostasis during metabolically stressful situations declines with age (2,4,12,14,27). For example, McDonald et al. (14) observed hypothermia and lower heat production in 26-mo-old male Fischer 344 (F344) rats exposed to acute cold exposure compared with similarly exposed younger rats. Enhancing muscle metabolism with a prior 6-mo exercise regime (23) or with injections of aminophylline (18,34) alleviated the drop in core body temperature normally observed in aged cold-exposed sedentary rats. These and other studies led several investigators to suggest that the failure to maintain homeothermy during cold stress may be partially explained by diminished thermogenic contributions from skeletal muscle (18,22,34).

Such a diminution could reflect a number of factors. Results from our laboratory suggest that blood flow to skeletal muscle does not differ among the age groups during acute cold exposure (21), that availability of serum substrates (glucose and lactate) is not lower in aged 26- vs. 6- or 12-mo-old rats (15), and that insulin-stimulated glucose uptake does not differ between 6-

and 26-mo-old F344 male rats (24). Taken together, these observations strongly suggest that substrate delivery, availability, and/or uptake do not limit skeletal muscle metabolism in the aging animal. However, we did observe increased serum lactate levels concomitant with diminished glycogen stores in the 26- vs. the 6-mo-old rats exposed to 5°C for 4 h (15). We thus postulated that acutely cold-exposed 26-mo-old male F344 rats metabolize available carbohydrate via nonoxidative pathways rather than oxidative pathways to a greater degree than do younger rats, and that incomplete oxidation of carbohydrate (as indicated by increased serum lactate) contributes to muscle's attenuated thermogenesis (15).

In this study, we have further examined the possibility of greater nonoxidative glucose metabolism in skeletal muscle of older rats by evaluating the effects of epinephrine (Epi) on skeletal muscle glycogenolysis in the isolated hindlimb of aged male F344 rats. In addition, the effects of forskolin (FSK), which stimulates glycogenolysis via postreceptor-mediated stimulation of adenylate cyclase, was used to assess receptor- vs. postreceptor-mediated stimulation of glycogenolysis in an isolated hindlimb preparation.

METHODS

Animals and animal care

Ninety male inbred (F344) rats, age 6, 12, and 26 mo, were obtained from the National Institute on Aging's animal colony maintained by Harlan Sprague-Dawley Laboratory (Indianapolis, IN). Rats were acclimated to our colony conditions, i.e., light cycle and temperature, for 2 wk before experimentation. Rats were housed individually in hanging wire bottom cages (20 × 25 × 18 cm) and kept on a 12:12-h light-dark cycle (lights on at 0600, off at 1800) at a temperature of 25°C. The rats were fed NIH-31 laboratory diet (Teklad Research Diets, Indianapolis, IN) and distilled water, ad libitum.

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. Internal tumors were observed on several of the 26-mo-old rats; however, no rats were excluded from the study.

Materials

FSK (initially dissolved in 1 ml 95% ethanol; ethanol < 0.1% final solution) and dextran (industrial grade, 60,000–90,000 g mol wt) were obtained from Sigma, St. Louis, MO. L-(–)-Epinephrine-(+)-bitartrate and [U-¹⁴C]-D-glucose (99.5% purity; 250–360 µCi/mmol) were purchased from ICN Biomedicals, Irvine, CA; heparin (1,000 units/ml) was obtained from Elkin-Sinn, Cherry Hill, NJ.

Surgical preparation

On the day of the experiment, food was removed from the rats at 0800, at least 4 h before surgery. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (65 mg/kg), and a cannula was placed in the left carotid artery. Both sciatic nerves were isolated and stimulated for 4 × 2.5 min at 4 Hz, 40 V, and 6 ms duration with 2-min rest periods between stimulations (Grass Instruments, Quincy, MA; model SD5 stimulator). A pilot study indicated that this procedure decreased the glycogen content of the hindlimb muscles by an average of 40%. After the last stimulation period, 1 ml of a solution containing 10 mM glucose, 500 µU insulin, and 5 µCi [U-¹⁴C]-D-glucose was infused through the carotid cannula at a rate of 1 ml/min. Then the hindlimb was surgically isolated for nonrecirculating perfusion utilizing a modification of the methods described by Rudderman et al. (29). Briefly, all vessels leaving the aorta or returning to the vena cava from areas other than the hindlimb skeletal muscles, including skin, abdominal muscles, bladder, reproductive organs, tail, kidney, and ilio-lumbar

