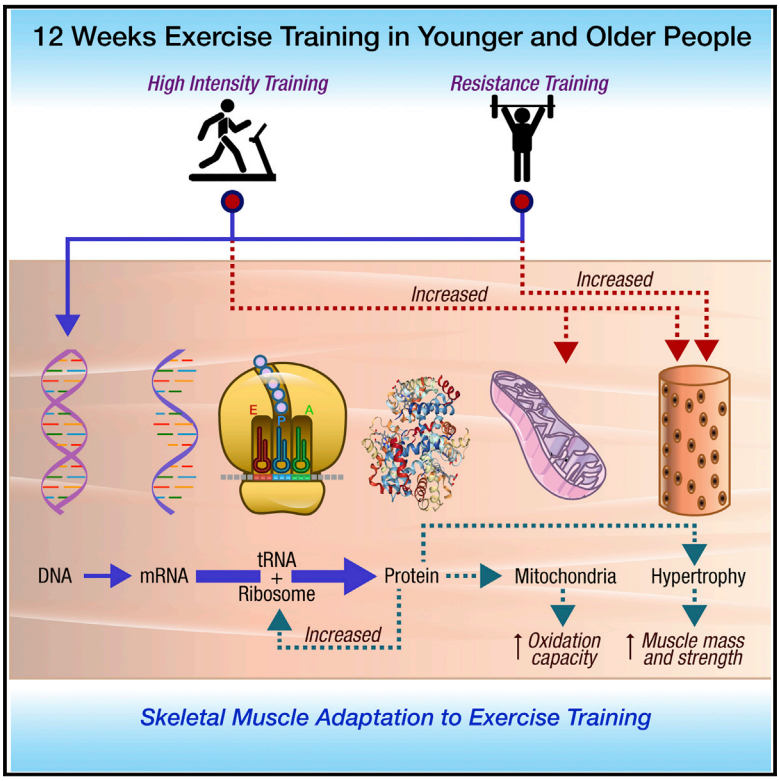
Clinical and Translational Report

Cell Metabolism

Enhanced Protein Translation Underlies Improved Metabolic and Physical Adaptations to Different Exercise Training Modes in Young and Old Humans

Graphical Abstract



Highlights

- High-intensity interval training improved age-related decline in muscle mitochondria
- Training adaptations occurred with increased gene transcripts and ribosome proteins
- Changes to RNA with training had little overlap with corresponding protein abundance
- Enhanced ribosomal abundance and protein synthesis explain gains in mitochondria

Authors

Matthew M. Robinson, Surendra Dasari, Adam R. Konopka, ..., Rickey E. Carter, Ian R. Lanza, K. Sreekumaran Nair

Correspondence

nair.sree@mayo.edu

In Brief

Robinson et al. assessed the effects of three different exercise modalities on skeletal muscle adaptations in young and older adults. While all enhanced insulin sensitivity, only HIIT and combined training improved aerobic capacity, associated with enhanced translation of mitochondrial proteins. HIIT effectively improved cardio-metabolic health parameters in aging adults.

Robinson et al., 2017, Cell Metabolism *25*, 1–12
March 7, 2017
http://dx.doi.org/10.1016/j.cmet.2017.02.009

CellPress

Please cite this article in press as: Robinson et al., Enhanced Protein Translation Underlies Improved Metabolic and Physical Adaptations to Different Exercise Training Modes in Young and Old Humans, Cell Metabolism (2017), http://dx.doi.org/10.1016/j.cmet.2017.02.009

Cell Metabolism
Clinical and Translational Report

Enhanced Protein Translation Underlies Improved Metabolic and Physical Adaptations to Different Exercise Training Modes in Young and Old Humans

Matthew M. Robinson,[1] Surendra Dasari,[2] Adam R. Konopka,[1] Matthew L. Johnson,[1] S. Manjunatha,[1] Raul Ruiz Esponda,[1] Rickey E. Carter,[2] Ian R. Lanza,[1] and K. Sreekumaran Nair[1,3,*]
[1]Division of Endocrinology, Diabetes and Nutrition
[2]Division of Biomedical Statistics and Informatics
Mayo Clinic, Rochester, MN 55905, USA
[3]Lead Contact
*Correspondence: nair.sree@mayo.edu
http://dx.doi.org/10.1016/j.cmet.2017.02.009

SUMMARY

The molecular transducers of benefits from different exercise modalities remain incompletely defined. Here we report that 12 weeks of high-intensity aerobic interval (HIIT), resistance (RT), and combined exercise training enhanced insulin sensitivity and lean mass, but only HIIT and combined training improved aerobic capacity and skeletal muscle mitochondrial respiration. HIIT revealed a more robust increase in gene transcripts than other exercise modalities, particularly in older adults, although little overlap with corresponding individual protein abundance was noted. HIIT reversed many age-related differences in the proteome, particularly of mitochondrial proteins in concert with increased mitochondrial protein synthesis. Both RT and HIIT enhanced proteins involved in translational machinery irrespective of age. Only small changes of methylation of DNA promoter regions were observed. We provide evidence for predominant exercise regulation at the translational level, enhancing translational capacity and proteome abundance to explain phenotypic gains in muscle mitochondrial function and hypertrophy in all ages.

INTRODUCTION

Health benefits of exercise are indisputable in combating age-related risks for disease and disability (Myers et al., 2002), and understanding the transducers of such benefits is of high national interest (Neufer et al., 2015). Aerobic exercise training leads to skeletal muscle protein remodeling and stimulates multiple molecular steps,including DNA methylation (Barrès et al., 2012) and synthesis of new proteins (Short et al., 2003). Many studies have demonstrated changes to mRNA content, but the extent to which transcriptional changes lead to changes in protein abundance remains inconclusive (Miller et al., 2016). Understanding the regulation of skeletal muscle molecular adaptations to diverse types of exercise training can help to develop future targeted therapies and exercise recommendations. There is a gap in knowledge about age effects on pathways regulating exercise adaptations in response to different exercise modalities.

Different types of exercise can stimulate variable, but specific, responses in muscle functions. Aerobic exercise training enhances mitochondrial oxidative enzymes' capacity (Holloszy, 1967) and coincides with improvements to insulin sensitivity with age (Lanza et al., 2008). It remains to be determined whether age-related decline in muscle mitochondrial protein synthesis (Rooyackers et al., 1996) is reversed by aerobic training. High-intensity aerobic interval training (HIIT) involves repeating short bouts of activity at near-maximal intensity, which rapidly and robustly increases aerobic capacity, mitochondrial respiration, and insulin sensitivity in young people (Burgomaster et al., 2008; Irving et al., 2011). Resistance training (RT) reverses sarcopenia and age-related declines in myosin heavy-chain gene transcripts and synthesis rates of muscle proteins (Balagopal et al., 2001), but a comprehensive gene transcripts and proteome comparison with aerobic training has not been performed. Combined training (CT) offers many benefits of both aerobic and resistance training, although the intensity of aerobic and resistance components are lower than either HIIT or standard RT programs (Irving et al., 2015). Lower exercise intensity may limit training adaptations (Ross et al., 2015), particularly of mitochondria (MacInnis et al., 2016). A comprehensive approach to different exercise programs and the specific physiological and molecular adaptations and the potential impact of age on these adaptations remain to be determined.

We performed comprehensive metabolic and molecular phenotyping of young and older adults in response to 12 weeks of aerobic training (using HIIT), RT, and 12 weeks of a sedentary period followed by CT of moderate-intensity aerobic plus resistance training. These measurements were performed 72 hr following the last bout of exercise to specifically determine the training effect. We hypothesized that skeletal muscle transcriptome, translation, and proteome would increase with training, and the pattern of responses would reflect the type of training modality and phenotype changes.

HIIT robustly improved cardio-respiratory fitness, insulin sensitivity, mitochondrial respiration, and fat-free mass (FFM) in both age groups. RT improved FFM and insulin sensitivity in

