Exercise training from late middle age until senescence does not attenuate the declines in skeletal muscle aerobic function

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ABSTRACT

We previously showed that 7 weeks of treadmill exercise training in late middle aged rats can reverse the modest reductions in skeletal muscle aerobic function and enzyme activity relative to values in young adult rats (Exp Physiol. 93.7: 863-871, 2008). The purpose of the current study was to determine if extending this training program into senescence would attenuate the accelerated decline in the muscle aerobic machinery normally seen at this advanced age. For this purpose, 29 mo old Fisher 344 Brown-Norway rats underwent 5 or 7 months of treadmill exercise training. Training resulted in greater exercise capacity during an incremental treadmill exercise test and reduced percent body fat in 34 mo old and 36 mo old rats, and improved survival. Despite these benefits at the whole body level, in situ muscle aerobic capacity and muscle mass were not greater in the trained groups at 34 mo or 36 mo of age. Similarly, the trained groups did not have higher activities of citrate synthase or complex IV in homogenates of either the plantaris (fast twitch) or the soleus (slow twitch) muscles at either age. Finally, protein expression of citrate synthase (a marker of mitochondrial content) and PGC-1 (relating to the drive on mitochondrial biogenesis) were not higher in the trained groups. Therefore, although treadmill training from late middle age into senescence had significant benefits on running capacity, survival and body fat, it did not prevent the declines in muscle mass, muscle aerobic capacity or mitochondrial enzyme activities normally seen across this age, revealing a markedly diminished plasticity of the aerobic machinery in response to endurance exercise at advanced age.

Key Words: aging, oxygen uptake, sarcopenia, mitochondria, endurance training
INTRODUCTION

Aging is accompanied by declines in skeletal muscle mass (13; 24), contractile function (12; 13; 60) and aerobic metabolic capacity (14; 16; 29) that contribute to health problems, impaired mobility and reduced quality of life. Furthermore, the declines in muscle mass (13; 29; 40) and muscle function (29; 33) accelerate dramatically between late middle age and senescence, both of which play an important role in the increased risk of frailty in advanced age (23). Age-related declines in physical activity do not entirely explain the decreases in aerobic capacity at the whole body level, since chronically trained older individuals and Masters athletes still show evidence of a decline with aging (27; 39; 46-48; 61). Nonetheless, many studies in middle to late middle aged individuals have shown a maintained capacity to increase whole body maximal oxygen uptake (VO₂ max) (28; 42; 43) and mitochondrial enzyme activity (14; 63) following exercise training relative to young adults. Very few studies have examined whole body aerobic responses to endurance training in very advanced age (≥80 y of age; senescence) (20; 41), and none has assessed adaptability of the skeletal muscle aerobic machinery specifically. Since improvements in muscle and whole body VO₂ max are accompanied by increases in mitochondrial enzyme activity in both young adult and late middle aged muscle (9; 14; 17), it is important to establish the efficacy of endurance training on the skeletal muscle aerobic machinery at these advanced ages because such adaptations are important to whole body aerobic capacity and mobility in the elderly.

The Fisher 344 Brown-Norway F1 hybrid rat (F344BN), a popular rodent model for studying skeletal muscle aging because it lives long enough to experience sarcopenia in a pattern similar to humans (40), experiences hindlimb muscle mass declines of 15-
20% between young adult and late middle age (28 mo), but more drastic declines (a further 30-60%) between late middle age and senescence (36 mo, 35% survival) (13; 29). Similarly, declines in peak tetanic force (13) and muscle mass-specific VO$_2$ (29) are modest ( < 20%) until late middle age, but decline 30-50% between late middle age and senescence. Given the fact that age-related declines are most severe after late middle age, it is critical that we focus our efforts in this part of the lifespan to combat these effects.

Recently, we showed that 7 weeks of treadmill training of 28 mo old male F344BN rats resulted in a 20% increase in muscle mass-specific VO$_2$ max and ~25% increase in mitochondrial enzyme activity, illustrating that at this age, this rat strain remained sufficiently responsive to a training program to restore skeletal muscle aerobic function to young adult levels (9). The efficacy of a prolonged exercise program from late middle age until senescence on muscle aerobic function and mitochondrial oxidative capacity is unknown. The purpose of this study was to determine if a program of exercise training starting at late middle age and continuing into senescence would better maintain skeletal muscle aerobic function and mitochondrial oxidative capacity. Based on our previous results showing treadmill exercise increased running capacity, muscle mass-specific VO$_2$ max and mitochondrial enzyme activity at late middle age (9), we hypothesized that the same exercise training program executed from late middle age until senescence would result in a higher running capacity, muscle mass-specific VO$_2$ max and muscle mitochondrial enzyme activity compared to sedentary control animals.

METHODS
Animals. 57 F344BN male rats (29 mo of age) were acquired from the National Institute of Aging (Bethesda, MD). All rats were allowed food and water *ad libitum* and were housed in the same room, with 2-3 animals per cage. 24 rats were randomly assigned to the training group and the remaining 33 rats were allocated to the sedentary control group. These sample sizes were chosen on the basis of several criteria. Firstly, because the decline in muscle mass and function rapidly accelerates between 29 and 36 mo of age in the F344BN rat (29), and because we wished to obtain insight into the trajectory of changes between these ages, we determined that we would subsample a group of rats for hindlimb contractile and metabolic performance, and *in vitro* muscle analyses at 34 mo of age (9 in each group), with the remaining animals studied at 36 mo of age (11 in the control group, 9 in the trained group). Secondly, our sample size was based upon survival curves for this strain of rat (62) and the expectation that there would be greater survival rates in the trained group since exercise has been shown to prolong mean lifespan (34; 35). All experimental procedures were approved by the University of Calgary Animal Care Committee.

Food Intake and Body Mass. To provide an estimate of food intake per animal, food consumption (grams) was measured weekly for each cage and divided by the number of animals in that cage. Body mass was also measured weekly throughout the entire experiment.

Graded Exercise Test. All animals were habituated to the treadmill (Columbus Instruments, Columbus, OH, USA) over five consecutive days, by walking on the
treadmill (10% grade) twice per day for 5 min at 5 m · min⁻¹. After this time, all animals performed a graded exercise test (GXT) to voluntary exhaustion which consisted of starting at 5 m·min⁻¹ for one minute and then increasing the speed by 1 m·min⁻¹ each minute until the animal could no longer keep up with the treadmill speed and continuously sat on the shock grid. This protocol was performed on all rats after 7 weeks (31 mo of age), 5 months (34 mo of age) and 7 months (36 mo of age), except that the 34 and 36 mo old rats began at 4 m·min⁻¹ because some of them had difficulty at 5 m·min⁻¹.

Training. Training was modeled after our recent study in late middle aged F344BN rats showing that 7 weeks of treadmill running increased mass specific VO₂ max and mitochondrial enzyme activity by 20-25% (9). Specifically, the training program consisted of running on the treadmill 5 days per week and progressively increasing the duration so that by week 3 the animals were running for 60 min per day (10% grade). Each training session was broken down into 6 bouts of 10 min with 2 min rest in between bouts. Within the 10 min segments, 8 min was performed at a base velocity and a higher velocity was used for the last 2 min. The base velocity was slowly increased from 5 m·min⁻¹ to 7 m·min⁻¹ at week 3, with increments of 0.5 m·min⁻¹ each week until the animals could not tolerate an increase in velocity (coincided with week 9). The 2 min interval was performed at 2.0 m·min⁻¹ faster than the base velocity. After 8 weeks the training sessions were reduced to 4 days per week with the goal being to maintain any adaptations that occurred during the initial training sessions and to allow more recovery time as the animals aged into senescence.
Surgical Procedures. Muscle mass specific VO₂ max was assessed by a hindlimb pump perfusion technique that permits matching skeletal muscle O₂ delivery for each animal so that differences in aerobic capacity reflect differences in the contracting muscles (7; 37). Animals were anesthetized (following at least 2 d rest after last exercise bout) with sodium-pentobarbital (50-65 mg kg⁻¹ i.p.) and supplemented with 10 mg kg⁻¹ as necessary throughout the surgical procedure. Body composition was measured in each animal by dual-energy x-ray absorptiometry (Hologic, Bedford, MA). Muscles from the right lower limb and quadriceps were removed, weighed and frozen in liquid nitrogen. The mass of the distal hindlimb muscles was used for the calculation of muscle blood flow for the perfusion experiments on the left leg. The left lower limb was prepared for hindlimb perfusion using a similar method to that used previously (31; 37), with the exception that the femoral artery and vein were surgically isolated to permit perfusion of only the distal hindlimb, as described by Baker et al. (8). All branching vessels from the femoral artery and vein prior to their entry into the gastrocnemius muscle were ligated with silk thread or cauterized. The portion of the Achilles tendon originating from the soleus muscle was separated from that of the gastrocnemius-plantaris muscles, and each portion of tendon was attached to individual force transducers (FT-10, Grass Instruments, West Warwick, RI) with 6.0 non-compliant silk thread such that force development could be independently measured in the soleus muscle versus the gastrocnemius-plantaris muscles. The femoral artery and vein were cannulated in the femoral area with Intramedic™ Polyethylene tubing (I.D. 0.58 mm, Becton Dickinson, Sparks, MD) that was advanced distally in each vessel to the apex of the gastrocnemius muscle to ensure the perfusate was going directly into the lower limb (8). As previously reported (29), the perfusion
medium consisted of isolated bovine erythrocytes reconstituted with Krebs-Henseleit bicarbonate buffer containing 4% bovine serum albumin, 5mM glucose, 100 mU ml\(^{-1}\) insulin, 1000 mU ml\(^{-1}\) heparin, 0.15 mM pyruvate to achieve a hematocrit of \(~45\%\).

Once the cannulation was complete, the distal hindlimb was perfused (0.5 mL min\(^{-1}\)), by a peristaltic pump (Gilson Minipuls 3, Villiers Le Bel, France) and the animal was sacrificed via cardiac removal. The sciatic nerve was cut proximal to the gastrocnemius muscle and was placed over a hook electrode to provide stimulation of the muscles in the distal hindlimb only. The hindlimb was loosely wrapped in saline-soaked gauze, cellophane and aluminum foil and was kept at 37\°C with a heat lamp and thermistor probe that was inserted inside the cellophane. A pressure transducer (PT-300 Grass Instruments), in-line with the hindlimb perfusion apparatus, was positioned at the height of the hindlimb muscles to determine total perfusion pressure. Net pressure was calculated by subtracting the pressure through the arterial tubing from the total pressure recorded during the muscle contractions.

**In situ Aerobic Capacity.** After at least 30 min of perfusion, perfusate flow was incrementally increased over a 10-12 min period to the desired flow rate, which was calculated from the masses of the entire distal hindlimb of the right leg to ensure similar mass-specific muscle blood flow for each animal. After 2 min at the peak perfusate flow, an arterial and venous resting blood sample was collected and the muscle length was adjusted to yield maximal force. The distal hindlimb muscles were stimulated (Grass S48, Grass Instruments, Warwick, RI) via the sciatic nerve with square wave electrical pulses (200 ms trains, 0.05 ms duration, 100 Hz) to induce tetanic contractions at a rate of
7.5, 15, 30 and 60 tetani min\(^{-1}\) for one min each, and 90 tetani min\(^{-1}\) for 2 min to elicit a maximal VO\(_2\) response, as reported previously (32). Force from the soleus and gastrocnemius-plantaris muscles, and total pressure were recorded continuously (DATAQ DI-720, DATAQ Instruments, Akron, OH). Venous blood was sampled every 30 s throughout the contraction bout and was analyzed for PO\(_2\), PCO\(_2\), O\(_2\) saturation (SO\(_2\)), hematocrit and hemoglobin concentration by a blood-gas analyzer (Rapidlab 865, Siemens, Deerfield, IL). Blood oxygen content was calculated using the formula: 
\[
[O_2] \times SO_2 \times 1.39 + 0.003 \times PO_2.
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VO\(_2\) across the distal hindlimb was calculated as the product of the rate of blood flow (held constant) and the arterio-venous O\(_2\) content difference, and was normalized to the mass of the contracting muscles of the distal hindlimb.

*Biochemistry.* The plantaris (primarily fast twitch) and soleus (primarily slow twitch) muscles were chosen based upon their contrasting phenotypes as representatives of the distal hindlimb musculature (3) and because these muscles adapt in response to treadmill running in rats (44). Citrate synthase and complex IV enzyme activities were assessed in crude homogenates of muscle, as we have done previously (30). The entire muscle was pulverized with mortar and pestle under liquid nitrogen and an aliquot of this powder was homogenized 1:20 (mass/volume) with a potassium phosphate buffer (pH 7.5). The homogenate was freeze-thawed three times to rupture cellular and mitochondrial membranes, centrifuged (900 G, 10 min, 4°C) and the supernatant aliquoted into eppendorf tubes. For citrate synthase activity, this homogenate was further diluted with the homogenizing buffer to achieve a final concentration of 1:400. Citrate synthase activity was measured according to the method of Srere (57). The rate of mercaptide ion...
formation was measured spectrophotometrically (412nm wavelength, 37°C; DU 800 Spectrophotometer, Beckman Coulter) for 3 min after the addition of 100 μL of homogenate, 650 μL Tris Buffer (pH 8.0), 50 μL 3mM Acetyl CoA, 100 μL 1 M DTNB and 100 μL of 0.5 mM oxaloacetate. Complex IV activity was determined by measuring the rate of cytochrome c oxidation spectrophotometrically (550nm, 37°C) for 3 min after the addition of 4 μL of homogenate, 30 μL of 1mM reduced cytochrome c to 970 μL of 10mM potassium phosphate buffer (pH 7.0, 37°C). Each sample was measured in duplicate and enzyme activities determined from the average change in absorbance over time and was normalized to the amount of tissue added to the cuvette.

Protein Expression. Frozen powder aliquots of plantaris and soleus muscle samples were mechanically homogenized (1 part muscle powder to 9 parts buffer) in an extraction buffer containing 50 mM Tris-HCl, 250 mM mannitol, 50 mM NaF, 5 mM NaPPi, 1 mM EDTA, 1 mM EGTA, 10% glycerol, 1% Triton X-100 with the following inhibitors added just prior to use (1 mM DTT, 1 mM benzamidine, 0.5 mM AEBSF, 100 uM leupeptin, 1 ug/ml soybean trypsin inhibitor). The samples were left on ice for 30 min, followed by centrifugation for 10 min at 700 g. Supernatants were removed and placed into an eppendorff tube and protein concentration was determined using a Bradford assay (11). Samples were then diluted by half with 2x sample buffer containing Glycerol, Tris pH 6.8, SDS, DTT, Bromophenol Blue and β-mercaptoethanol. Equal quantities of protein for each sample, and a pre-stained marker (Fermentas), were loaded onto 10 % SDS-PAGE mini-gels and separated by electrophoresis at 100 V for 90 minutes. Proteins were then electro-transferred to nitrocellulose membranes in a transfer buffer containing
48 mM TRIS, 39 mM Glycine, and 20 % methanol at 100 V for 80 minutes. Blots were blocked for 1 hr at room temperature with 5 % non-fat milk PBS-0.05 % Tween solution, followed by overnight incubation (4°C) with antibodies against peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) (1:1000, Calbiochem), citrate synthase (1:10,000, kind gift from J. Holloszy) and α-actin (1:2000, Santa Cruz). The following day, blots were washed three times for 15 min each with PBS-0.05 % Tween and probed with an appropriate HRP-conjugated secondary antibody (1:2000, Pierce, Rockford, IL) for 1 hr at room temperature. Blots were washed a further two times for 15 min each with PBS-0.05 % Tween, a final wash of PBS (no Tween) and then treated with chemilluminescent developing solution (Pierce). Chemiluminescence was digitally captured (Syngene Bio-Imager, Frederick, MD) and densitometry measured using the Bio-imager software (Syngene Tools, Frederick, MD). Protein data was normalized to α-actin, and expressed relative to the 34 mo sedentary group.

Statistics. Comparisons within groups over time were performed with one-way repeated measures ANOVA. Comparisons of food intake between groups over time were assessed by a two-way ANOVA (Training x Time), with a Student-Neuman Keul post-doc multiple comparison test. All other comparisons were by two-way ANOVA (Age x Training), with a Student-Neuman Keul post-hoc multiple comparison test. Values are expressed as means ± SE.

RESULTS.
Body and Muscle Masses. Body mass was lower in the trained groups compared to the control groups at 31, 34 and 36 mo (Fig 1). This was largely due to the lower body fat percentage in the trained animals versus the control animals, with no difference between the 34 and 36 mo old age groups within either trained or sedentary animals. It was not possible to obtain body composition at 29 or 31 mo. However, previous results in our lab found the percent body fat in 30 mo old sedentary animals to be 31 ± 1% (D.J. Baker, unpublished), meaning that the trained rats halved their body fat with training, whereas there was little change in the sedentary group. The body mass in the 36 mo trained animals was significantly lower than the 34 mo trained animals (406 ± 13 and 464 ± 13 g, respectively) due to lower muscle and fat masses, since the body fat percentage was the same (Table 1). While only the masses of the distal hindlimb muscles is reported here, significantly smaller muscles masses were observed in the 36 mo trained group compared to the 36 mo control group for the gastrocnemius, plantaris, soleus, tibialis anterior, extensor digitorum longus, adductor longus and rectus femoris muscles (A.C. Betik and M.M. Thomas, unpublished). With the exception of the 31 mo old control group, the initial body mass (29 mo) of both groups was significantly higher than all other groups and time points (583 ± 6 g).

Food Consumption. Absolute food consumption in the trained group tended to be slightly lower than the control group throughout the entire experiment. However, when food consumption was normalized to body mass, food intake was actually higher at some time points in the trained animals (Figure 2).
Survival. The survival was tracked from the beginning of the study (29 mo of age) until 34 mo of age, at which point some animals were culled from both groups for muscle performance, biochemistry and molecular measurements. The trained group had a higher rate of survival (82%) compared to the control group (52%) between 29 and 34 mo of age.

Graded Exercise Tests. Treadmill training improved performance on the GXT at 31 mo, with no change in the control group. After 7 weeks of training, the trained group increased exercise endurance time by 25% (9.2 vs 7.4 min for pre-training versus 7 week training points, respectively) and peak velocity by 17% (13.7 vs 11.8 m min\(^{-1}\) for pre-training versus 7 week training points, respectively). However, performance declined in the trained group at 34 and 36 mo compared to pre-training (36 mo compared to 29 mo, velocity -18%, total time -12%) but was always better than the control group (Figure 3). There was no difference in the control group after 7 weeks, but their performance was lower at 34 and 36 mo, declining by ~35% in peak velocity and total time at 36 mo compared to 29 mo.

In situ Aerobic Capacity. In situ aerobic capacity was assessed using a modified hindlimb perfusion model in which the muscles of the lower limb were perfused at rates proportional to their muscle masses between groups at a given age, such that convective O\(_2\) delivery was matched at a given age between control and trained groups (Table 2). At both 34 and 36 mo of age, muscle VO\(_2\) max did not differ between the trained and
sedentary groups (34 mo: 212 ± 31 vs 220 ± 21; 36 mo 212 ± 39 vs 209 ± 19 µmol min⁻¹100g⁻¹ for control and trained groups respectively, Figure 4). The response to the increasing frequency of the contractions was the same between all groups, resulting in a peak VO₂ between 4 and 4.5 min. Similarly, there were no differences between the control and trained groups in peak lactate concentration, peak specific force of the gastrocnemius-plantaris complex, peak specific force of the soleus muscle, or the force at the end of the stimulation protocol for either muscle, in support of a lack of training benefit for VO₂ max (Table 3).

**Biochemical Analyses.**

**Citrate Synthase.** For the plantaris muscle, CS activity normalized to muscle mass was lower in the trained groups (Figure 5A), but this difference was eliminated after normalizing to muscle protein (Figure 5C). For the soleus muscle, there was no difference in CS activity between trained and control groups at 34 or 36 mo at both the whole muscle level (Figure 5B) and after normalizing to muscle protein. Within a given group there were no differences for CS activity for either muscle between 34 and 36 mo of age.

**Complex IV.** Complex IV activity was not different between control and trained groups at 34 and 36 mo of age in either muscle and this was the same regardless of whether the activity was expressed relative to whole muscle mass or muscle protein. As for CS activity, within a given group there was no difference between 34 and 36 mo for either muscle (Figure 6).
**CS and PGC-1 Protein Content.** CS protein content was not different between trained and sedentary control groups for either muscle, regardless of age (Figure 7 A+B). Only in the plantaris muscle was there an effect of age, as CS protein was lower (p<0.05) in the 36 mo control group compared to the 34 mo control group only (not seen in trained groups). Similarly, PGC-1 protein content in plantaris muscle was not different in the trained groups compared to the control groups, regardless of age. On the other hand, for the soleus muscle, PGC-1 protein was lower in the 36 mo trained group compared to the 36 mo control group (p<0.05). Within each group there was no difference in PGC-1 protein between 34 and 36 mo old animals for either muscle (Figure 5 C+D). Muscle actin protein content was not affected by age or training status (Figure 5 E + F).

**DISCUSSION**

The purpose of this study was to determine the efficacy of a chronic exercise program starting in late middle age and continuing into senescence on the aerobic function of skeletal muscle. Since this period of the lifespan is associated with accelerated declines in muscle mass and mass-specific oxidative capacity (29), it was hypothesized that regular exercise would attenuate the declines in whole body exercise capacity, muscle oxidative enzyme activity, muscle VO₂ max and muscle mass. Previously, we have shown that 7 weeks of the same training program at late middle age increased mitochondrial enzyme activity and muscle VO₂ max (20-25%). Our current results show that while body fat was lower in the trained groups and whole body exercise capacity was higher at all time points after the initiation of training in the trained animals compared to
the sedentary groups, whole body exercise capacity still declined (after an initial improvement over the first 2 mo of training) in the trained groups, and neither 5 mo nor 7 mo of training benefited muscle oxidative enzyme activity, muscle VO₂ max or muscle mass in this critical period of the lifespan. Indeed, muscle mass declined to a greater extent in the trained animals by 36 mo of age.

The current study design differs from our previous study (9) in that we reduced the training frequency from 5 days per week to 4 days per week after the initial 8 weeks of training. As such, it is possible that the reduction of weekly training frequency could account for some loss of training benefits after the initial 8 week period (at which point it is reasonable to assume the muscle adaptations were similar to our previous study because the training stimulus was identical). However, it is unlikely that this alone would completely abrogate the training benefits for the skeletal muscle aerobic machinery, particularly relative to the sedentary control animals which were cage-bound and had no training stimulus. That the trained animals maintained a significantly lower % body fat and superior whole body running performance versus the sedentary controls to the end of the study supports this view. Therefore, we have combined measurements at the whole body level, muscle level and cellular level to demonstrate a diminished plasticity of the skeletal muscle aerobic machinery in response to endurance exercise training in very old age and to underscore the need to identify effective interventions at this advanced age.

Systemic Responses to Endurance Training

Despite the lack of improvement in muscle VO₂ max, there are other measures that demonstrate the exercise was beneficial. Firstly, the higher exercise intensity and
longer exercise duration in the graded exercise test for the trained animals demonstrates a higher capacity for exercise. The higher exercise capacity at 34 and 36 mo despite no difference in muscle mass specific VO$_2$ max is likely explained by the lower body mass of the trained animals. The trained animals had ~ 20% less body mass to support while treadmill running, meaning that at the whole body level, the same muscle VO$_2$ max would translate to a higher VO$_2$ max per body mass in the trained animals. However, despite significant improvements in running capacity after 2 mo of training, between 31 and 36 mo of age the declines in the training group (-27%) were of a similar rate as the control group (-33%), suggesting that exercise cannot prevent the aging-related decline at this critical period of the life span, and that the decline during this period is due to aging processes. This is in support of human studies that have tracked chronically trained and/or Masters athletes and observed a decline in whole body aerobic and running capacity with age despite a maintained exercise program (39; 47; 48; 61). A second benefit of the training program was an improved life expectancy, as there were fewer deaths in the trained group over the 5 mo of training from 29 to 34 mo of age. An improvement in mean lifespan has been shown in male and female rats that voluntarily exercised (running wheel in cage) throughout their entire lifespan (34; 35), and in humans who are habitually active (2). Significantly, this study demonstrates that improvements in survival can be achieved even if the training only begins at late middle age. A third benefit of the exercise training program was a lower body fat percentage. Specifically, the trained rats demonstrated a body fat that was about half that seen in sedentary control animals, a change that likely has positive implications on health risk factors that are related to obesity (e.g., insulin resistance, cardiovascular function, atherosclerosis). For
example, surgical removal of adipose tissue via liposuction improves whole body insulin
sensitivity (26; 51). In our study, the trained rats did not increase their food intake
sufficiently to maintain body mass, a typical response of male rats that are chronically
exercising (18; 35). Although absolute food intake was slightly lower in the trained
animals, when normalized to body mass (since body mass was lower in the trained
groups at all points after the initiation of training) food intake was actually higher in the
trained animals for some time points. Therefore, the higher exercise capacity, greater
survival and lower body fat show valuable benefits from the exercise program.

Impact of Endurance Training on Skeletal Muscle Aerobic Function

There are many human (28; 43; 45; 54), and rodent studies (14; 53; 63) that have
shown beneficial improvements from endurance exercise training in late middle age, both
in terms of whole body aerobic capacity and muscle mitochondrial enzyme activity. We
have also previously shown that treadmill exercise training in late middle aged F344BN
rats can restore muscle aerobic function and enzyme activity levels to young adult levels
(9). Only a few studies have investigated endurance or resistance exercise training effects
in very old humans (≥79 yrs) and although there are clear benefits to exercise training
(22), overall these studies suggest that in very old age (20; 41; 56) and in frail elderly (21;
25) there is a diminished capacity for adaptation at these advanced ages. For example,
Slivka and colleagues recently demonstrated a complete lack of adaptation to resistance
training at the single fiber level in men ≥ 80 years of age (56). Blough and Linderman
(10) also observed an inability of 36 mo old F344BN rat plantaris muscle to adapt to a
functional overload (gastrocnemius muscle ablation) in which young adult rats
experienced a 50% increase in muscle mass, again suggesting an impaired muscle adaptability at very old age. It is noteworthy, however, that no prior studies have examined the plasticity of the muscle aerobic response specifically in response to endurance exercise training in the transition from late middle age to senescence. We have previously shown that exercise training in late middle aged F344BN rats was able to increase muscle VO$_2$ max and muscle oxidative enzyme capacity (9), essentially restoring these values back to the levels seen in young adult rats (30). The exercise capacity from the treadmill running tests and training intensity of the rats reported here was nearly identical to our previous study (9), and thus it is reasonable to assume that the initial improvement in muscle VO$_2$ and muscle enzyme activity were also similar to the previous study over the first 2 mo of the training period. In contrast to the demonstrated benefits of exercise training for skeletal muscle aerobic function at late middle age, the data in the present study shows that continuing exercise into senescence does not attenuate the decline in muscle aerobic function, and at the muscle level there is no apparent benefit from exercise compared to the sedentary controls. In light of the findings from our previous study (9), the adaptations that occurred in the first 2 mo were completely lost after 5 mo of training (34 mo of age).

The fact that muscle VO$_2$ max was not higher in the trained animals was surprising and unexpected given the plethora of endurance training studies in young adult to late middle aged subjects showing improvements in muscle oxidative capacity (43; 49; 54). However, no one to our knowledge has undertaken a training study that starts in late middle age and ends in senescence, and thus the effects of chronic exercise throughout this part of the lifespan were previously unknown. One particular human study on
octogenarians showed only subtle improvements in arterial–venous O2 difference in response to 3 months of endurance training, implying minimal improvements in muscle oxidative capacity, and leaving the authors to conclude that this age group has a diminished capacity for adaptation (20). However, as noted above, no prior study has considered the impact of endurance training on the muscle aerobic machinery specifically at this advanced age. Other aging studies showing positive adaptations to endurance training were only short term in duration (less than 6 mo) and did not involve very old/senescent humans.

It is possible to approximate the ages and training duration of this study relative to ages in humans based on survival data on humans (4) and the F344BN rat (62). In relation to human years, 7 mo of training in this study with rats corresponds to 18 years for a human, which would roughly equate to starting training at 65 years of age and ending around 83 years of age. Clearly conducting a human study over a similar age-range would be a major undertaking. The significance of examining this age range is that both humans (40) and rats (13; 29) exhibit a marked acceleration of sarcopenia and functional decline across this age range. Animal endurance exercise training studies, like the human studies, have also shown an adaptive capacity at older ages; however, these have also largely examined late middle aged animals (14; 53; 63). Thus the main findings from this study suggesting that beyond late middle age, the capacity for endurance exercise training to attenuate the declines in aerobic function is lost, is novel in both the study design and the result, and does not refute any prior work. Another unique and advantageous aspect of this study is the use of the hindlimb perfusion technique to match oxygen delivery to the muscles between groups at a given age, allowing for the
assessment of skeletal muscle aerobic function without the confounding influence of changes in central effects such as cardiac output (7). Using this approach we observed that there is no benefit of endurance exercise training from late middle age to senescence at the muscle level specifically. This does not diminish the significance of the other systemic benefits, such as the improved exercise performance, greater survival and lower body fat that we observed.

Effect of Endurance Training on Mitochondrial Enzyme Activities

There was no training benefit for CS or complex IV activity in either the plantaris or soleus muscles, which represent mixed fast twitch and predominantly slow twitch muscles, respectively (3), showing that the responses observed are not specific to a particular fiber type. CS and complex IV are key enzymes of the Kreb’s cycle and the electron transport chain, respectively, and reflect the oxidative capacity of the mitochondria. Maximal activity of these enzymes increases after training in late middle aged rodents (9; 19; 44; 53) and humans (17; 45; 54). Similar to the point raised above, we are unaware of any study examining mitochondrial enzyme adaptations in response to endurance training in senescent muscle, but it was expected that these would be higher in the trained group compared to sedentary controls, as we have shown at late middle age for this strain of rat (9). In further support of the lack of mitochondrial proliferation with training, we also measured CS protein as a marker for mitochondrial content, and it too, was not affected by training in either muscle. The fact that mitochondrial content was not increased with exercise training plays an important role in explaining why muscle specific VO2 max was also not higher with training compared to sedentary controls.
In attempting to explain why we did not observe the expected increases in mitochondrial enzyme activity from the exercise training, we measured peroxisome proliferator-activated receptor gamma co-activator 1 (PGC-1) protein in the muscles to determine if this was up-regulated by the exercise training. PGC-1, a master regulator of mitochondrial biogenesis (36), increases significantly following both a single bout of muscle activation (5; 59) and after chronic muscle activation (1; 38; 54; 58). However, in this study, PGC-1 protein was not higher in the 34 or 36 mo trained groups compared to the sedentary groups, which provides a credible explanation for why the trained rats did not demonstrate higher mitochondrial content versus controls. We are unaware of any other study investigating PGC-1 protein with endurance training in aged skeletal muscle, although one study in humans observed similar increases in PGC-1 mRNA across a range of ages from 21 to 87 y of age following 16 weeks of endurance exercise training (54). Note, however, that there were only two subjects aged 80 y or older in this prior study (54) and it appears that these two subjects had smaller increases in transcripts related to mitochondrial biogenesis with endurance training (e.g., PGC-1, NRF-1, Tfam). Thus, these data do not refute our point that the aerobic machinery in senescent muscle has a blunted response to endurance exercise training.

Previous studies have observed lower PGC-1 mRNA (6) and protein (15) when compared to young adult muscle. Whereas Short et al. (54) did not find PGC-1 mRNA to decline with age, in a later investigation the same group found transcript levels of some mitochondrial proteins were reduced in skeletal muscle with aging (55). A reduced rate of skeletal muscle mitochondrial protein synthesis has been observed with aging in humans (52), which is consistent with lower mitochondrial biogenesis with aging. It has been
shown that AMP-activated protein kinase (AMPK) activation is diminished in late middle aged muscle (50), and since AMPK is an activator of PGC-1 (38; 64), this may partly explain why PGC-1 was not up-regulated with training in our senescent animals. Although reducing the training frequency from 5 to 4 days per week could have modestly attenuated the protective effects of the training, it is unlikely that this would be sufficient to explain the complete lack of benefit for skeletal muscle aerobic function and mitochondrial adaptation in the trained group relative to cage-bound sedentary rats.

PERSPECTIVES

In contrast to previous findings of a skeletal muscle metabolic adaptation to exercise training at late middle age, during the phase of the lifespan from late middle age and into senescence there is growing evidence of a diminished plasticity in the skeletal muscles. A key element in this study was that we combined measures at the whole muscle level (aerobic function in response to electrically-evoked muscle contractions, biochemistry in muscle homogenates) and at the cellular level (molecular signals involved in mitochondrial biogenesis) to describe the muscle adaptations with endurance training in senescence. To that end, all of these measures support the notion that the aerobic machinery in senescent muscle becomes much less responsive to endurance exercise training. Skeletal muscle specific VO$_2$ max was not higher in the trained muscles, which is explained at the mitochondrial level by no greater CS or Complex IV enzyme activity, or CS protein content. The significance of these findings is that they underscore the point that exercise training alone may be insufficient to prevent age-related declines in skeletal muscle mass and function in advanced age. As such, the
current results suggest that further work determining whether other perturbations can
induce skeletal muscle mitochondrial biogenesis at very advanced age is warranted.
Despite the lack of adaptation at the muscle level, training did result in better maintained
exercise capacity, greater survival and lower body fat, showing that significant benefits of
endurance training can be obtained in senescence independent of a diminished plasticity
in skeletal muscle.
ACKNOWLEDGEMENTS. This work was supported by an operating grant from the Canadian Institutes of Health Research (MOP 57808). Mr. Betik was supported by a scholarship from the Canadian Institutes of Health Research funded training program for Alberta Bone and Joint Health. Ms. Thomas was supported by a PGS-D award from the Natural Sciences and Engineering Research Council. Dr. Hepple was supported by an Alberta Heritage Foundation for Medical Research Senior Scholar award.
### TABLE 1. Percent body fat (% Fat), and muscle masses of the lower limb.

<table>
<thead>
<tr>
<th></th>
<th>Fat (%)</th>
<th>Gas (mg)</th>
<th>Plan (mg)</th>
<th>Sol (mg)</th>
<th>TA (mg)</th>
<th>EDL (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>34 mo Con</td>
<td>25 ± 1</td>
<td>1265 ± 90</td>
<td>247 ± 15</td>
<td>143 ± 8</td>
<td>550 ± 31</td>
<td>142 ± 8</td>
</tr>
<tr>
<td>34 mo Train</td>
<td>16 ± 2^a,b</td>
<td>1216 ± 70</td>
<td>250 ± 11</td>
<td>138 ± 9</td>
<td>473 ± 26</td>
<td>134 ± 4</td>
</tr>
<tr>
<td>36 mo Con</td>
<td>25 ± 1</td>
<td>1252 ± 33</td>
<td>236 ± 11</td>
<td>124 ± 4^a</td>
<td>551 ± 40</td>
<td>148 ± 2</td>
</tr>
<tr>
<td>36 mo Train</td>
<td>15 ± 1^a,b</td>
<td>910 ± 64^a,b,c</td>
<td>197 ± 12^a,b,c</td>
<td>111 ± 6^a,b,c</td>
<td>339 ± 29^a,b,c</td>
<td>104 ± 6^a,b,c</td>
</tr>
</tbody>
</table>

Gas=Gastrocnemius; Plan=Plantaris; Sol=Soleus; TA=Tibialis Anterior; EDL = Extensor Digitorum Longus; Significantly different (p<0.05) from ^a 34 mo control group, ^b 36 mo control group, ^c 34 mo trained group.
Table 2. Perfusion conditions for hindlimb perfusion experiments.

<table>
<thead>
<tr>
<th></th>
<th>34 mo Con</th>
<th>34 mo Train</th>
<th>36 mo Con</th>
<th>36 mo Train</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contracting/Perfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mass (mg)</td>
<td>3261 ± 259</td>
<td>3074 ± 148</td>
<td>3303 ± 122</td>
<td>2429 ± 171</td>
</tr>
<tr>
<td>Blood flow (ml.min⁻¹)</td>
<td>1.72 ± 0.10</td>
<td>1.64 ± 0.06</td>
<td>2.33 ± 0.14*</td>
<td>1.70 ± 0.21+</td>
</tr>
<tr>
<td>Muscle blood flow</td>
<td>0.53 ± 0.02</td>
<td>0.53 ± 0.01</td>
<td>0.71 ± 0.05*</td>
<td>0.70 ± 0.06*</td>
</tr>
<tr>
<td>(ml.min⁻¹.g⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Net Perfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pressure (Torr)</td>
<td>99 ± 7</td>
<td>95 ± 10</td>
<td>161 ± 13*</td>
<td>131 ± 23+</td>
</tr>
<tr>
<td>Arterial O₂ content</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(volume %)</td>
<td>21.6 ± 0.3</td>
<td>20.8 ± 0.3</td>
<td>21.7 ± 0.4</td>
<td>22.1 ± 0.3</td>
</tr>
<tr>
<td>Muscle QO₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µmol.min⁻¹.g⁻¹)</td>
<td>4.5 ± 0.2</td>
<td>4.3 ± 0.1</td>
<td>6.1 ± 0.5*</td>
<td>6.2 ± 0.5*</td>
</tr>
</tbody>
</table>

Significantly different (p<0.05) from * 34 mo group of the same training status or +control group within same age.

Table 3. Contractile and metabolic characteristics from the hindlimb perfusion VO₂ max tests.

<table>
<thead>
<tr>
<th></th>
<th>34 mo Con</th>
<th>34 mo Train</th>
<th>36 mo Con</th>
<th>36 mo Train</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak VO₂ (µmol.min⁻¹.100g⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>212 ± 31</td>
<td>221 ± 21</td>
<td>212 ± 39</td>
<td>209 ± 19</td>
</tr>
<tr>
<td>Peak force (N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-P</td>
<td>14.4 ± 2.0</td>
<td>16.5 ± 2.5</td>
<td>15.5 ± 3.3</td>
<td>10.2 ± 0.6*+</td>
</tr>
<tr>
<td>Sol</td>
<td>0.94 ± 0.16</td>
<td>0.96 ± 0.16</td>
<td>0.96 ± 0.17</td>
<td>0.80 ± 0.11</td>
</tr>
<tr>
<td>Peak force (N.g⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-P</td>
<td>9.2 ± 1.0</td>
<td>11.0 ± 1.2</td>
<td>9.8 ± 1.8</td>
<td>9.5 ± 0.6</td>
</tr>
<tr>
<td>Sol</td>
<td>6.1 ± 0.9</td>
<td>6.6 ± 0.9</td>
<td>7.0 ± 1.0</td>
<td>7.3 ± 0.8</td>
</tr>
<tr>
<td>Final Force (N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-P</td>
<td>4.9 ± 0.7</td>
<td>5.4 ± 0.4</td>
<td>4.1 ± 0.6</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td>Sol</td>
<td>0.78 ± 0.18</td>
<td>0.70 ± 0.11</td>
<td>0.80 ± 0.15</td>
<td>0.62 ± 0.08</td>
</tr>
<tr>
<td>Final Force (% of peak)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-P</td>
<td>34 ± 2</td>
<td>36 ± 4</td>
<td>31 ± 8</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>Sol</td>
<td>78 ± 7</td>
<td>78 ± 8</td>
<td>83 ± 5</td>
<td>78 ± 3</td>
</tr>
<tr>
<td>Peak lactate (mmol.L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.2 ± 0.7</td>
<td>5.4 ± 0.5</td>
<td>4.8 ± 0.9</td>
<td>4.5 ± 0.3</td>
</tr>
</tbody>
</table>

Force values are from the gastrocnemius-plantaris muscle (G-P) complex and from the soleus (Sol). Significantly different (p<0.05) from * 34 mo group of the same training status and +control group for the same age.
Figure 1. Body mass of sedentary control and trained groups at four time points.  
Significantly different ($p<0.05$) from * 29 mo group and +trained group for the same age.

Figure 2. Food intake normalized per animal (A) or per kg body mass (B) in sedentary 
control and trained groups throughout the seven months of the study. *Significantly 
different from sedentary control group.
Figure 3. Running performance during graded exercise test in sedentary control and trained groups. *Significantly different from 29 mo old group and +from sedentary control group of same age.

Figure 4. Hindlimb muscle VO$_2$ max achieved during increasing electrical stimulation test (pump-perfusion experiments).
Figure 5. Citrate synthase enzyme activity normalized per g muscle (A, B) or per mg protein (C, D) for the plantaris (A,C) and soleus (B, D) muscles for 34 and 36 mo age in the sedentary control and trained groups. *p<0.05.

Figure 6. Complex IV enzyme activity normalized per g muscle (A, B) or per mg protein (C, D) for the plantaris (A,C) and soleus (B, D) muscles for 34 and 36 mo age in the sedentary control and trained groups. *p<0.05

Figure 7. CS protein for the plantaris (A) and soleus (B), PGC-1α protein for the plantaris (C) and soleus (D), and Actin protein for the plantaris (E) and soleus (F) for 34 and 36 mo age in the control and trained groups.


58. Taylor EB, Lamb JD, Hurst RW, Chesser DG, Ellingson WJ, Greenwood LJ, Porter BB, Herway ST and Winder WW. Endurance training increases skeletal


B

CS Protein (Fold of 34 mo Control)

Age (months)

34 36

Control Trained