muscles were double ligated to ensure complete isolation. Heparin (0.2 ml, 1,000 unit/ml) was injected into the portal vein, and the portal vein, celiac artery, and superior mesenteric artery were ligated. This procedure took ~ 45 min, allowing enough time for maximal reincorporation of glycogen stores. Our pilot data indicated that in 12-mo-old rats, reincorporation of 100–110% of prestimulation glycogen concentrations was accomplished within 30 min postinfusion. The descending aorta and vena cava were isolated and cannulated, and the nonrecirculating perfusion was started at a rate of 10 ml/min with a red blood cell (RBC)-free perfusate containing (in mM) 115 NaCl, 1.2 MgCl₂, 1.2 Na₂SO₄, 1.2 Na₂PO₄, 5.9 KCl, 2.5 CaCl₂, 25 NaHCO₃, and 6% dextran, warmed to 37°C and saturated with 95% O₂-5% CO₂. Rats were killed by pneumothorax, and a temperature sensor (Dig-Sense temperature controller, Cole-Parmer, Chicago, IL) was inserted intramuscularly and connected to a heat lamp to maintain the muscle at 37°C. Vascular pressure was monitored throughout the perfusion with a Cole-Parmer (Chicago, IL) pressure transducer.

Experimental protocol

Both hindlimbs were perfused for 20 min to allow for the clearance of unincorporated [¹⁴C] glucose and RBCs. Weights of perfused tissue were calculated as one-sixth of the total body weight of rats (29): 6 mo, 65.3 ± 3.4 g; 12 mo, 73.7 ± 6.1 g; 26 mo, 68.2 ± 6.9 g, and did not significantly differ among the age groups. Maximum clearance was defined as the time point at which steady-state concentrations of total ¹⁴C in the perfusate had occurred. This time point was determined in a separate group of animals (5 per age group) and occurred consistently between 15 and 18 min of perfusion. After the 20-min [¹⁴C]glucose washout period, the soleus (SOL), gastrocnemius (GAS), and the extensor digitorum longus (EDL) muscles of the left leg were quickly excised, frozen between tungsten clamps cooled in liquid nitrogen, and stored at -70°C for subsequent analysis of [¹⁴C]glycogen content, creatine phosphate, protein, and water. The left femoral artery and vein were ligated and perfusion flow rate to the right hindlimb was decreased to 5 ml/min. The next 10 min of the perfusion consisted of an additional infusion (0.50 ml/min) via a model 22 Harvard infusion apparatus (Harvard Apparatus, South Natick, MA) of 0.0 (perfusate), 0.25, 0.50, or 0.75 μM Epi or 40 μM FSK. The Epi concentrations were selected such that they would stimulate ¹⁴C release into the effluent perfusate without altering vascular pressure. Vascular pressure was: 6 mo, 61.5 ± 0.3 mmHg; 12 mo, 61.0 ± 0.3 mmHg; and 26 mo, 61.3 ± 0.3 mmHg and did not differ significantly among the age groups or during the 50-min perfusion. The 10-min infusion of Epi or FSK was followed by an additional 20-min period in which no drug was infused. At the end of the 50-min perfusion period, the SOL, GAS, and the EDL of the right leg were removed as described above. Effluent perfusate was collected every 60 s for the entire 50-min perfusion and immediately analyzed for ¹⁴CO₂. An additional aliquot was frozen at -70°C for analysis of ¹⁴C-labeled lactate, alanine, and glucose via ion-exchange chromatography (25). The percent of ¹⁴C substrates recovered from the ion-exchange columns ranged from 75–100%. Values of PO₂, PCO₂, and pH of the effluent perfusate were also measured (using an IL blood gas analyzer, model 1304). An additional five animals per age group were perfused for the 50-min period without Epi or FSK infusion and served as a baseline control group (Table 1).

Analytic procedures

Effluent perfusate was analyzed for total ¹⁴C content via β-scintillation counting; skeletal muscle was monitored for ¹⁴C-glycogen content by methods developed in our laboratory. Briefly, muscles were dissolved in 30% KOH, ethanol was added to precipitate the glycogen, the precipitate was resuspended in deionized water, and aliquots of the resulting suspension were analyzed for [¹⁴C]glycogen activity using a β-scintillation counter. Due to inadequate mass of the SOL and EDL muscles, only the GAS muscle was used for determination of water, creatine phosphate, and ATP concentration. Creatine phosphate and ATP concentrations were analyzed via a method described by Heinz and Weiber (11), protein content using a Bradford