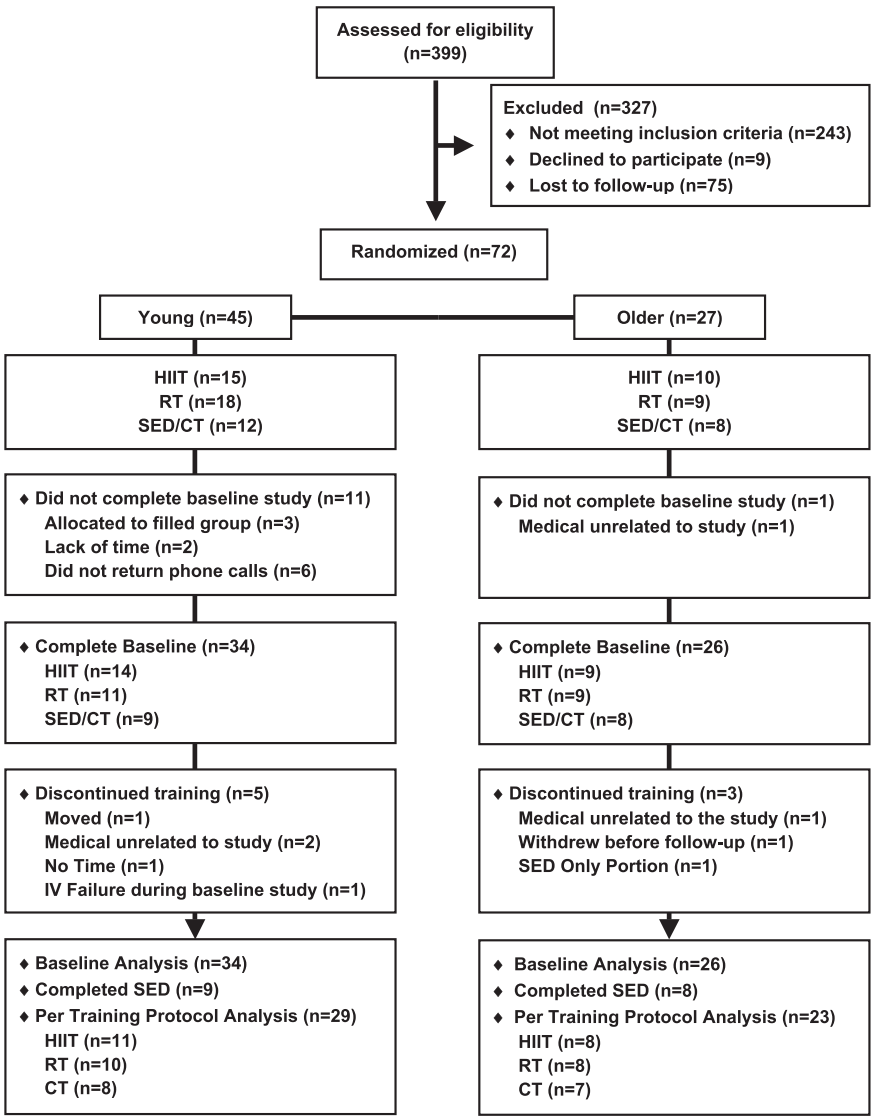


Figure 1. Study Recruitment

Study recruitment flow chart and final group sizes for high-intensity aerobic interval training (HIIT), resistance training (RT), or combined training (CT) that included a 12 week sedentary control period (SED). Five young adults dropped out of study due to time constraints (2), health unrelated to study (2), and IV failure (1). Three older adults dropped out due to health unrelated to study (1), did not want to perform follow up testing (1), and completed sedentary-only portion (1).

both age groups while CT had lesser gains, perhaps due to differences in training intensity. RNA sequencing of muscle biopsies revealed robust increases in mRNA expression with HIIT, more so than RT or CT, particularly of mitochondrial transcripts. Quantitative proteomics in response to HIIT revealed larger proteomic changes, particularly in mitochondrial and ribosome proteins, as well as reversal of many age-related changes. We report relatively small changes (<10%) to methylation of DNA promoter regions and low overlap between transcriptional and proteomic changes. Thus, our findings indicate that the regulation of exercise adaptation is tightly linked with protein translation and translational machinery.

RESULTS AND DISCUSSION

Study Overview

The prospective exercise training study (Figure 1) was approved by the Mayo Clinic Institutional Review Board, registered at https://clinicaltrials.gov (#NCT01477164) and conducted in accordance with the Declaration of Helsinki. All participants provided informed written consent. Participants were recruited into two distinct age groups: young (18–30 years) or older (65–80 years) with a goal of an equal number of men and women. The final groups were approximately balanced for sex, and all women in the older group were postmenopausal. Exclusion criteria were structured regular exercise (>20 min, twice weekly), cardiovascular disease, metabolic diseases (type 2 diabetes mellitus, fasting blood glucose > 110 mg/dL, and untreated hypothyroidism or hyperthyroidism), renal disease, high body mass index (BMI > 32 kg/m^2), implanted metal devices, pregnancy, smoking, and history of blood clotting disorders. Exclusionary medication included anticoagulants, insulin, insulin sensitizers, corticosteroids, sulfonylureas, barbiturates, peroxisome proliferator-activated receptor γ agonists, β blockers, opiates, and tricyclic antidepressants.

Following baseline measurements, the participants were randomized to three groups (HIIT, RT, or CT) using gRand (v1.1, Peter A. Charpentier) following a permuted block strategy with block length of 15 and 2 factors (age and sex). HIIT was 3 days per week of cycling (4 × 4 min at >90% of peak oxygen consumption [$VO_{2\ peak}$] with 3 min pedaling at no load) and 2 days per week of treadmill walking (45 min at 70% of $VO_{2\ peak}$). RT consisted of lower and upper body exercises (4 sets of 8–12 repetitions) 2 days each per week. CT participants first underwent a 12-week sedentary period (SED) and wore accelerometers to record any structured activity. Following SED, participants underwent metabolic studies and began CT of 5 days per week cycling (30 min at 70% $VO_{2\ peak}$) and 4 days per week weight lifting with fewer repetitions than RT. Both baseline and post-training studies were performed in all participants.

Baseline subject characteristics show that older participants had higher body fat percentage, BMI, and fasting plasma glucose concentrations despite similar fasting insulin concentrations (Table 1). During training, the weekly energy expenditure of exercise per FFM was highest with HIIT (Young: 26 ± 3;

Table 1. Baseline Differences between Young and Older Participants

	Young			Older			ANOVA p value		
	HIIT	Resistance	Combined	HIIT	Resistance	Combined	Age	Group	Age × Group
N	14 (7 M/7 F)	11 (5 M/6 F)	9 (5 M/4 F)	9 (4 M/5 F)	9 (5 M/4 F)	8 (5 M/3 F)	N/A	N/A	N/A
Age	25.4 y (4.3 y)	23.7 y (3.5 y)	26.3 y (2.7 y)	70.7 y (4.6 y)	70.3 y (3.9 y)	68.6 y (3.4 y)	N/A	N/A	N/A
Height	174.5 cm (6.9 cm)	172.3 cm (14 cm)	173.4 cm (8.7 cm)	170.7 cm (10.7 cm)	168.9 cm (10.8 cm)	169.5 cm (9.7 cm)	0.1458	0.8745	0.9987
Weight	75 kg (9.7 kg)	73.8 kg (14.3 kg)	77.9 kg (17.1 kg)	80.3 kg (17 kg)	76.4 kg (13.7 kg)	77 kg (16 kg)	0.5534	0.8475	0.8041
BMI	24.6 kg/m^2 (2.3 kg/m^2)	24.7 kg/m^2 (2.7 kg/m^2)	25.6 kg/m^2 (3.3 kg/m^2)	27.3 kg/m^2 (3.3 kg/m^2)	26.6 kg/m^2 (2 kg/m^2)	26.6 kg/m^2 (3.8 kg/m^2)	0.0138	0.8377	0.6606
Body Fat	33.5% (7.1%)	28.3% (8.9%)	31.9% (4.7%)	36.2% (4.6%)	38.5% (4.5%)	37.9% (6.1%)	0.0002	0.5553	0.1088
Fasting Insulin	5.6 μIU/mL (2 μIU/mL)	5.7 μIU/mL (3.8 μIU/mL)	5.5 μIU/mL (2.6 μIU/mL)	5 μIU/mL (3.1 μIU/mL)	5.8 μIU/mL (2.1 μIU/mL)	4.3 μIU/mL (1.8 μIU/mL)	0.5698	0.8185	0.4807
Fasting Glucose	97 mg/dL (4 mg/dL)	96 mg/dL (6 mg/dL)	96 mg/dL (7 mg/dL)	104 mg/dL (11 mg/dL)	103 mg/dL (10 mg/dL)	105 mg/dL (8 mg/dL)	0.0003	0.6446	0.9092

Body composition was measured by dual energy X-ray absorptiometry. Data are mean with SD. BMI, body mass index; FFM, fat-free mass; HIIT, high-intensity interval training; N/A, not applicable.

Older: 18.5 ± 2, $p < 0.001$) followed by CT (Young: 22.8 ± 2; Older 16.9 ± 1, $p < 0.05$) and lowest with RT (Young: 9.6 ± 2; Older 7.3 ± 1, $p < 0.0001$). All baseline comparisons are mean ± SD.

Cardio Respiratory Fitness, Muscle Mass, and Insulin Sensitivity Improve with Training

$VO_{2\ peak}$ during a graded exercise test was determined at baseline and following training. There was a high correlation (r^2 = 0.988, $p < 0.0001$) and low variability between pre- and post-SED $VO_{2\ peak}$ even though measurements were separated by 12 weeks (Young: Pre = 2,643 ± 649, Post = 2,517 ± 603; Old: Pre = 1,646 ± 567, Post = 1,627 ± 550 mL/min, Figure S7). The respiratory exchange ratio (RER) for SED group was also consistent for both young (Pre: 1.2 ± 0.1, Post: 1.2 ± 0.1) and older adults (Pre: 1.2 ± 0.1, Post: 1.2 ± 0.1), indicating that $VO_{2\ peak}$ measurements were done during identical conditions.

Compared to young, older adults had ~30% lower $VO_{2\ peak}$ relative to body weight (Figure 2A). Absolute $VO_{2\ peak}$ (mL/min) significantly increased in the younger group following HIIT (mean[95%CI]: +637[462–812] $p < 0.0001$) with lesser but significant increase with RT (+185[1–368] $p = 0.048$) and CT (+429 [223–634] $p = 0.0001$). In the older group, absolute $VO_{2\ peak}$ also increased following HIIT (278[72–483] $p = 0.0091$) and CT (+295[75–514] $p = 0.0096$); however, the increase in absolute $VO_{2\ peak}$ of the older RT group did not reach statistical significance (+203[−3–409] $p = 0.053$). In the young group, HIIT produced the highest increase of ~28% in relative $VO_{2\ peak}$ (+8.3 [6.2–10.3] $p < 0.0001$ mL/kgBW/min) followed by ~17% with CT (+5.3[2.9–7.6] $p < 0.0001$) (Figure 2B) without any significant increase with RT. In the older group, relative $VO_{2\ peak}$ increased ~17% with HIIT (+3.5[1.2–5.9] $p = 0.0042$) and ~21% with CT (+4.4[1.8–6.9] $p = 0.0011$) without any significant change following RT (+2.3[−0.1–4.6] $p = 0.06$) (Figure 2B).

Frailty with age is largely due to muscle wasting and weakness or sarcopenia (Goodpaster et al., 2006). Declines in FFM and muscle quality (e.g., force per muscle mass) with age contribute to decreased exercise capacity (Delmonico et al., 2009). We investigated the response of muscle mass and quality to different exercise modalities. Baseline whole-body FFM was similar between young and older groups (Figure 2C). Whole-body FFM increased in all training groups, with the greatest increase in young RT (2.2 kg; +4%, $p < 0.0001$; Figure 2D). Leg strength was lower in older humans in absolute terms or relative to leg FFM (Figure 2E, Young: 15.8 ± 3.8, Older: 13 ± 4.1 one-repetition maximum [1RM]/kg leg FFM, $p = 0.017$), suggesting lower muscle quality with age. The training groups with resistance training (RT and CT) had increased leg strength per change in leg mass, indicating an increase in the capacity for a given mass of muscle to produce force (Figure 2F). Leg strength did not change significantly with HIIT, possibly due to training specificity associated with cycling versus leg press exercises. Alternatively, the increase in strength was related to increase in muscle mass. These results demonstrate that both muscle strength and mass robustly improved with CT and RT in both younger and older adults. Collectively, the gains in whole-body FFM suggest that a high-intensity aerobic stimulus can induce both aerobic and hypertrophy adaptations.

Exercise intensity is a strong influence on adaptations. CT had lower-intensity aerobic and resistance components than HIIT and RT, respectively. Approaches to improve exercise responses will have positive benefits on public health, and raising exercise intensity can increase the number of exercise responders (Ross et al., 2015). A previous work in younger adults demonstrated that 12 weeks of HIIT increased $VO_{2\ peak}$ and muscle citrate synthase activity to a similar extent as longer duration of lower-intensity aerobic exercise training (Gillen et al., 2016). We demonstrate that HIIT is a feasible approach to increase exercise intensity in healthy younger and older adults. Younger adults demonstrated more robust increase of $VO_{2\ peak}$ in response to HIIT unlike older adults who responded equally to HIIT and CT (Figure 2B).

Older adults are at risk for developing insulin resistance associated with sedentary lifestyle and gains in adiposity (Karakelides et al., 2010). Exercise can improve insulin sensitivity, and we sought to clearly define the age effect on different types of exercise training and age on insulin sensitivity. For this, we measured peripheral insulin sensitivity as the glucose rate

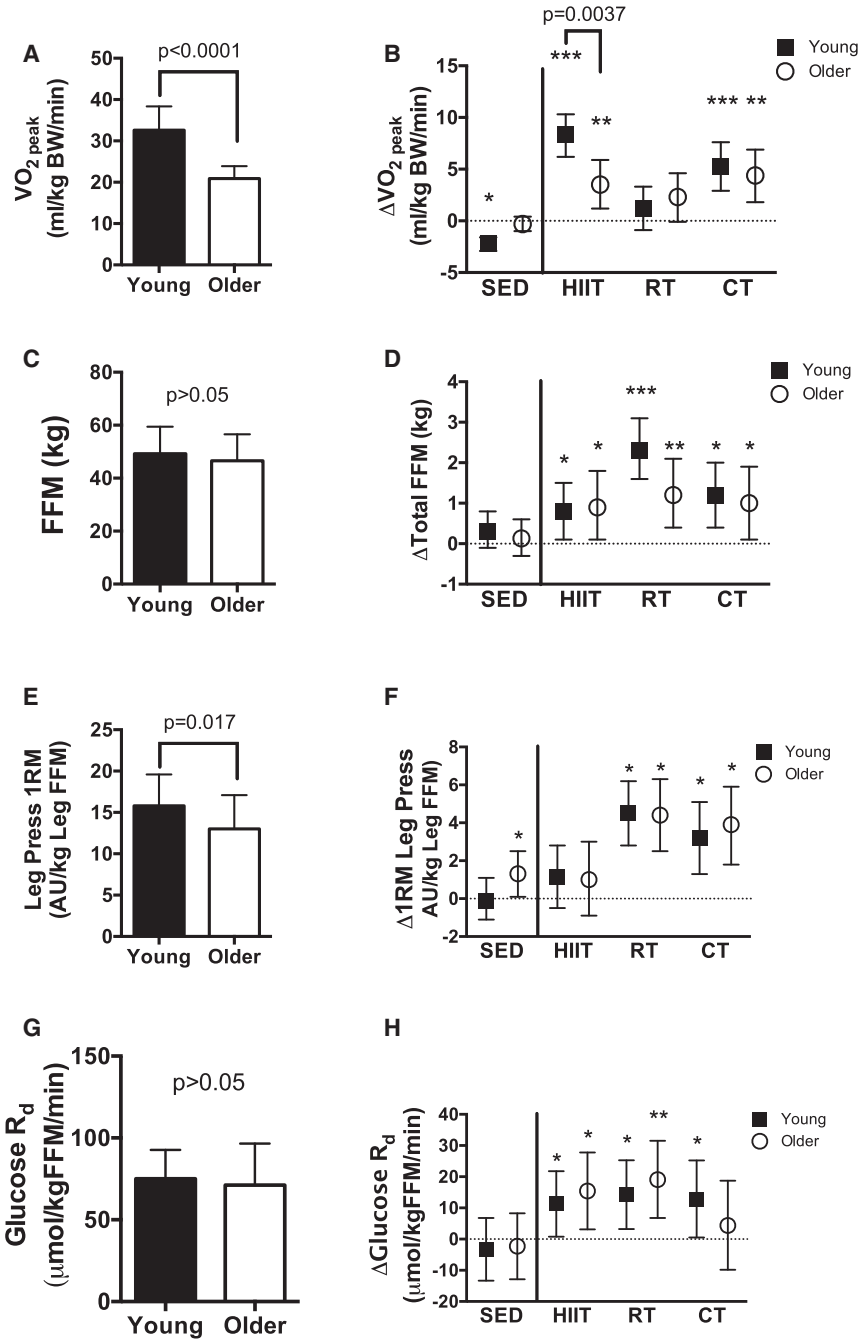


Figure 2. Baseline and Training Differences in Aerobic Fitness, Skeletal Muscle Mass, and Insulin Sensitivity

(A) Peak aerobic capacity per kg body weight ($VO_{2\ peak}$) was measured by indirect calorimetry during a graded exercise test and was lower in older adults at baseline.

(B) Exercise training improved the aerobic phenotype with pronounced gains following 12 weeks of aerobic training using high-intensity interval training (HIIT) compared to resistance training (RT) or combined training (CT) with sedentary (SED) control period.

(C) Fat-free mass (FFM) was measured by dual-energy X-ray absorptiometry and was similar between age groups at baseline.

(D) FFM increased in all training groups, particularly in young RT.

(E) Maximal leg strength, as 1 repetition maximum (1RM) leg press, was lower in older adults at baseline.

(F) 1RM increased in all training groups, with greatest gains following RT and CT.

(G) Insulin sensitivity was measured by a hyperinsulinemic-euglycemic clamp. The glucose rate of disappearance (R_d) during hyperinsulinemia was similar between age groups at baseline.

(H) Glucose R_d increased across training group with non-significant changes following CT in older. Changes during SED were analyzed separately and included in graphs for comparison. Data from baseline comparisons are displayed as mean ± SD with p values for unpaired t test. Changes with training are presented as least square adjusted mean with Tukey honest significant difference (HSD) 95% confidence intervals with the horizontal dotted line set at zero (no change from baseline). Within a training group, a difference between young and old is displayed as exact p value. Statistical significance from baseline is indicated as *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$. Exact p values are reported in Table S8.

of disappearance (R_d [glucose rate of disappearance] μmol/kgFFM/min) during a two-stage hyperinsulinemic-euglycemic clamp (mean ± SD steady state glucose was 88 ± 6 mg/dL) at baseline and after exercise training in all groups (Figures S1 and S3). At baseline, young and older adults had similar insulin sensitivity (Figure 2G). R_d increased in all training groups, except in older CT (Figure 2H). Fasting insulin and glucose did not change with training in either age group (Figure S1). Predominant fates of glucose in skeletal muscle are either oxidative as fuel or non-oxidative for storage as glycogen. Non-oxidative glucose disposal increased with training (Figure S3), indicating greater storage rather than oxidation, which is a useful training adaptation for promoting exercise performance. These results are consistent with a previous cross-sectional study showing chronically trained older and younger adults have similar measurements of insulin sensitivity (Lanza et al., 2008). We did not detect any changes to hepatic insulin sensitivity (Figures S2 and S3), indicating that improvements were predominantly in skeletal muscle metabolism, suggesting that the previous cross-sectional data showing enhanced insulin sensitivity to endogenous glucose production represent long-term (≥4 years) exercise training effect (Lanza et al., 2008).

Mitochondrial Decline with Age and Improvement with Training

A major exercise effect to skeletal muscle metabolism is mitochondrial oxidative capacity. Declines in mitochondrial content

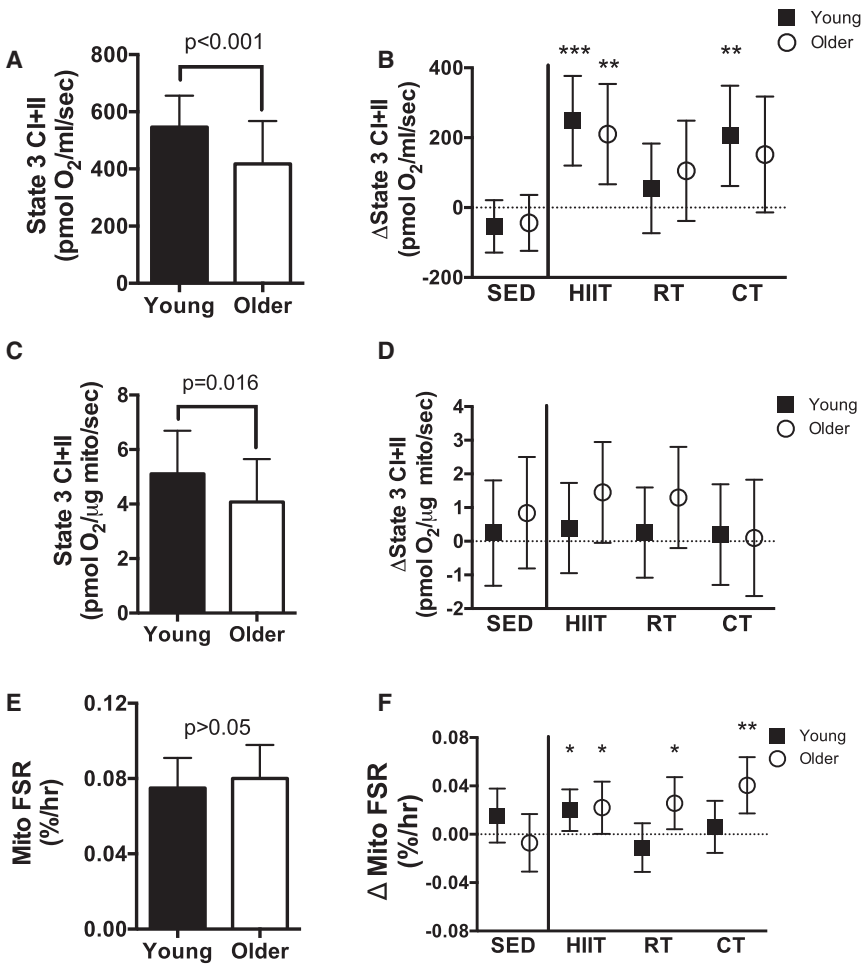


Figure 3. Baseline and Training Differences in Skeletal Muscle Mitochondria Respiration and Protein Synthesis Rates

(A) Mitochondrial respiration was measured using high-resolution respirometry and was lower per tissue content in older adults at baseline.

(B) Mitochondrial respiration per tissue increased with HIIT and CT groups, although less so in older CT.

(C) Mitochondrial respiration per mitochondrial protein content was lower at baseline in older people.

(D) Mitochondrial respiration per mitochondrial protein content did not change with training.

(E) The fractional synthesis rate of mitochondrial proteins (Mito FSR) was measured as the incorporation of [$^{13}C_6$ *ring*]-phenylalanine into mitochondrial proteins isolated from serial muscle biopsies. Mito FSR was similar between age groups at baseline.

(F) Mito FSR increased with HIIT in both age groups and then with RT and CT in the older group. Changes during sedentary (SED) control period were analyzed separately and included in graphs for comparison. Data from baseline comparisons are displayed as mean ± SD with p values for unpaired t test. Changes with training are presented as least square adjusted mean with Tukey HSD 95% confidence intervals with the horizontal dotted line set at zero (no change from baseline). Within a training group, a difference between young and old is displayed as exact p value. Statistical significance from baseline is indicated as *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$. Exact p values are reported in Table S8.

with age are closely linked to reduced cardiorespiratory fitness (Short et al., 2005). Decreased resting mitochondrial ATP production has been implicated in the development of insulin resistance with aging (Petersen et al., 2003). Indeed, a relationship between insulin-resistant states and decreased oxidative enzymes in skeletal muscle has been previously reported in obesity and type 2 diabetes (Simoneau and Kelley, 1997). However, this relationship is not always observed (Karakelides et al., 2010). We investigated aging and exercise training effects on isolated mitochondria from skeletal muscle biopsy samples collected in the resting and fasting state and then determined maximal mitochondrial oxygen consumption by high-resolution respirometry.

At baseline, maximal respiration was lower in older adults compared to young for the respiratory complexes (Complex I+II displayed in Figures 3A and 3C), expressed in either absolute units or normalized to mitochondrial protein content. HIIT increased maximal absolute mitochondrial respiration in young (+49%) and older adults (+69%), whereas a significant increase following CT was observed in young (+38%), but not older adults (Figures 3B and 3D). RT did not increase mitochondrial respiration significantly in either age group. The intrinsic functions of mitochondria, including coupling efficiency and reactive oxygen species production, were not different either between age groups or in response to training (Figures S4A–S4D). Older adults had lower mtDNA copy number when normalized to nDNA, consistent with a decline in mitochondria content with age (Figure S4E). HIIT and RT increased mtDNA content in older adults, with non-significant gains following CT (Figure S4F).

Collectively, the mitochondrial data in our cohort of sedentary, but otherwise healthy, adults indicate that a change in mitochondrial protein content was a predominate contributor to the loss of mitochondrial respiratory capacity with age and gains with training. There was no difference in insulin sensitivity at baseline despite differences in mitochondrial respiration. These results are in agreement with our previous work showing that differences in insulin sensitivity are more related to changes in exercise status and adiposity rather than mitochondrial capacity (Karakelides et al., 2010). Insulin resistance is associated with decreased mitochondrial respiratory chain efficiency and increased reactive oxygen species (ROS) production (Anderson et al., 2009), which can be restored in insulin-resistant women by aerobic training to those of a lean phenotype (Konopka et al., 2015). Our current study of healthy older adults with insulin sensitivity similar to younger adults showed no difference in respiratory chain efficiency or ROS production despite lower mitochondrial capacity than the younger group, supporting a notion that reduced insulin sensitivity is not associated with reduced mitochondrial coupling efficiency.

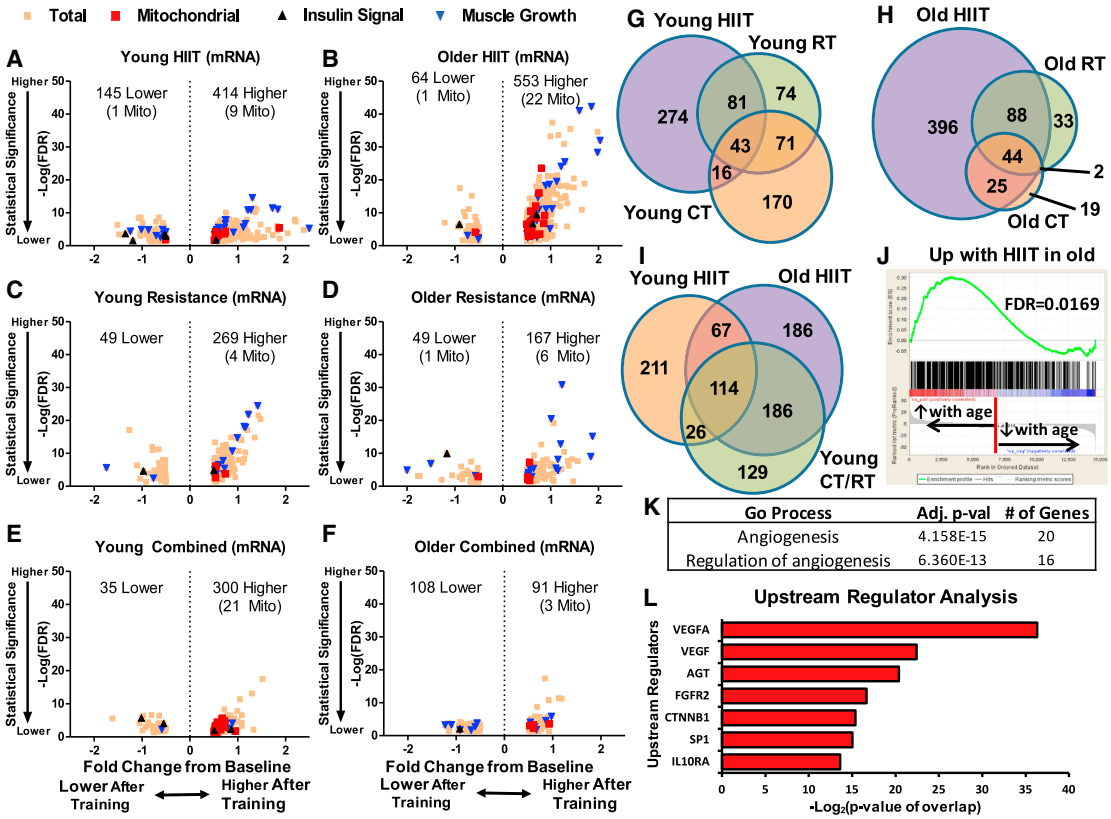


Figure 4. Muscle Gene Expression Changes with Exercise Training

(A–F) Genes that were differentially expressed following high-intensity interval training (HIIT) in the young (A) or older (B), resistance training in the young (C) or older (D), and combined training in the young (E) or older (F) using an adjusted p value of ≤ 0.05 and an absolute fold change of ≥ 0.5 were annotated according to their mitochondrial specificity (using MitoCarta) and molecular function (using KEGG). Mito stands for mitochondrial.

(G and H) Overlap of genes upregulated with different modes of exercise training in younger (G) and older (H) participants.

(I) Overlap of genes that were upregulated with HIIT in older adults and any type of exercise training in younger adults.

(J) Gene set enrichment analysis of baseline gene expression differences between young and old participants against genes that were upregulated with HIIT in older participants. Genes that increased expression with age were more likely to increase their expression with HIIT in older participants.

(K) A "universal exercise training response gene set" was derived by looking for genes that increased with exercise with an adjusted p value of ≤ 0.05 and an absolute fold change of ≥ 0.3 in all groups. Gene Ontology (GO) process annotations enriched for this universal exercise training response gene set was derived using MetaCore software configured with an adjusted p value threshold of ≤ 0.05.

(L) Ingenuity Pathway Analysis (QIAGEN) was used to detect up stream regulators of the "universal exercise training response gene set."

Exercise Training Enhances Skeletal Muscle Gene Expression Regardless of Age

We investigated the extent to which changes in mRNA coincided with phenotypes to further understand the regulation of skeletal muscle changes with age and adaptations to exercise. We performed RNA sequencing on baseline and post-exercise training skeletal muscle biopsies to assess whether transcript levels account for aging or training phenotypes of mitochondria, muscle hypertrophy, and insulin sensitivity. At baseline, when compared to young, 267 gene transcripts were lower and 166 were higher in older people (Figure S5A). Several mitochondrial-, insulin signaling-, and muscle growth-related genes were downregulated with age (Figure S5A). In contrast, among all training regimens, HIIT increased the expression of the largest number of genes in both young and older, especially in mitochondrial, muscle growth, and insulin signaling pathways in older adults (Figures 4A and 4B). Older HIIT increased 22 mitochondrial genes, including those involved with translational regulation (ribosomes *MT-RNR1* and *2*) and mitochondrial tRNA transferase for methionine (*MT-TG*), leucine (*MT-TL1*), valine (*MT-TV*), glycine (*MT-TG*), and arginine (*MT-TR*). When compared to HIIT, RT increased 35% and 70% fewer genes in young and old, respectively (Figures 4C and 4D), and CT increased 28% and 84% fewer genes in young and old, respectively (Figures 4E and 4F). These data demonstrate a varied response of gene transcripts based on exercise mode between young and older adults, and the greatest increase was following HIIT in older adults.

Next, we determined whether training-induced gene sets are specific to training modes in young and older adults. The young had 274, 74, and 170 genes uniquely increased by HIIT, RT, and CT, respectively (Figure 4G). The older had 396, 33, and 19 genes uniquely increased by HIIT, RT, and CT, respectively (Figure 4H). Taken together, these data show that HIIT induced the largest gene expression change regardless of age. In older adults, the changes in gene expression with HIIT completely

subsumes CT and RT changes. Given that older HIIT produced the largest gene expression change, we assessed whether these genes were unique or overlapping with the younger training groups. One-third of the older HIIT genes (181 out of 553) were also shared by the young HIIT group, and 114 of these were shared with young RT and CT groups (Figure 4I). Another third of older HIIT genes were unique to that group (186 out of 553; Figure 4I). Taken together, these data suggest that a large portion of older HIIT genes is age specific.

HIIT had a robust effect on increasing gene transcript content, and we next considered whether training in older individuals reversed age-related loss of muscle gene transcripts, potentially contributing to changes in metabolic phenotypes. To test this, we rank ordered young versus old baseline gene transcript changes from most upregulated to most downregulated with age. A gene set enrichment analysis (GSEA) was performed using genes that were upregulated with either old HIIT (Figure 4J) or young HIIT (Figure S5B). We observed that a majority of genes that were upregulated with HIIT in both groups also were upregulated with age (enrichment false discovery rate [FDR] = 0.0169 for old HIIT and FDR < 0.0001 for young HIIT). Table S1 shows the expression relationship of individual genes that were different with age at baseline and then changed after HIIT in the old. These data support the hypothesis that HIIT is more likely to enhance expression of genes that are also increased with age. There were 11 genes that were significantly decreased in older adults and then were upregulated in older HIIT (Table S1), indicating that HIIT reversed a few specific genes that were decreased with age. Collectively, the gene overlap and GSEA demonstrate that exercise training did not reverse all the age-related declines in gene transcripts per se but induced specific patterns of genes in both young and older adults.

Finally, we were interested in whether there is a common group of genes that are upregulated in all exercise training types and both age groups (i.e., a universal gene set induced with training). A total of 55 genes were upregulated across all training types and in both age groups (Table S2). Gene ontology analysis revealed that these genes are primarily involved in angiogenesis and regulation of angiogenesis (Figure 4K; Table S3). An upstream regulator analysis of the universal gene set identified major transcriptional regulators, like vascular endothelial growth factor, angiotensinogen, fibroblast growth factor, and interleukin 10-receptor subunit (Figure 4L; Table S4). Taken together, the universal exercise training gene set involves cardiovascular remodeling across training and age groups.

Skeletal Muscle Methylation Is Not Significantly Affected by Training

We wanted to test whether the observed exercise training-induced transcriptional changes are related to methylation of DNA. Previous studies show that acute bouts of exercise can alter DNA methylation (Barrès et al., 2012) and influence mRNA expression; yet, the effects of exercise training are less known. Global DNA methylation analysis was performed at baseline and after exercise training in all groups. At baseline, a total of 3,874 promoter CpG sites were differentially methylated between young and old groups (Figure S6). However, we observed statistically insignificant changes in gene promoter methylation due to exercise training in both age groups (Figure S6). These data show that the large gene expression changes observed after 12 weeks of training are not fully explained by a concurrent change in gene promoter methylation.

Previous work was able to detect an ~10% ($p < 0.05$) decrease in methylation of DNA within 20 min of acute exercise (Barrès et al., 2012). Nitert et al. also reported changes to DNA methylation was altered within 48 hr after exercise following 6 months of lower-intensity aerobic training in middle age adults (Nitert et al., 2012). The acute changes to methylation coincide with time course studies showing mRNA content peaks within several hours after exercise followed by a general return to baseline at 24 hr (Louis et al., 2007). The current study demonstrated relatively small changes (<10%) in DNA methylation in comparison to a more substantial increase in mRNA content following exercise training, while studies by others demonstrate changes to DNA methylation in select genes using either shorter times to biopsy sampling (Barrès et al., 2012) or longer training interventions (Nitert et al., 2012). We cannot exclude the possibility of acute changes to DNA methylation, as shown by others (Barrès et al., 2012), that may contribute to dynamic changes in transcription, but our results show no robust differences in DNA methylation by 72 hr.

Training Induces Proteome-wide Expression Changes in Skeletal Muscle

Exercise exerts widespread influence on many muscle proteins (Lanza et al., 2008), yet there is controversy regarding the transcriptional and translational regulation of exercise adaptations. Exercise-induced mRNA do not necessarily translate into proteomic changes (Miller et al., 2016). Hence, we were interested in determining the overlap between mRNA and protein abundance at 72 hr post-exercise. The CT group was not included in the analysis due to attenuated gene expression changes. An intensity-based, label-free proteomics analysis was performed to detect differentially expressed proteins at baseline and post-intervention muscle samples, with significance at an adjusted p value ≤ 0.05 and absolute log2 fold change ≥ 0.5. The SED group had low variability in the control period with only five upregulated proteins, supporting that the changes that we observed in the exercise groups were not time-related changes but occurred in response to exercise. We also considered the pathways of genes and proteins that were expressed with exercise training to provide insight into potential regulatory mechanisms.

Baseline proteomic analysis revealed lower protein abundance in older adults for many proteins but specifically of 33 mitochondrial proteins (Figure 5A), which is consistent with the decreased mitochondrial respiratory capacity in the older group at baseline. Mitochondrial protein abundance increased following RT in both young and old (Figures 5B and 5C), but HIIT produced the largest increase in protein abundance particularly in the older (Figures 5D and 5E), which was consistent with the large changes in gene transcripts in the older with HIIT. HIIT in the older group induced pathways that are reflective of an oxidative phenotype, while both HIIT and RT induced pathways related to protein translation, including aminoacyl-tRNA biosynthesis and tRNA aminoacylation (Table S5). Of the proteins that changed with training, only 35 in the younger and 38 in the older were simultaneously upregulated in both HIIT and RT (Figures 5F and 5G). The gains in mitochondrial protein abundance occurred

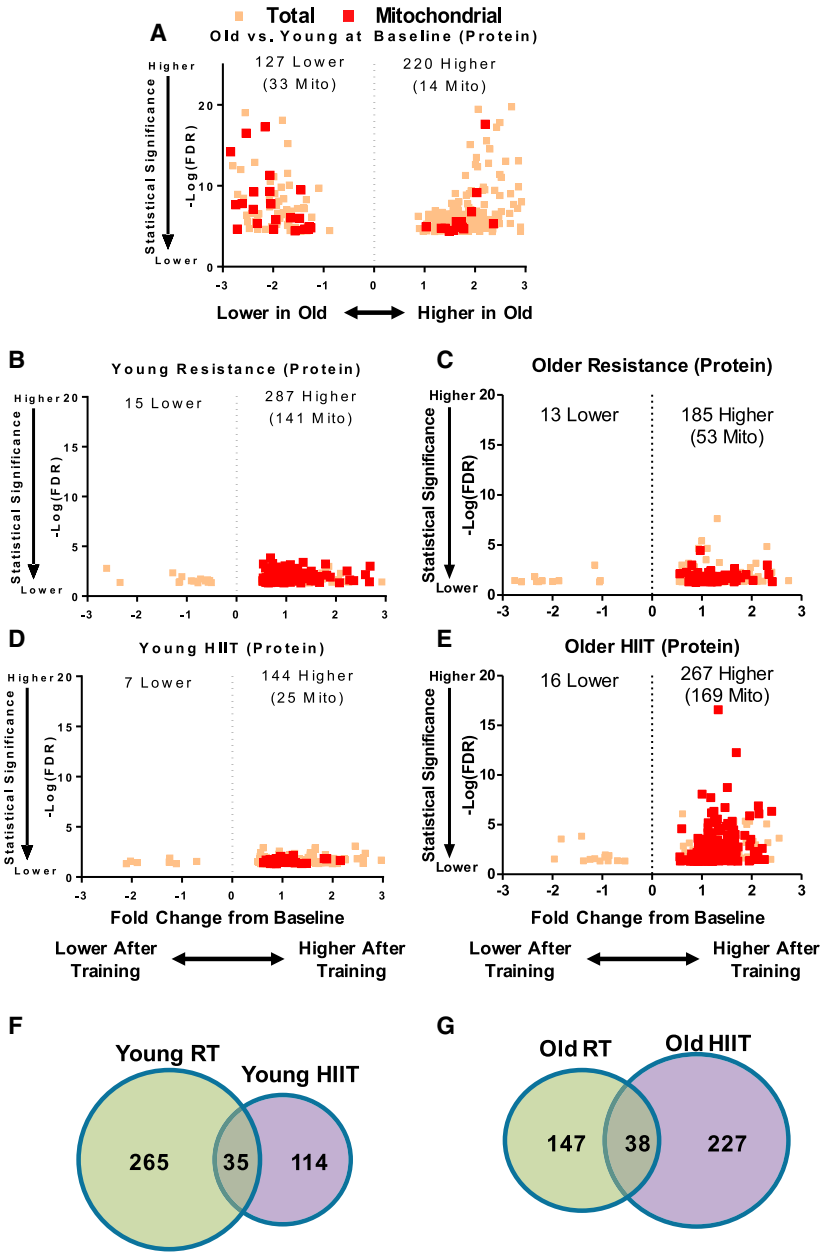


Figure 5. Muscle Protein Abundance with Age and Changes with Exercise Training

(A–E) Baseline differences in muscle protein abundance between young and older adults revealed decreased expression of 33 mitochondrial proteins (A). MaxQuant software configured to process label-free data was used to detect differentially expressed proteins with an adjusted p value of $\leq$0.05 and an absolute fold change of $\geq$0.5 following resistance training in the young (B) or older (C) or high-intensity interval training (HIIT) in the young (D) or older (E). MitoCarta database was used to highlight mitochondrial proteins. Fold change in skeletal muscle protein expression following 12 weeks of resistance (RT) or high-intensity aerobic interval training (HIIT) in young and older adults (B–E). Mitochondrial protein abundance increased in both RT and HIIT modalities with pronounced gains following HIIT in older adults.

(F and G) Overlap of proteins that increased abundance with RT and HIIT in younger (F) and older (G) subjects, respectively.

despite relatively lower changes in mRNA (compare to Figures 4B–4D) and demonstrate a dissociation between mRNA and protein abundance.

Our data suggest that exercise training in older humans can induce a strong upregulation of mitochondrial proteins, predominantly with HIIT. Also, RT regulated a different set of proteins than HIIT in the older group, which is in contrast with high overlap (Figure 4H) between genes induced in older HIIT and RT. Thus, there was a dissociation between proteomic changes compared to the transcriptome. Combined with the increase in protein synthesis rates and overall translational machinery, our data indicate a robust post-transcriptional regulation of protein abundance with exercise training. The common pathways that were induced in all training groups have important roles in protein translation, including tRNA amino acylation and branched-chain amino acid synthesis, as well as upregulation of ribosomal proteins. Collectively, these increases are consistent with increased protein translation capacity, and we next considered whether changes to mRNA were consistent with changes to protein abundance.

We considered whether changes to the synthesis rate of mitochondrial proteins could contribute to the changes in mitochondrial proteome, respiration, or DNA content. We used a stable isotope tracer-based approach to measure protein synthesis rates of skeletal muscle mitochondria. At baseline, there was no difference in protein synthesis rates between young and older groups (Figure 3E). HIIT increased ($p < 0.05$) mitochondrial protein synthesis in both young and older. RT and CT also increased mitochondrial protein synthesis in older, but not younger, adults (Figure 3F). The increase in mitochondrial protein synthesis across training groups in older people demonstrated that mitochondrial adaptations occurred with both aerobic and resistance training protocols. The increase in protein synthesis rates indicates greater translation of mitochondrial proteins and is consistent with results of mitochondrial proteome.

Of the 553 mRNA and 267 proteins that increased with HIIT in the older adults, only 12 were increased at both the mRNA and protein levels. The discrepancy indicates that changes in mRNA do not necessarily lead to changes in protein abundance. At the mRNA level, genes involved in translation to proteins and protein catabolism were significantly downregulated after training in older HIIT (Figure 6A). Yet, at the protein level, pathway

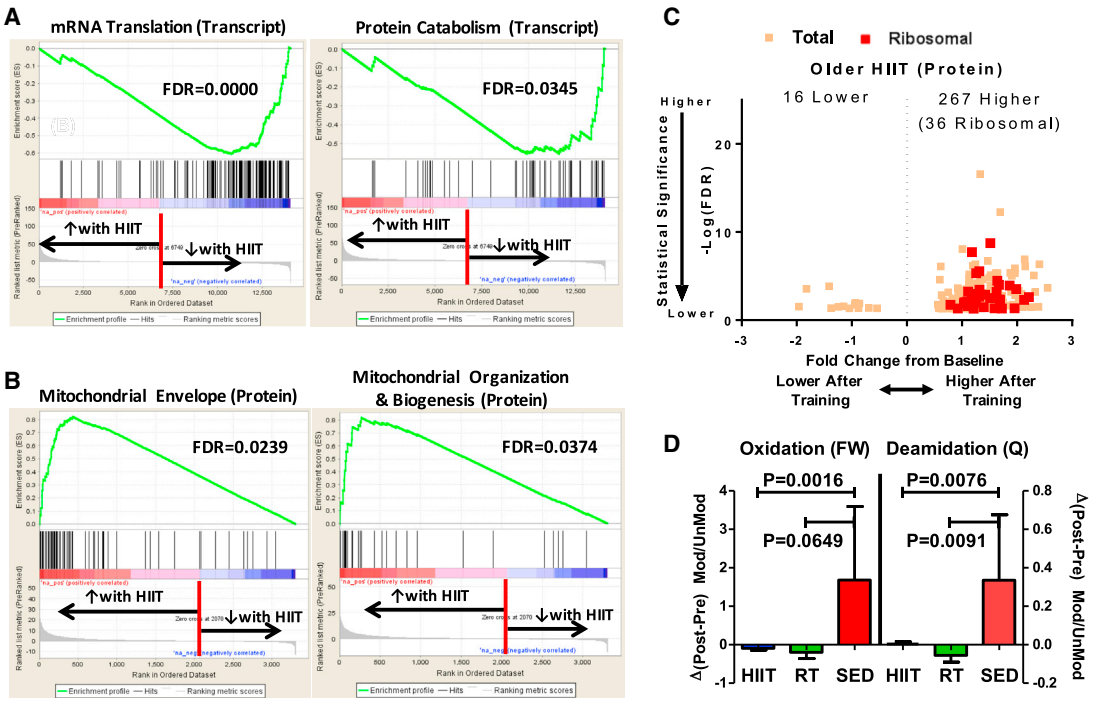


Figure 6. Downregulation of mRNA while Protein Abundance Increased for Mitochondrial and Ribosomal Protein with HIIT in Older Adults

(A) Gene set enrichment analysis of transcripts reveal downregulation of mRNA for translational and protein catabolism pathways between pre-HIIT and post-HIIT in older cohort (adjusted p value < 0.05 and an absolute log2 fold change ≥ 0.5).

(B) Gene set enrichment analysis of proteins demonstrate an increased abundance of mitochondrial proteins between pre-HIIT and post-HIIT in older cohort (adjusted p value < 0.05 and an absolute log2 fold change ≥ 0.5). The software was configured to find enrichment from predefined gene sets in MSigDB.

(C) Ribosomal protein abundance increased with HIIT in older, supporting a role for greater translational capacity. MaxQuant software configured to process label-free data from older HIIT group was used to detect differentially expressed proteins with an adjusted p value of ≤0.05 and an absolute fold change of ≥0.5. Mitochondrial ribosomal proteins are highlighted in red and detailed in Table S7.

(D) Post-translational modification analysis of older subjects showed decreased oxidative damage to proteins after exercise training when compared to sedentary (SED) state. For this, MS1 intensities of peptides with phenylalanine oxidation, tryptophan oxidation, and glutamine deamidation were used to compute the ratio of modified (Mod) to unmodified (UnMod) quantities. Distribution of ratio of Mod/UnMod for all peptides was plotted and (two-sided) p values were computed using Mann-Whitney rank-sum test.

analysis revealed that proteins involved with the mitochondrial envelope and mitochondrial biogenesis were increased with HIIT (Figure 6B). A list of additional mitochondrial pathways is included in Table S6. Previous work in younger men identified 31 differentially expressed proteins that are involved in respiration and citric acid cycle following 7 days of aerobic training (Egan et al., 2011). Our results provide evidence that gains of the mitochondrial proteome occur in response to both aerobic and resistance training, and the response to training persists with longer training duration involving upregulation of the translational machinery, including ribosomal proteins, mitochondrial organization, and biogenesis (synthesis). Of interest, these changes occur robustly in older adults who have reduced mitochondrial biogenesis (Rooyackers et al., 1996) and proteome abundance (Short et al., 2005).

The dissociation between proteome and transcriptome may be explained on the basis of many factors. There are technical issues in comparing mass spectrometry-based proteome and RNA sequencing-based transcriptome with variable sensitivity and precisions of these measurements. Many transcriptomes that might enhance following acute exercise bouts may contribute to translation of proteins, and those transcripts may not show increase at 72 hr following exercise bout. In other words, the half-life of transcriptome and proteome may be different. Moreover, protein abundance is also influenced by the degradation of proteins. Indeed, resistance exercise increases activation of the ubiquitin proteasome and autophagy lysosome pathways that regulate protein degradation in young and older adults, particularly within several hours after exercise (Fry et al., 2013). However, measuring the activation of such pathways does not provide information into which individual proteins are being degraded. We cannot exclude the possibility that changes to individual protein degradation occurred with exercise and therefore may influence the dissociation between mRNA content and protein abundance.

At the protein level, ribosomal proteins were significantly upregulated in old HIIT and, to a lesser degree, in old RT (Figure 6C; Table S7) and provide a mechanism to contribute to the increased mitochondrial protein synthesis. Increases in protein synthesis, and subsequently improved protein turnover, may provide a protective effect against accumulation of proteins with irreversible post-translational modifications. Consistently, there was significantly lower protein oxidation and deamidation observed after training in old HIIT and old RT when compared

to the old SED group (Figure 6D). All of these data support the hypothesis that the changes observed in protein abundance after exercise training were most likely due to translational regulation rather than transcriptional.

CONCLUSION

We assessed the effects of three different exercise modalities on skeletal muscle adaptations in young and older adults and explained on the basis of changes in transcriptome, translational regulation, and proteome abundance. HIIT training in young and older adults increased $VO_{2\ peak}$, insulin sensitivity, mitochondrial respiration, FFM, and muscle strength. In contrast, RT increased insulin sensitivity and FFM, but not $VO_{2\ peak}$ or mitochondrial function. CT involved lower intensity than HIIT or RT groups and resulted in modest gains in FFM and $VO_{2\ peak}$, with modest gains in insulin sensitivity, primarily in young people. Supervised HIIT appears to be an effective recommendation to improve cardio-metabolic health parameters in aging adults.

We were interested in understanding the molecular transducers of exercise adaptations and performed RNA sequencing to determine changes to gene transcripts in skeletal muscle biopsies. HIIT robustly increased gene expression, particularly in older adults, while RT and CT had less pronounced effects in both age groups. Of interest, a set of gene transcripts were increased with HIIT in both young and older groups despite select genes having either greater or lower content at baseline in older adults. These data demonstrated that HIIT induced a pattern of gene expression regardless of age. Finally, HIIT also had robust increases in transcriptional and translational regulation of muscle growth and mitochondrial pathways.

Our study was powered to detect relevant effect sizes at the proteomic level, which demonstrated robust gains, particularly in proteins regulating translation. There were also robust effect sizes for training groups on metabolic phenotypes. For example, HIIT training in older adults had strong effect sizes in multiple outcomes, including mitochondrial respiration (1.7), aerobic fitness (0.99), insulin sensitivity (0.5), and smaller effect sizes for 1RM leg press (0.3) and FFM (0.1). Other parameters, such as DNA methylation, did not detect differences, and we cannot exclude the possibility of type II error. Additionally, a source of variability between mRNA content and protein abundance is the potential for splice variants to generate peptides that may not be annotated in mass spectrometry libraries. A high degree of alternative splicing could impact our datasets and potentially underestimate the relationship between protein abundance and mRNA content, as multiple splice variants may lead to the same peptide.

The increases in specific muscle proteins were greater relative to changes in mRNA content, particularly in mitochondrial and ribosomal proteins, and demonstrate a lack of direct relation between transcriptional and proteomic adaptations. DNA methylation is a regulatory point for transcription and had relatively small changes. Collectively, these data suggest that exercise adaptations are regulated to a greater extent at the post-transcriptional level. Increased ribosome protein content and other proteins involved in the translational machinery were detected following HIIT and provide for increased translational capacity. Mitochondrial protein synthesis was increased with HIIT as directly measured by isotope incorporation (representing translation). These data demonstrate an increase in both the protein translation machinery and synthesis rates of proteins. We also found a lowering of post-translational protein damages (oxidation and deamidation) following exercise training that may improve the functional quality of proteins. The increased mitochondrial protein synthesis, along with proteomic gains, despite differences in mRNA transcripts, support the hypothesis that translational level regulation is a predominant factor of mitochondrial biogenesis in human in response to exercise training. Further support for the above notion is provided by the increase in ribosomal protein content despite a fall in ribosomal transcript levels. The increases in specific proteins in muscle were greater relative to the changes in mRNA content, particularly in mitochondrial proteins and ribosomal proteins, and this demonstrates a lack of direct relation between transcriptional and proteomic abundances when measured 72 hr following the last bout of exercise. Together, the current results demonstrated a predominant regulation of exercise adaptations at the post-transcriptional level.

EXPERIMENTAL PROCEDURES

Study procedures are summarized here and detailed in the Supplemental Experimental Procedures.

$VO_{2\ peak}$ and Body Composition

$VO_{2\ peak}$ was measured with indirect calorimetry (Medgraphics Diagnostics) and an electronically braked cycle ergometer (Lode Medical Technologies). $VO_{2\ peak}$ was defined as reaching a perceived exertion > 17 on the Borg scale with RER > 1.1 (mean ± SD [Range] for Young: 1.2 ± 0.1 [1.1–1.43]; Older 1.2 ± 0.1 [0.98–1.36]) and achieving a heart rate within 10% of age-predicted maximal heart rate. Body composition was measured after an overnight fast with dual-energy X-ray absorptiometry as previously described (Nair et al., 2006).

Exercise Training

A 3-month, supervised, exercise-training program was conducted at the Dan Abraham Healthy Living Center. The Supplemental Experimental Procedures include the complete exercise list. 1RM leg press was determined as previously described (Irving et al., 2015). Aerobic exercise training zones were prescribed as the heart rate at a percentage of $VO_{2\ peak}$ and maintained ±5 beats/minute. $VO_{2\ peak}$ was measured during week 6 in HIIT and CT groups to increase training zones due to gains in aerobic fitness.

Metabolic Measurements

All pre- and post-intervention metabolic measurements were performed after 3 days of weight-maintenance meals (20% protein, 50% carbohydrates, and 30% fat). Participants refrained from exercise for 72 hr prior to metabolic study days.

Insulin Sensitivity

Hepatic and peripheral insulin sensitivity was measured using a two-stage euglycemic clamp (85–95 mg/dL) with somatostatin (Rizza et al., 1981). Glucose kinetics were determined using [6,6]-$^2H_2$-glucose and indirect calorimetry (Lalia et al., 2015). Insulin was infused in two 3 hr stages at low and high rates (0.62 and 2.3 mU/kg FFM/min, respectively). Plasma glucose, tracer enrichment, and hormone concentrations for both age groups are provided (Figures S1–S3). Hepatic insulin sensitivity was computed as suppression of endogenous glucose production from basal to low insulin infusion. Peripheral insulin sensitivity was computed as glucose disposal during the final 60 min of the high-insulin stage.

Muscle Protein Synthesis

Fractional synthesis rate (FSR) of muscle proteins from biopsies of the current cohort was measured using isotopic tracer methodology. Participants were

fasted overnight and a resting biopsy of the vastus lateralis (with 2% lidocaine) was collected at 0700 hr followed by a primed continuous infusion of *ring*-[$^{13}C_6$]-phenylalanine (1.5 mg/kg FFM prime and 1.5 mg/kg FFM/hr infusion). Biopsies were collected at 1,000 hr and 1,500 hr during isotopic steady state, and FSR (%/hr) was calculated as $[\Delta E/(E_p \times t)] \times 100$, where t was hours between biopsies, ΔE was the tissue protein enrichment between biopsies (ΔE), and E_p was tissue fluid precursor enrichment.

Mitochondrial Function

Mitochondria were isolated from the 0700 hr biopsy and analyzed by high-resolution respirometry (Lanza and Nair, 2009). Mitochondria were added to a 2 mL chamber (Oxygraph-2K, Oroboros) followed by sequential additions of glutamate, malate, ADP, succinate, and inhibitors. Mitochondrial membrane integrity was verified with cytochrome-c.

Omics Measurements and Bioinformatics

Gene Expression Analysis

We measured mRNA expression as previously described (Lanza et al., 2012) with slight modifications. In brief, total RNA was isolated from the biopsy collected at 1,000 hr, and sequencing libraries were prepared with TruSeq RNA Sample Prep Kit v2. Libraries were sequenced on a HiSeq 2000 sequencer using TruSeq SBS sequencing kit version 3 and HCS version 2.0.12.0 software. Genes with a FDR-corrected p value of ≤ 0.05 and an absolute log2 fold change of ≥ 0.5 (where 0.0 signifies no change) were considered for further analysis.

Proteomics

Proteins were extracted from the 1,000 hr muscle biopsy sample and were subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Lanza et al., 2012). Label-free LC-MS/MS data were acquired using a high-resolution QExactive MS (Thermo Fisher Scientific). A total of 9.3 million MS/MS were collected from all analyses. MaxQuant software (v.1.5.1.2) was configured to match MS/MS against RefSeq human sequence database (v.58) and identify peptides and proteins at 1% FDR. Between two experimental groups, protein groups with a differential expression (corrected) p value of ≤ 0.05 and an absolute $\log_2$ fold change (FC) of ≥ 0.5 were considered for further analysis. Sample level protein oxidation and deamination were quantified by obtaining peptide-wise ratios of modified to unmodified (Mod/UnMod) intensities (Martin et al., 2016).

Methylation Analysis

Methylation analysis on the 1,000 hr skeletal muscle samples was performed using Infinium 450K array (Illumina). Raw chip data were processed using GenomeStudio (v.2011.1) to remove background, normalize for chip-to-chip variation, and compute β (% methylation) values for each CpG site. Partek Genomics Suite (v.6.13.0612; Partek) analyzed the CpG beta values between any two groups using ANOVA models. CpG sites that had a corrected p value $\leq$ 0.05 and an absolute $\Delta\beta$ value of $\geq 0.05\%$ were considered as significant. CpG sites that were within the promoter regions (defined as 2,000 bp $\leq$ transcription start site $\leq$ 500 bp) of known genes were considered for further analysis.

Pathway and Gene Set Enrichment Analysis

Genes or proteins that were statistically up- or downregulated with different phenotypes (age at baseline and exercise training) were subjected to pathway analysis using WEBGESTALT software. Genes that were upregulated with all exercise training types in both age groups ("universal exercise response gene set") were subjected to Gene Ontology (GO) process enrichment using MetaCore software (Thompson Reuters) and upstream regulator analysis using Ingenuity Pathway Analysis (QIAGEN). Gene set enrichment analysis was performed using Broad's GSEA software. All enrichment p values were FDR corrected (using Benjamini-Hochberg procedure), and entities with p values $\leq$ 0.05 were reported.

Statistics

Metabolic metrics were analyzed using JMP 10 (SAS Institute). Continuous baseline metrics between young and old groups were compared using two-way ANOVA (age × training). The SED group served as a no-treatment control, and pre- and post-intervention values were compared with a paired t test and p values from post hoc comparisons in the ANOVA models were corrected with Tukey's procedure. Training effect (HIIT, RT, and CT) on metabolic metrics was evaluated by computing delta change in the metrics with intervention and comparing them using two-way ANOVA (age × training). Metabolic data in graphs are shown as least-squares adjusted means (type III population estimates) with 95% confidence intervals corrected with Tukey's procedure. The power was based on a two-sample t test with 20 per exercise training group (60 in total). The sample size was estimated a priori from a cross-sectional comparison of myosin synthesis rates that decline with age (mean [SD] for young, 0.64 [0.3]; older, 0.36 [0.4]). The detectable effect size for two-sided test with $\alpha = 0.05$ and 80% power was 0.9 SD.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and eight tables and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2017.02.009.

AUTHOR CONTRIBUTIONS

M.M.R., A.R.K, M.L.J., R.R.E., S.M., and I.R.L. performed exercise testing and sample collection. M.M.R, S.D., R.E.C., and K.S.N. analyzed and interpreted the data. M.M.R. wrote the manuscript, and all authors contributed to editing. K.S.N. provided project oversight, final approval of the manuscript, and funding acquisition.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

We thank the participants for their time and enthusiasm during the project. We acknowledge the skillful assistance of Melissa Aakre, Roberta Soderberg, Jill Schimke, Dan Jakaitis, Lynne Johnson, Katherine Klaus, Dawn Morse, Dr. Brian Irving, and Mandie Maroney-Smith, along with the staff of the Dan Abraham Healthy Living Center and Clinical Research Unit at Mayo Clinic. Funding was provided by R01AG09531 (K.S.N.), T32DK7352 (M.M.R. and A.R.K.), and T32 DK007198 (M.L.J.). Additional support was provided by the Mayo Foundation, Kogod Aging Center, and the Murdock-Dole Professorship (to K.S.N.). This publication was made possible by the Mayo Clinic Metabolomics Resource Core through grant number U24DK100469 from the National Institute of Diabetes and Digestive and Kidney Diseases and the Mayo Clinic CTSA grant UL1TR000135 from the National Center for Advancing Translational Sciences (NCATS).

Received: August 16, 2016
Revised: December 7, 2016
Accepted: February 16, 2017
Published: March 7, 2017

REFERENCES

Anderson, E.J., Lustig, M.E., Boyle, K.E., Woodlief, T.L., Kane, D.A., Lin, C.T., Price, J.W., 3rd, Kang, L., Rabinovitch, P.S., Szeto, H.H., et al. (2009). Mitochondrial H2O2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. J. Clin. Invest. *119*, 573–581.

Balagopal, P., Schimke, J.C., Ades, P., Adey, D., and Nair, K.S. (2001). Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise. Am. J. Physiol. Endocrinol. Metab. *280*, E203–E208.

Barrès, R., Yan, J., Egan, B., Treebak, J.T., Rasmussen, M., Fritz, T., Caidahl, K., Krook, A., O'Gorman, D.J., and Zierath, J.R. (2012). Acute exercise remodels promoter methylation in human skeletal muscle. Cell Metab. *15*, 405–411.

Burgomaster, K.A., Howarth, K.R., Phillips, S.M., Rakobowchuk, M., Macdonald, M.J., McGee, S.L., and Gibala, M.J. (2008). Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. J. Physiol. *586*, 151–160.

Delmonico, M.J., Harris, T.B., Visser, M., Park, S.W., Conroy, M.B., Velasquez-Mieyer, P., Boudreau, R., Manini, T.M., Nevitt, M., Newman, A.B., and

Goodpaster, B.H.; Health, Aging, and Body (2009). Longitudinal study of muscle strength, quality, and adipose tissue infiltration. Am. J. Clin. Nutr. *90*, 1579–1585.

Egan, B., Dowling, P., O'Connor, P.L., Henry, M., Meleady, P., Zierath, J.R., and O'Gorman, D.J. (2011). 2-D DIGE analysis of the mitochondrial proteome from human skeletal muscle reveals time course-dependent remodelling in response to 14 consecutive days of endurance exercise training. Proteomics *11*, 1413–1428.

Fry, C.S., Drummond, M.J., Glynn, E.L., Dickinson, J.M., Gundermann, D.M., Timmerman, K.L., Walker, D.K., Volpi, E., and Rasmussen, B.B. (2013). Skeletal muscle autophagy and protein breakdown following resistance exercise are similar in younger and older adults. J. Gerontol. A Biol. Sci. Med. Sci. *68*, 599–607.

Gillen, J.B., Martin, B.J., MacInnis, M.J., Skelly, L.E., Tarnopolsky, M.A., and Gibala, M.J. (2016). Twelve weeks of sprint interval training improves indices of cardiometabolic health similar to traditional endurance training despite a five-fold lower exercise volume and time commitment. PLoS ONE *11*, e0154075.

Goodpaster, B.H., Park, S.W., Harris, T.B., Kritchevsky, S.B., Nevitt, M., Schwartz, A.V., Simonsick, E.M., Tylavsky, F.A., Visser, M., and Newman, A.B. (2006). The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. J. Gerontol. A Biol. Sci. Med. Sci. *61*, 1059–1064.

Holloszy, J.O. (1967). Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. J. Biol. Chem. *242*, 2278–2282.

Irving, B.A., Short, K.R., Nair, K.S., and Stump, C.S. (2011). Nine days of intensive exercise training improves mitochondrial function but not insulin action in adult offspring of mothers with type 2 diabetes. J. Clin. Endocrinol. Metab. *96*, E1137–E1141.

Irving, B.A., Lanza, I.R., Henderson, G.C., Rao, R.R., Spiegelman, B.M., and Nair, K.S. (2015). Combined training enhances skeletal muscle mitochondrial oxidative capacity independent of age. J. Clin. Endocrinol. Metab. *100*, 1654–1663.

Karakelides, H., Irving, B.A., Short, K.R., O'Brien, P., and Nair, K.S. (2010). Age, obesity, and sex effects on insulin sensitivity and skeletal muscle mitochondrial function. Diabetes *59*, 89–97.

Konopka, A.R., Asante, A., Lanza, I.R., Robinson, M.M., Johnson, M.L., Dalla Man, C., Cobelli, C., Amols, M.H., Irving, B.A., and Nair, K.S. (2015). Defects in mitochondrial efficiency and H2O2 emissions in obese women are restored to a lean phenotype with aerobic exercise training. