

# Myosin Heavy Chain Plasticity in Aging Skeletal Muscle With Aerobic Exercise Training

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To assess myosin heavy chain (MHC) plasticity in aging skeletal muscle with aerobic exercise training, MHC composition was measured at the messenger RNA (mRNA) level and protein level in mixed-muscle homogenates and single myofibers. Muscle samples were obtained from eight nonexercising women ( $70 \pm 2$  years) before and after 12 weeks of training (20–45 minutes of cycle exercise per session at 60%–80% heart rate reserve, three to four sessions per week). Training elevated MHC I mRNA ( $p < .10$ ) and protein ( $p < .05$ ) in mixed-muscle ( $54\% \pm 4\%$  to  $61\% \pm 2\%$ ) and single myofibers ( $42\% \pm 4\%$  to  $52\% \pm 3\%$ ). The increase in MHC I protein was positively correlated ( $p < .05$ ) with improvements in whole muscle power. Training resulted in a general downregulation of MHC IIa and IIx at the mRNA and protein levels. The training-induced increase in MHC I protein and mRNA demonstrates the maintenance of skeletal muscle plasticity with aging. Furthermore, these data suggest that a shift toward an oxidative MHC phenotype may be beneficial for metabolic and functional health in older individuals.

**Key Words:** Endurance training—Myofiber—Physical activity—Elderly—Sarcopenia.

Received January 26, 2011; Accepted April 12, 2011

Decision Editor: Rafael de Cabo, PhD

**S**ARCOPENIA, the age-related loss of skeletal muscle mass, is associated with an increase in disability and a decrease in independent living (1,2). This loss of skeletal muscle mass is mediated by a reduction in total number of myofibers (3,4), decreased size of fast-twitch myosin heavy chain (MHC) IIa fibers (3–5), and altered MHC morphology (6,7). These maladaptations create negative metabolic and functional implications within aging skeletal muscle that impede healthy aging.

Previous investigations have shown that myofibers from skeletal muscle of young individuals are capable of altering their MHC profile in response to various perturbations, such as prolonged unloading (8,9), resistance (10,11), and aerobic exercise training (12,13). Conversely, research focusing on the plasticity of aging skeletal muscle in response to exercise training is heterogeneous (14–18). These equivocal findings may be a result of the techniques that have been implemented to assess the MHC distribution in aging skeletal muscle and/or the characteristics of the aging population examined. For example, after an aerobic exercise program consisting of walking and jogging for 9–12 months, older participants (age  $64 \pm 3$  years) demonstrated a shift toward a more oxidative fiber type profile when analyzed with mATPase histochemistry (16). However, when utilizing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) to determine MHC composition from mixed-muscle homogenates of older (age 60–87 years) men and women, Short and colleagues (17) reported no change in the

proportion of MHC protein isoforms despite alterations at the messenger RNA (mRNA) level, which may suggest a distinct translational regulation. The variety of participant population and methodology used to detect MHC composition most likely accounts for the ambiguity after aerobic exercise training in aging skeletal muscle.

To our knowledge, no investigation has examined the MHC distribution of individual myofibers from aging skeletal muscle (>65 years) after an aerobic exercise training program. Therefore, the primary focus of the present study was to provide a profile of MHC composition at the mRNA, mixed-muscle homogenate, and single muscle fiber levels after a 12-week progressive aerobic exercise training program. We have previously reported that this aerobic training program induced hypertrophy of the quadriceps femoris group (11%) and MHC I myofibers (16%) as well as augmented MHC I myofiber power (21%) and improved aerobic capacity (30%) of older women (19,20). Therefore, we hypothesized that after the aerobic training program, MHC I mRNA expression and the percentage of MHC I protein at the mixed-muscle homogenate and single-fiber level would be elevated in a similar cohort of older women ( $70 \pm 2$  years).

## METHODS

Eight older women (Table 1) volunteered to participate in this investigation. All participants were cleared to participate by the study physician following a thorough physical

Table 1. Participant Characteristics

	Pre	Post
Age (years)	70 ± 2	
Weight (kg)	66.6 ± 4.6	66.3 ± 4.4
VO <sub>2</sub> max (ml·min <sup>-1</sup> ·kg <sup>-1</sup> )	15.5 ± 0.9	19.8 ± 0.6*
Knee extensor power (watts)	265 ± 63	326 ± 65*

Notes: Data from overlapping cohorts have been previously published (19,20). Data are presented as mean ± SE. \**p* < .05 vs PRE.

examination and remained weight stable for the duration of the investigation. Volunteers were excluded based on the following criteria: body mass index  $\geq 28$  kg/m<sup>2</sup>; type 1 or type 2 diabetes; uncontrolled hypertension; active cancer, cancer in remission, or having received treatment for any form of cancer in the previous five years; coronary artery disease; cardiovascular disease (eg, peripheral artery disease, peripheral vascular disease); abnormal thyroid function; nonsedentary defined as one who has completed regular aerobic or resistance exercise more than one time per week for 20 minutes or longer during the previous year; chronic and/or regular non-steroidal anti-inflammatory drug consumption, and any condition that presents a limitation to exercise training (eg, severe arthritis, chronic obstructive pulmonary disease, neuromuscular disorder, moderate or severe cognitive impairment, dizziness) (19). Written informed consent, approved by the Institutional Review Boards of Ball State University and Ball Memorial Hospital, was obtained from each participant prior to study participation.

#### Experimental Design and Methodology

Each participant completed the experimental protocol over a period of approximately 15 weeks consisting of several visits to the laboratory for baseline measurements of aerobic capacity, whole muscle function, a muscle biopsy, and 42 exercise training sessions (19). Baseline measurements were repeated after the 12-week training protocol.

#### Aerobic Exercise Training Protocol

Participants performed 12 weeks of aerobic training on a cycle ergometer with 100% exercise adherence (Stairmaster Stratus 3300 CE) as previously described in detail (19). A total of 42 exercise sessions were performed (Table 2). Duration (20–45 minutes), intensity (60%–80% heart rate reserve [HRR]), and frequency (three or four sessions per week) of exercise were progressively increased throughout the 12 weeks. The last 5 weeks consisted of four 45-minute sessions at 80% intensity per week.

#### Aerobic Capacity

Participants performed a physician-supervised graded exercise test for the assessment of VO<sub>2</sub>max before and after the 12-week aerobic training intervention as previously described (19). During the test, participant's heart rate, blood pressure, rating of perceived exertion, and electrocardiogram

Table 2. Outline of the Aerobic Exercise Training Program

Week	Duration (min)	Intensity (% HRR)	Frequency (sessions per week)
1	20	60	3
2	30	60	3
3	40	60	3
4	40	70	3
5	40	75	3
6	40	75	3
7	40	75	4
8	45	80	4
9	45	80	4
10	45	80	4
11	45	80	4
12	45	80	4

Note: HRR = heart rate reserve.

were monitored, and ventilation and expired air samples were measured by a metabolic cart (TrueOne 2400 Metabolic System; ParvoMedics, Inc.) for the determination of VO<sub>2</sub>. Participants resting and maximum heart rate were used to determine proper exercise intensity (% HRR) during the training protocol.

#### Whole Muscle Function

Peak power of the knee extensor muscle group was assessed before and after the 12-week aerobic training intervention using an inertial ergometer (Inertial Technology, Sweden) connected to a strain gauge load cell and potentiometer interfaced with a personal computer (Gateway E-4200). Following multiple orientation sessions with the knee extensor device, participants performed three identical sessions separated by at least 2 days. All tests were bilateral. Prior to any testing, participants performed a 10-minute warm-up on a stationary bicycle followed by small loads on the resistance apparatus. Participants completed three submaximal repetitions followed by three maximal attempts with 3-minute rest between sets. The concentric power output was recorded throughout the full range of motion. Whole muscle power data are presented with an *N* = 7.

#### Skeletal Muscle Biopsy

Before and after the 12-week aerobic training intervention, a muscle biopsy was obtained from the vastus lateralis of each participant. The posttraining biopsy sample occurred ~48 hours after the last exercise session to avoid any transient alterations from the last training session. Tissue was obtained following local anesthetic (Lidocaine HCl 1%) using a 5-mm Bergstrom needle with suction (21). One ~15 mg piece was placed in RNAlater and stored at -20°C until RNA extraction, one ~15 mg bundle was placed in cold skinning solution and stored at -20°C until fiber isolation, whereas the remaining sample was immediately frozen and stored in liquid nitrogen.

Table 3. Summary of mRNA Primers Used for Real-Time Polymerase Chain Reaction

Protein Isoform	mRNA		Sequence	Gene Bank No.	Amplicon Size (bp)	mRNA Region (bp)
MHC I	MYH7	Sense	5'-TGGGGCTGATGCGCCTATTGAGAA-3'	NM_000257	129	1,953-2,081
		Antisense	5'-TGGGGATGGGTGGAGCGCAAGTT-3'			
MHC IIa	MYH2	Sense	5'-GGAGGCTGAGGAACAATCCAACACCAA-3'	NM_017534	129	5,795-5,923
		Antisense	5'-TCCCGGCTCTCACCCGAGTTT-3'			
MHC IIx	MYH1	Sense	5'-AGTTGCTGACAAGGCAGCCTATCTCCAA-3'	NM_005963	97	1,241-1,337
		Antisense	5'-GACATACTCATTGCCGACCTTGACCCTA-3'			

Note: bp = base pairs; MHC = myosin heavy chain; mRNA = messenger RNA.

### Gene Expression

**Total RNA extraction and RNA quality check.**—Total RNA was extracted in TRI reagent (Molecular Research Center, Cincinnati, OH). The quality and integrity (RNA integrity number of  $8.4 \pm 0.1$ ) of extracted RNA ( $158.1 \pm 17.2$  ng/ $\mu$ L) was evaluated using an RNA 6000 Nano Lab-Chip kit on Agilent 2100 Bioanalyzer.

**Reverse transcription and real-time polymerase chain reaction.**—Oligo-primed first-strand complementary DNA was synthesized (150 ng total RNA) using SuperScript II RT (Invitrogen, Carlsbad, CA). Quantification of mRNA transcription (in duplicate) was performed in a 72-well Rotor-Gene 3,000 Centrifugal Real-Time Cycler (Corbett Research, Mortlake, NSW, Australia). GAPDH was used as a reference gene (22). The validation of GAPDH was performed to ensure that its expression was unaffected by the experimental treatment as we have previously described (23,24). All primers used in this study were mRNA specific (on different exons and over an intron) and were designed for SYBR Green chemistry using Vector NTI Advance 9 software (Invitrogen). Each primer sequence is provided in Table 3. A melting curve analysis was generated validating that only one product was present. The details regarding reverse transcription and polymerase chain reaction parameters have been reported previously (22,24).

**Relative quantitative polymerase chain reaction data analysis.**—Relative gene expression analysis comparing the expression of a gene of interest in relation to a reference gene is based on the distinct cycle (CT) differences. The  $2^{-\Delta C_T}$  method (25) was used in this study as described in detail previously (22,26).

### Mixed-Muscle Homogenate MHC Composition

MHC composition was determined from mixed-muscle fiber homogenates by sodium dodecyl sulfate–polyacrylamide gel electrophoresis as described previously (27). Briefly, 100 ng of protein were run overnight at 4°C on a Hoeffer SE 600 gel electrophoresis unit utilizing a 3.5% acrylamide stacking gel with a 5% separating gel. After electrophoresis, gels were silver stained according to Giulian and colleagues (28). MHC

isoforms (I, IIa, IIx) were identified according to migration rate, and the relative proportion of each MHC isoform was determined by densitometry (FluorChem SP, Alpha Innotech).

### Single Muscle Fiber MHC Distribution

The MHC isoform profile for each fiber was determined by isolating individual fibers under a microscope and performing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SE 600 series; Hoefer, San Francisco, CA) analysis (15). Individual muscle fibers were dissected within 4 weeks of muscle biopsy collection. Following isolation, fibers were solubilized in 80  $\mu$ L of 1% sodium dodecyl sulfate sample buffer (10% sodium dodecyl sulfate, 6 mg/mL ethylenediaminetetraacetic acid, 0.06 M Tris [pH 6.8], 2 mg/mL bromphenol blue, 15% glycerol, and 5%  $\beta$ -mercaptoethanol). These samples were stored at  $-80^\circ\text{C}$  until analyzed for MHC content. Samples were loaded on a 3.5% loading and a 5% separating gel and ran 12 hours at 4°C. The gels were silver stained (28) allowing for the MHC isoform profile (I, I/IIa, I/IIa/IIx, IIa, IIa/IIx, and IIx) for each individual fiber to be determined. The fibers that qualified for each respective profile were taken relative to the total number of fibers per participant to determine the percentage of single-fiber MHC isoforms.

### Statistical Analysis

Pre- and posttraining values for all variables were analyzed using a paired two-tailed student's *t* test. Pearson product–moment correlation coefficients were utilized to examine relationships between selected variables. Significance was accepted as  $p < .05$ . All values are presented as mean  $\pm$  standard error.

## RESULTS

### MHC—mRNA

The mRNA expression for MHC IIx was reduced ( $p < .05$ ) after aerobic training (Figure 1A). MHC I mRNA demonstrated a trend to increase ( $p < .10$ ), whereas the MHC IIa mRNA expression was unaltered after 12 weeks of aerobic exercise training.

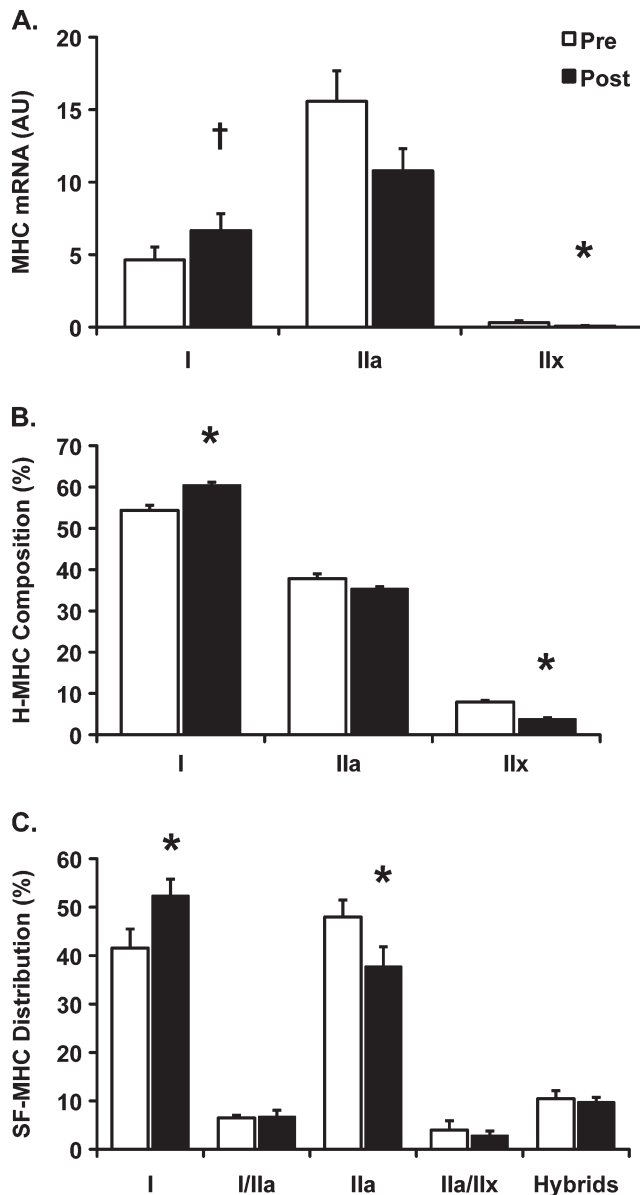


Figure 1. (A) MHC mRNA, (B) mixed-muscle homogenate composition (H-MHC), and (C) single-fiber distribution (SF-MHC) within skeletal muscle of older women pre- and post-aerobic exercise training. Hybrids in (C) signify the collective representation of all hybrid fibers (I/Ila and Ila/Ilx). Data are presented in mean  $\pm$  SE. \* $p < .05$ , <sup>†</sup> $p < .10$  compared with Pre.

#### MHC—Mixed-Muscle Homogenate

Examining MHC composition in the mixed-muscle homogenate revealed an increase ( $p < .05$ ) in MHC I protein from pre- ( $54\% \pm 4\%$ ) to posttraining ( $61\% \pm 2\%$ ; Figure 1B). The percentage of MHC IIX protein decreased ( $p < .05$ ) after training ( $4\% \pm 1\%$ ) compared with pretraining ( $8\% \pm 1\%$ ), whereas the MHC Ila protein proportion was unaffected (pre:  $38\% \pm 4\%$  and post:  $35\% \pm 2\%$ ). Furthermore, the increase in MHC I proportion from a mixed-muscle homogenate was correlated ( $R^2 = .78$ ,  $p < .05$ ) to the improvements

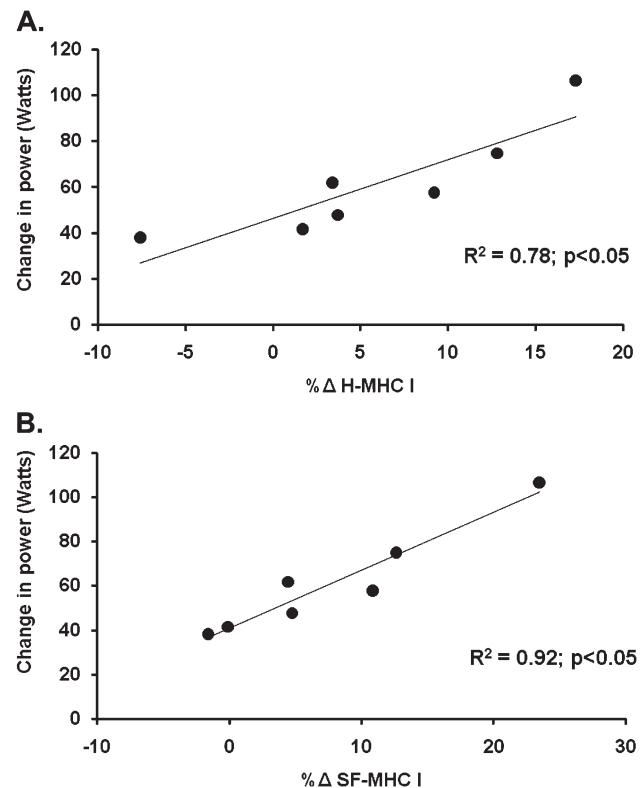


Figure 2. Correlation between the absolute change in whole muscle knee extensor power with the change of myosin heavy chain (MHC) I content in (A) the mixed-muscle homogenate and (B) individual myofibers, with aerobic exercise training in older women ( $R^2 = .78$  and  $.92$  respectively,  $p < .05$ ).

in whole muscle power after aerobic exercise training in older women (Figure 2A).

#### MHC—Single Muscle Fiber

A total of 1,921 single muscle fibers were isolated for MHC analysis. On average, 120 single muscle fibers were examined for myofiber distribution before and after training for each participant. Aerobic training was associated with an increased ( $p < .05$ ) MHC I fiber distribution ( $42\% \pm 4\%$  to  $52\% \pm 3\%$ ) and a reduction ( $p < .05$ ) in MHC Ila fiber distribution ( $48\% \pm 3\%$  to  $38\% \pm 4\%$ ; Figure 1C). The increase in MHC I fibers and concomitant decrease in MHC Ila fibers were highly correlated ( $R^2 = .92$ ,  $p < .05$ ; Figure 3). There were no changes in the percentages of any hybrid isoform profiles, and no pure MHC IIX single fibers were identified. Moreover, there was an association ( $R^2 = 0.92$ ,  $p < .05$ ) between the increase in MHC I fiber distribution and the improvement in whole muscle power after aerobic exercise training (Figure 2B).

#### DISCUSSION

The global purpose of this investigation was to examine the skeletal muscle response to aerobic exercise training in older women. Specifically, we used a multifaceted approach



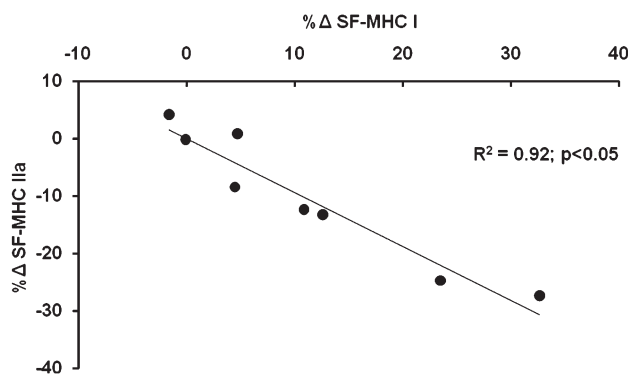


Figure 3. The inverse relationship between the percent change of myosin heavy chain (MHC) I and MHC IIa single-fiber distribution (SF-MHC) after aerobic exercise training in older women ( $R^2 = .92$ ,  $p < .05$ ).

to examine alterations in MHC distribution after an aerobic exercise training program. The main findings from this study were that older women increased MHC I and decreased MHC IIx protein composition, which was accompanied by a trend to upregulate MHC I mRNA and reduce MHC IIx mRNA expression. Additionally, aerobic training evoked an increase in the percentage of individual muscle fibers containing MHC I with a concomitant reduction in the percentage of myofibers containing MHC IIa isoforms. Collectively, reduced fast MHC isoforms (IIa or IIx) with concomitantly increased MHC I isoforms support the notion that aerobic exercise creates an oxidative phenotype in aging skeletal muscle. The shift toward greater relative amounts of MHC I isoforms demonstrates the maintenance of skeletal muscle plasticity with aging and may also indicate a muscle phenotype that is beneficial for metabolic health and functionality in the aging population.

Numerous investigations have suggested that the loss of skeletal muscle mass with age is characterized by a decline in the total number of muscle fibers and specific atrophy of MHC IIa muscle fibers (3,4,29,30). We have previously reported that aerobic exercise training can improve whole muscle size and function in older women (19,20). Interestingly, these improvements occur primarily due to adaptations of MHC I fiber size and contractile function with marginal adaptations in MHC IIa fibers. Therefore, to fully identify the myocellular adaptations to aerobic exercise, it is critical to understand the regulation and distribution of MHC isoforms in aging skeletal muscle.

There have been several factors identified in the regulation of MHC protein composition (31,32), including MHC gene expression (33–35). Therefore, measuring the MHC transcripts is important to recognize the basis for MHC composition in aging skeletal muscle. Aerobic exercise training reduced MHC IIx mRNA expression and tended to elevate MHC I mRNA expression in older women. Additionally, the translated product of these transcripts demonstrated an associated reduction in MHC IIx and increased MHC I protein isoform composition in muscle homogenates. These

data support evidence that link the MHC gene family in regulating the relative MHC protein composition, even after 3 months of exercise training in aging skeletal muscle. Previous reports have suggested that aging skeletal muscle contains an inability to synthesize new proteins from mRNA at rest (36) and/or after exercise training (17,37). This may be due to a possible decline of skeletal muscle protein synthesis with age, although collectively these data are ambiguous (38–40). Additionally, after a 16-week aerobic exercise training protocol conducted by Short and colleagues (17), MHC protein composition at the homogenate level did not change nor reflect alterations in MHC mRNA in older individuals. A principal difference between Short and colleagues (17) and the current study is that our protocol induced MHC I myofiber hypertrophy (19). This is relevant because the electrophoretic assessment of MHC protein composition of a mixed-muscle homogenate has been correlated to percent fiber area of the muscle (41). Thus, when utilizing this index as a reflection of the proportion of myosin isoforms within the whole muscle, it is fundamental to note the influence of myofiber size. It is therefore likely that the considerable increase in the proportion of MHC I protein isoform is due to both an increase in MHC I myofiber size and distribution.

To our knowledge, this is the first investigation to report single-fiber MHC distribution after aerobic exercise training in aging skeletal muscle. Utilizing individually dissected single muscle fibers provides an accurate representation of the fiber type diversity in a given muscle (15,28). Employing either mATPase histochemistry or examining mixed-muscle homogenates to determine percent fiber type area provides difficulties in properly evaluating hybrid fibers that coexpress two or more MHC isoforms, which are common in aging skeletal muscle (7,15). The prevalence of hybrid fibers in aging skeletal muscle is most likely due to physical inactivity and/or denervation, which may render these fibers responsive to exercise training (15).

As hypothesized, we observed an increase in the percentage of MHC I and a concomitant reduction in MHC IIa single fibers, which is similar to observations in younger individuals (13). Given the greater power production of MHC IIa fibers relative to MHC I, this shift to a slow-twitch or oxidative fiber type profile may appear less than optimal for maximal improvements in whole muscle function. However, we have previously demonstrated that this training regimen has provided robust enhancements in whole muscle power (20) accompanied with an increased MHC I myofiber power (19). Therefore, within aging skeletal muscle, the transition to greater MHC I fiber composition appears to create a myocellular milieu that is positive for improvements in whole muscle function after aerobic exercise training. In support of this notion, there was a direct correlation between the increase in MHC I content (both homogenate and single fiber) and whole muscle power with aerobic training in the current study (Figure 2). This relationship is

intriguing as increased MHC IIa myofiber function would likely translate into more robust improvements in whole muscle function because MHC IIa fibers are functionally superior (five to six greater power production) to MHC I fibers (5). However, our laboratory has observed greater plasticity in MHC I fibers in response to resistance (18) and aerobic training (19), which is potentially an age-related adaptation to exercise training in skeletal muscle of older individuals (age  $74 \pm 2$  and  $70 \pm 2$  years, respectively). This response to exercise training in older individuals would most likely result in a more efficient skeletal muscle profile to withstand the exercise training and activities of daily living (42). These observations are supported by Coggan and colleagues (16) who observed a shift toward a slower more oxidative fiber type utilizing mATPase histochemistry methodology in older individuals (age  $64 \pm 3$  years). These data suggest that aging skeletal muscle remains malleable and modifies its phenotype to sustain the functional demands of an aerobic training program.

In conclusion, the beneficial cardiovascular and metabolic health implications of aerobic exercise have been well characterized in young and old individuals. Interestingly, from the comprehensive assessment of MHC distribution after aerobic training, we continue to provide support for the improvement in health of older individuals. After aerobic training, older women demonstrate a greater proportion of slow-twitch MHC I proteins/fibers with a reciprocal decrease in fast-twitch MHC IIx protein and IIa fibers. This shift has potential clinical relevance as high amounts of MHC IIx isoforms or low proportions of MHC I isoforms have been associated with conditions, such as poor insulin sensitivity (43), diabetes (44), obesity (45), and cardiac heart failure (46). Additionally, a large percentage of MHC I fibers correlates with greater oxidative enzyme capacity (47), satellite cell content (48), weight loss (45), and fatigue resistance (42), which are factors that contribute to greater functionality. Moreover, possessing a greater quantity of MHC I fibers may be advantageous because they are responsive to both aerobic and resistance exercise training in older adults (<80 years). Collectively, these data provide evidence that aerobic exercise training creates a MHC profile within skeletal muscle that may be conducive to the overall health in older adults.

#### FUNDING

This investigation was supported by the National Institute of Aging grant AG-32127 to M.P.H.

#### ACKNOWLEDGMENTS

The authors would like to thank Matthew Douglass and Paul Reidy for their assistance during data collection and analysis for this study and Kiril Minchev for technical assistance.

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