protein assay kit from Bio-Rad (Richmond, CA), and water content via pre- vs. postlyophilization weights of gastrocnemius muscle samples. $^{14}\text{CO}_2$ in the effluent perfusate was measured by injecting 0.5 ml of perfusate into an airtight tube containing 0.5 ml of 2 N HCL in the bottom and a hanging center well filled with 0.2 ml of concentrated hyamine hydroxide. These tubes were then incubated for 12 h at 37°C releasing $^{14}\text{CO}_2$ from the perfusate, and capturing the $^{14}\text{CO}_2$ in the hanging center well. The center wells were then analyzed for $^{14}\text{CO}_2$ activity using a β -scintillation counter. In a separate group of 6- and 26-mo-old rats ($n = 5$ per age group) muscles were harvested for determination of fiber type. Before dissection, animals were anesthetized with pentobarbital sodium. The skin was removed from the hindlimb, and the SOL, EDL, and GAS muscles were removed. These muscles were chosen because they represent the three broad categories of muscle type: slow oxidative, SOL; fast glycolytic, EDL; and mixed, GAS. Sections of the muscle were mounted on cork for histology sectioning and frozen in isopentane cooled in liquid N_2 . Samples were stored at -70°C until histochemical analysis.

Serial cross sections, 10 μm thick, were placed on glass slides and dried at room temperature before staining. Percent fiber type was determined after staining the sections for adenosinetriphosphatase activity according to the methods of Armstrong and Phelps (1) and Dubowitz and Brooke (6).

Statistics

We used analysis of variance (ANOVA) with a factorial design [age and drug (Epi or FSK) as main effects] to evaluate data. When a significant main effect was found, Fisher's least significant difference post hoc test was used to determine differences between the groups. Differences were considered significant at $P \leq 0.05$.

RESULTS

Integrity of the hindlimb perfusion preparation

Although there was no effect of age on percent water in the gastrocnemius muscle, there was an ~3% increase in water content in the gastrocnemius muscle after 20 min of perfusion (Table 1). This increase was comparable in all age groups and did not significantly change after another 30 min of perfusion. Age of subject or length of perfusion did not significantly affect creatine phosphate or ATP content. The pH of the effluent perfusate ranged from an initial value of 7.4 to a final value of ~7.3 and was independent of drug infusion. The decrease in pH did not differ significantly with age. Protein (mg/g tissue) was not significantly affected by length of the experiment. Protein content of the hindlimb muscle declined significantly with age (Table 1).

Muscle fiber population

The fiber type distribution in SOL and EDL muscle of young 6- and old 26-mo-old rats did not significantly differ (Table 2). In contrast, the percent type I (slow oxidative) fibers increased and type II (fast glycolytic) decreased significantly in the old vs. young GAS muscle.

Oxygen consumption

Effluent perfusate gases were monitored, and O_2 consumption ($\text{ml} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$) was calculated from measures of PO_2 . Values of PO_2 ranged from 500 Torr in the influent perfusate to 100–200 Torr in the effluent perfusate. Calculated baseline values of hindlimb O_2 consumption (in the absence of Epi and FSK) were 4.81 ± 0.01 (6 mo), 5.12 ± 0.06 (12 mo), and 5.08 ± 0.09 $\text{ml} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ (26 mo) and did not differ. Age significantly affected the Epi-induced increase in O_2 consumption ($P < 0.05$; Fig. 1), with greater increase occurring in the 26- vs. the 6- and

12-mo-old groups at the 0.50 and 0.75 μM dose. Although FSK-induced O_2 consumption increased significantly from baseline values, this increase was not affected by age.

Composition of effluent perfusate

There was no effect of age on total ^{14}C (disintegrations/min) released in response to Epi or to 40 μM FSK infusion (Fig. 2). There was a significant main effect of the drug on total ^{14}C released during the 10-min infusion period ($P < 0.0001$). That is, total ^{14}C released was greater in the presence than the absence of the drug; there was no difference in response among the individual doses of Epi (0.25 to 0.75 μM).

There was a significant effect of drug on total $^{14}\text{CO}_2$ released (Fig. 3). This reflected the generally greater $^{14}\text{CO}_2$ released in the 6- and 26-mo-old rats. However, there was no effect of age on $^{14}\text{CO}_2$ released into the effluent perfusate.

In contrast to total ^{14}C and $^{14}\text{CO}_2$, there was a significant effect of age on ^{14}C released as lactate ($P < 0.0001$; Fig. 4). This reflected the generally greater lactate production observed in the 6- vs. 12- and 26-mo-old rats. The pattern of lactate release among the age groups during FSK infusion was similar to that observed during Epi infusion.

There was no significant effect of age or drug on release of ^{14}C -amino acids or ^{14}C -glucose, which remained at ~ 10 and 20%, respectively, throughout the 10-min infusion period, regardless of infused drug concentration (Table 3).

$[^{14}\text{C}]$ glycogen concentration

Baseline (0 drug) preperfusion concentrations (left leg, after 20-min washout period) of muscle $[^{14}\text{C}]$ glycogen were always greater than postinfusion concentrations (right leg, after 50 min perfusion period; Fig. 5A). For ease of reading only the data from the SOL muscle of the 6-mo-old animals are presented. The pattern of response of the other age groups and muscle types was similar. Drug infusion (Epi and FSK) did not significantly affect the $[^{14}\text{C}]$ glycogen concentration (Fig. 5A). However, there was a significant effect of muscle type on the initial (left leg, no drug infusion) $[^{14}\text{C}]$ glycogen content. That is, the amount of glycogen reincorporation into the fast glycolytic fibers in the EDL muscle was greater than that of the mixed fiber type in the GAS, which in turn was greater than that of the slow-oxidative in the SOL (EDL > GAS > SOL; Fig. 5B). In addition, there was a significant interaction between age and muscle type on initial $[^{14}\text{C}]$ glycogen content. In SOL (slow oxidative) and GAS (mixed) muscle, there was no significant effect of age on initial $[^{14}\text{C}]$ glycogen content; in the EDL (fast glycolytic) muscle, initial $[^{14}\text{C}]$ glycogen content was lower with increasing age.

DISCUSSION

Non-RBC perfusates have been used by previous investigators to study the metabolism of skeletal muscle (32,33). Because using a non-RBC perfusate requires assurance that appropriate muscle integrity and/or oxygenation is maintained, we monitored several variables throughout the entire perfusion period to make certain that muscle integrity remained intact (Table 1). These variables, which included water content, creatine phosphate, ATP, and protein, were largely unchanged during the perfusion and are consistent with the literature in which RBC perfusate preparations are used (3,29,30). In fact, percent water was increased by only 3% during the 50-min perfusion, consistent with changes observed in RBC perfusate preparations (7,26) and less than those observed by others in non-RBC perfusate preparations (32). Together, these data indicate that the hindlimb was viable and retained the ability to metabolize substrate via oxidation (Fig. 1).

Our observed rates of O₂ consumption were lower than those of Ruderman et al. (30), reflecting our proportionately lower flow rates (30). Ruderman et al. have shown that rates of oxygen consumption increased with increasing flow rates, with the former reaching a maximum plateau at a flow rate of ~ 40 ml/min. In pilot studies, we found severe edema and detected lactate dehydrogenase (LDH; used as a measure of cell integrity) in effluent perfusate at flow rates > 10 ml/min. Therefore, we decided to use the lower rate of 5 ml/min. This protocol resulted in minimal edema, no detection of LDH, and adequate O₂ delivery and muscle integrity (Table 1, Fig. 1).

Our data indicate that the diminished metabolic response observed in aged male F344 rats during cold exposure is not associated with alterations in skeletal muscle's response to adrenergic stimulation. This conclusion is based on the observation that the concentration of total ¹⁴C substrate released during Epi (receptor mediated) and/or FSK (postreceptor mediated) stimulation of glycogenolysis did not significantly differ with age. Thus the ability of the adrenergic receptor to respond to stimulation and to transduce the stimulatory signal to intracellular messengers is unaffected by age. The literature has conflicting data on this point. For example, Elfellah et al. (8) found no significant differences in β-adrenergic receptor affinity or density in the rectus abdominus muscles of young vs. old humans. In contrast, Scarpace (31) observed an age-related decrease in the activation of β-adrenergic receptors in the rat myocardium. One possibility is that age-related alterations in β-adrenergic receptor function are tissue specific. Our data suggest that skeletal muscle β-adrenergic receptor function is not significantly affected by senescence.

Even though the rate of Epi-stimulated skeletal muscle glycogenolysis did not change with aging, as indicated by the lack of significant differences in total ¹⁴C substrates released, the ability of the aged skeletal muscle to synthesize glycogen does appear to be altered with age. Although the pattern of [¹⁴C]glycogen reincorporation among the age groups was as expected (i.e., greater stores in glycolytic compared with oxidative muscle), the glycogen content was significantly lower in 26- vs. 6- and 12-mo-old animals. These data are consistent with those of Dall'Aglia et al. (5) who observed an age-related decline in glycogen synthase activity in 24- vs. 12-mo-old Sprague-Dawley rats. It is possible, therefore, that attenuated metabolic responses of the aged animal to long-term exposure to a stressor, such as cold exposure, may reflect a more rapid depletion of glycogen stores. This hypothesis is supported by our previous data that describe significantly greater depletion of glycogen stores in the liver and GAS muscle of 26- vs. 6-mo-old male F344 rats during 4 h of acute cold exposure (15).

In addition to the observed decline in reincorporated [¹⁴C]glycogen stores, we also observed a decline in metabolically active tissue (skeletal muscle protein) in the older 26-mo-old rats (Table 1). One would expect with this decrease in metabolically active tissue that Epi-stimulated O₂ consumption would also be attenuated. However, this was not the case. Several possible explanations exist: 1) 26-mo-old rats have greater response to Epi stimulation than do the 6- and 12-mo-old animals (not supported by total ¹⁴C release data) and/or 2) the older rats have an age-related decline in type II (glycolytic) fibers and a preservation of type I (oxidative fibers) (9,16,17,19). This suggestion is supported in part by our data, which show an increase in type I fibers of the GAS muscle. An age-related decline in glycolytic fibers could force the older animal to increase usage of oxidative fibers, thereby oxidizing glucose more completely. Thus for the same amount of glycogen the old rats would exhibit greater rates of O₂ consumption.

Changes in muscle fiber composition could also explain the observed differences in the metabolic end products released into the effluent perfusate in response to adrenergic stimulation. For example, we find that the older rats released less [¹⁴C]lactate into the perfusate

in response to Epi stimulation than did the younger animals. These data also support the idea of a shift to a more oxidative fiber type.

Glycogen concentrations in the baseline (no Epi) group were not significantly different from the Epi- and FSK-stimulated groups. However, the increased ^{14}C substrate released during the 10-min infusion of drug indicates that the muscle was responding to stimulation, and since there was no substrate available in the perfusate, the baseline (no Epi) group would need to use some of its glycogen stores to maintain viability. The absence of such a decline suggests that the Epi/FSK-stimulated muscles may be retaining or removing lactate from perfusate and reincorporating it into glycogen stores to conserve potential energy. Further studies are needed to evaluate the possibility of differential substrate cycling in the skeletal muscle of aged rats compared with younger age groups.

We also observed differences between the response of our whole animal (15) and our hindlimb isolation data with respect to oxidative pathways. The blunted Epi-induced lactate production and the higher Epi-induced O_2 consumption seen in the hindlimb preparations of the 26- vs. the 6- and 12-mo-old groups suggests that these animals are metabolizing their glycogen stores via oxidative rather than nonoxidative glycolytic pathways. These data are in contrast to our whole animal work where we observed higher lactate production in the cold-exposed 26- vs. 6- and 12-mo-old animals. Several possibilities exist that could explain these differences observed in whole animal vs. isolated hindlimb. First, in the hindlimb isolation procedure the levels of Epi seen by the adrenergic receptors are comparable in all age groups. In the intact animal, cold exposure might elevate circulating Epi to a greater extent in the aged rat, leading to increased stimulation of skeletal muscle glycogenolysis and a greater amount of lactate generated. Several investigators have, in fact, suggested increased sympathetic tone in the resting (10,28) and metabolically stressed (20) older vs. younger animals.

Another possible explanation for the differences observed in lactate production in whole animal vs. hindlimb perfusion preparations is an age-related decrease in the conversion of lactate to glucose by the aging liver. Such a decrease would result in elevated serum lactate levels that could be interpreted as increased anaerobic glycolytic metabolism in aging skeletal muscle, when in fact the aging muscle is in an oxidative state with minimal lactate production. Khandelwal et al. (13) have observed reduced activities of liver glycogen synthase and phosphorylase in aged male Sprague-Dawley rats, suggesting decreased turnover of glycogen in liver during aging.

In conclusion, the age-related decrease in metabolism in response to environmental stressors cannot be explained by alterations in skeletal muscle glycogenolysis in response to adrenergic stimulation. Moreover, because we observed lower lactate production and greater O_2 consumption in the aged vs. younger skeletal muscle during stimulation with Epi, we suggest that aging skeletal muscle may utilize available substrate via more oxidative pathways than younger skeletal muscle during metabolically stressful situations.

Acknowledgments

We thank Denise Bilbaeno, Thu Huan, Anthony Jackson, Carol Murtagh, Mike Pagliassotti, Rod Ruhe, and Hanson Quan for their technical assistance.

This research was supported in part by National Institute on Aging Grants AG-06665 and AG-00495.

REFERENCES

1. Armstrong RB, Phelps RO. Muscle fiber type composition of the rat hindlimb. *Am. J. Anat* 1984;171:259–272. [PubMed: 6517030]

2. Baker GT, Sprott RL. Biomarkers of aging. *Exp. Gerontol* 1988;23:223–239. [PubMed: 3058488]
3. Berger M, Hagg S, Ruderman NB. Glucose metabolism in perfused skeletal muscle: interaction of insulin and exercise on glucose uptake. *Biochem J* 1975;146:231–238. [PubMed: 807202]
4. Collins KJ, Dore C, Exton-Smith AN, Fox RH, MacDonald IC, Woodward PM. Accidental hypothermia and impaired temperature homeostasis in the elderly. *Br. Med. J* 1977;1:353–356. [PubMed: 837095]
5. Dall'Aglia E, Chang H, Reaven GM, Azhar S. Age-related changes in rat muscle glycogen synthase activity. *J. Gerontol* 1987;42:168–172. [PubMed: 3102591]
6. Dubowitz, V.; Brooke, MH. *Muscle Biopsy: A Modern Approach*. Philadelphia, PA: Saunders; 1973. p. 20-33.
7. Eifert KC, Stern JS, Horwitz BA, Larkin LM, McDonald RB. Effect of age and high sucrose of 2-deoxyglucose uptake in perfused hindlimb. *FASEB J* 1991;5:A1298.
8. Elfellah MS, Dalling R, Kantola IM, Reid JL. Beta-adrenoceptors and human skeletal muscle characterization of receptor subtype and effect of age. *Br. J. Clin. Pharmacol* 1989;27:31–38. [PubMed: 2565118]
9. Essen-Gustavsson B, Borges O. Histochemical and metabolic characteristics of human skeletal muscle in relation to age. *Acta Physiol. Scand* 1986;126:107–114. [PubMed: 3953298]
10. Halter, JB.; Pfeifer, MA. Reff, ME.; Schneider, EL., editors. *Biological Markers of Aging*. Bethesda, MD: US Dept. of Health and Human Services; 1982. Aging and autonomic nervous system function in man. p. 168-176. (NIH Publication No. 82–2221)
11. Heinz, F.; Weiber, H. Creatine phosphate. In: Bergmeyer, HU., editor. *Methods of Enzymatic Analysis*. New York: Academic; 1985. p. 507-513.
12. Iwase S, Mano T, Watanabe T, Saito M, Kobayashi F. Age-related changes of sympathetic outflow to muscles in humans. *J. Gerontol* 1991;46:1–5.
13. Khandelwal RL, Enno TL, Narayanan N. Effects of age on glycogen synthase and phosphorylase activities in rat liver. *Mech. Ageing. Dev* 1984;28:13–22. [PubMed: 6439952]
14. Kirkwood, TBL.; Holliday, R. *Principles of Mammalian Aging*. Englewood Cliffs, NJ: Prentice Hall; 1979.
15. Larkin LM, Horwitz BA, McDonald RB. Effect of cold on serum substrate and glycogen concentration in young and old Fischer 344 rats. *Exp. Gerontol* 1992;179–190. [PubMed: 1521593]
16. Larsson L. Histochemical characteristics of human skeletal muscle during aging. *Acta Physiol. Scand* 1983;117:469–471. [PubMed: 6880808]
17. Larsson L, Sjödin B, Karlsson J. Histochemical and biochemical changes in human skeletal muscle with age in sedentary males, age 22–65 years. *Acta Physiol. Scand* 1978;103:31–39. [PubMed: 208350]
18. Lee TF, Wang LCH. Improving cold tolerance in elderly rats by aminophylline. *Life Sci* 1985;36:2025–2032. [PubMed: 3999913]
19. Lexell J, Henriksson-Larsén K, Winblad B, Sjöström M. Distribution of different fibre types in human skeletal muscles: effects of aging studied in whole muscle cross sections. *Muscle Nerve* 1983;6:588–595. [PubMed: 6646161]
20. McCarty R. Aged rats: diminished sympathetic-adrenal medullary responses to acute stress. *Behav. Neural Biol* 1981;33:204–212. [PubMed: 7305814]
21. McDonald RB, Hamilton JS, Stern JS, Horwitz BA. Regional blood flow of exercise-trained younger and older cold-exposed rats. *Am. J. Physiol* 1989;256:R1069–R1075. [PubMed: 2719150] (Regulatory Integrative Comp. Physiol. 25)
22. McDonald RB, Horwitz BA, Stern JS. Cold-induced thermogenesis in younger and older Fischer 344 rats following exercise training. *Am. J. Physiol* 1988;254:R908–R916. [PubMed: 3381916] (Regulatory Integrative Comp. Physiol. 23)
23. McDonald RB, Stern JS, Horwitz BA. Thermogenic responses of younger and older rats to cold exposure: comparison of two strains. *J. Gerontol* 1989;44:37–42. [PubMed: 2553792]
24. Murtagh CM, Horowitz BA, McDonald RB. Effect of age and cold exposure on glucose uptake in brown adipose tissue. *FASEB J* 1992;48:A2033.

25. Pagliassotti MJ, Donovan CM. Influence of cell heterogeneity on skeletal muscle lactate kinetics. *Am. J. Physiol* 1990;258:E625–E634. [PubMed: 2185646](Endocrinol. Metab. 21)
26. Pagliassotti MJ, Donovan CM. Role of cell type in net lactate removal by skeletal muscle. *Am. J. Physiol* 1990;258:E635–E642. [PubMed: 2110420](Endocrinol. Metab. 21)
27. Robbins AS. Hypothermia and heat stroke: protecting the elderly patient. *Geriatrics* 1989;44:73–80. [PubMed: 2910771]
28. Rowe JW, Troen BR. Sympathetic nervous system and aging in man. *Endocr. Rev* 1980;1:167–178. [PubMed: 6263602]
29. Ruderman NB, Houghton CRS, Hems R. Evaluation of the isolated perfused rat hindquarter for the study of muscle metabolism. *Biochem. J* 1971;124:639–651. [PubMed: 5135248]
30. Ruderman NB, Kemmer FW, Goodman MN, Berger M. Oxygen consumption in perfused skeletal muscle: effect of perfusion with aged, fresh and aged-rejuvenated erythrocytes on oxygen consumption, tissue metabolites and inhibition of glucose utilization by acetoacetate. *Biochem. J* 1980;190:57–64. [PubMed: 7447936]
31. Scarpace PJ. Forskolin activation of adenylate cyclase in rat myocardium with age: effects of guanine nucleotide analogs. *Mech. Ageing Dev* 1990;52:169–178. [PubMed: 2325432]
32. Shiota M, Sugano T. Characteristics of rat hindlimbs perfused with erythrocyte- and albumin-free medium. *Am. J. Physiol* 1986;251:C78–C84. [PubMed: 3524256](Cell Physiol. 20)
33. Stohfeldt P, Kettl H, Weinges KF. Perfusion of the isolated rat hindlimb with a synthetic medium. *Horm. Metab. Res* 1974;6:167–168. [PubMed: 4828278]
34. Wang LCH, Joudan ML, Lee TF. Mechanisms underlying the supra-maximal thermogenesis elicited by aminophylline in rats. *Life Sci* 1989;44:927–934. [PubMed: 2927250]

Fig. 1.

Change in hindlimb O₂ consumption from baseline values in response to 10-min infusion of epinephrine (Epi) or forskolin (FSK, 40 μM) of 6- (solid bars), 12- (gray bars), and 26-mo-old (hatched bars) rats [4.81 ± 0.01 , 5.12 ± 0.06 , and 5.08 ± 0.09 ml·g⁻¹·h⁻¹, respectively]. Values are means $\pm$ SE. There was a significant effect of age on O₂ consumption ($P < 0.05$).

*Significantly different in 6- and 12-mo-old animals ($P < 0.05$). **Significantly different from 6-mo-old animals ($P < 0.05$).

Fig. 2. Total ^{14}C (disintegrations/min; dpm) released during a 10-min infusion of Epi or FSK ($40\ \mu\text{M}$) of 6- (solid bars), 12- (gray bars), and 26-mo-old (hatched bars) rats. Values are means $\pm$ SE. There was a significant effect of Epi concentration on total ^{14}C released ($P < 0.0001$).

Fig. 3.
¹⁴CO₂ (dpm) released during a 10-min infusion of Epi or FSK (40 μM) of 6- (solid bars), 12- (gray bars), and 26-mo-old (hatched bars) rats. Values are means ± SE. There was a significant effect of Epi concentration on total ¹⁴C released ($P < 0.05$).

Fig. 4. [^{14}C]lactate (dpm) released during a 10-min infusion of Epi or FSK (40 μM) of 6- (solid bars), 12- (gray bars), and 26-mo-old (hatched bars) rats. Values are means $\pm$ SE. There was a significant effect of age and Epi concentration on total ^{14}C released ($P < 0.0001$ and $P < 0.05$, respectively).

Fig. 5.

A: [^{14}C]glycogen concentration (100 dpm/g muscle) in SOL muscle immediately before (solid bars) and 20 min after (hatched bars) a 10-min infusion of Epi or FSK (40 mM) in hindlimb preparations from 6-mo-old rats. B: SOL, GAS, and EDL muscle immediately before the 20-min drug infusion in 6- (solid bars), 12- (gray bars), and 26-mo-old (hatched bars) rats. Values are means $\pm$ SE. Values sharing a common lower case superscript letter do not significantly differ ($P < 0.5$).

Table 1

Water, creatine phosphate, ATP, and protein concentration in the gastrocnemius muscles in perfused hindlimbs of male F344 rats at 0, 20, and 50 min of perfusion and with no infusion of Epi or FSK

	Age, mo		
	6	12	26
Edema, % Water			
<i>minute 0</i>	73.3 ± 0.1 ^a	73.3 ± 0.1 ^a	72.4 ± 0.1 ^a
<i>minute 20</i>	77.0 ± 0.3 ^b	76.4 ± 0.5 ^b	76.0 ± 0.8 ^b
<i>minute 50</i>	77.4 ± 0.2 ^b	77.7 ± 0.2 ^b	77.3 ± 0.3 ^b
Creatine phosphate, μmol/g tissue			
<i>minute 0</i>	4.28 ± 0.26	4.04 ± 0.48	4.65 ± 0.26
<i>minute 20</i>	5.65 ± 0.36	6.20 ± 0.35	5.21 ± 0.45
<i>minute 50</i>	5.66 ± 0.26	6.19 ± 0.63	5.44 ± 0.43
ATP, μmol/g tissue			
<i>minute 0</i>	5.75 ± 0.19	5.48 ± 0.13	5.22 ± 0.17
<i>minute 20</i>	4.35 ± 0.35	4.33 ± 0.25	3.90 ± 0.23
<i>minute 50</i>	4.46 ± 0.35	4.41 ± 0.49	3.57 ± 0.21
Protein, mg/g tissue			
<i>minute 20</i>	175.4 ± 12.1 ^A	80.4 ± 3.0 ^B	65.6 ± 10.8 ^B
<i>minute 50</i>	181.2 ± 11.8 ^A	103.9 ± 3.1 ^B	81.4 ± 9.9 ^B

Values are means ± SE; *n* = 5 rats/age group and time. Epi, epinephrine; FSK, forskolin. *Minute 0* values (*n* = 5/age) were determined in a different group of male Fischer 344 (F344) rats, then values for the 20- and 50-min time points. Protein values were not analyzed in the *minute 0* group. Within an age group, values with no lower case superscripts or those sharing a common lower case letter superscript do not significantly differ (*P* < 0.05). At a given time point, values with no upper case letter superscripts or those sharing a common upper case letter superscript are not significantly different (*P* < 0.05).

Table 2

Mean fiber population (percent) of soleus, extensor digitorum longus, and gastrocnemius muscle isolated from young (6 mo) and old (26 mo) F344 rats

	Age, mo	
	6	26
Soleus		
Type I (slow oxidative)	86.0 ± 1.0	85.8 ± 1.3
Type II (fast glycolytic)	14.0 ± 1.0	14.2 ± 1.3
Extensor Digitorum Longus		
Type I (slow oxidative)	5.7 ± 0.5	3.4 ± 0.3
Type II (fast glycolytic)	94.3 ± 0.5	96.5 ± 0.3
Gastrocnemius		
Type I (slow oxidative)	16.8 ± 2.2	23.8 ± 3.0 *
Type II (fast glycolytic)	83.2 ± 2.2	76.2 ± 3.0 *

Values are means ± SE for 5 rats per group.

* Significantly different from young animals.

Table 3

¹⁴C-labeled amino acid and glucose appearance in perfusate in response to 10-min infusion of Epi or FSK in male F344 rats

	Age mo		
	6	12	26
Amino acid, disintegrations/min			
Epinephrine, μ M			
0	9,753 $\pm$ 2,428	14,755 $\pm$ 2,092	15,482 $\pm$ 10561 ^a
0.25	10,676 $\pm$ 1,007 ^A	15,299 $\pm$ 1,927 ^A	20,048 $\pm$ 4,137 ^{bB}
0.50	12,595 $\pm$ 2,731	17,889 $\pm$ 3,235	12,183 $\pm$ 1,361 ^a
0.75	16,924 $\pm$ 2,217 ^A	9,143 $\pm$ 700.6 ^B	14,748 $\pm$ 3033 ^{aA}
Forskolin (40 μ M)	15,892 $\pm$ 2,650	12,065 $\pm$ 2,446	13,271 $\pm$ 2,162 ^a
Glucose, disintegrations/min			
Epinephrine, μ M			
0	20,846 $\pm$ 5,135 ^a	25,474 $\pm$ 1,580	25,161 $\pm$ 4,479
0.25	38,215 $\pm$ 4,305 ^b	21,713 $\pm$ 2,211	23,054 $\pm$ 1,431
0.50	36,520 $\pm$ 2,260 ^{bA}	24,528 $\pm$ 2,536 ^B	24,017 $\pm$ 3,067 ^B
0.75	36,573 $\pm$ 4,922 ^b	26,912 $\pm$ 3,743	25,030 $\pm$ 1,937
Forskolin (40 μ M)	25,900 $\pm$ 1,887 ^{aA}	19,794 $\pm$ 2,991 ^B	17,367 $\pm$ 703 ^B

Values are means $\pm$ SE; $N = 4-8$ /group. At a given Epi concentration, values not sharing common upper case letter superscripts are significantly different ($P < 0.005$). Within an age group, values not sharing common lower case letter superscripts are significantly different ($P < 0.05$).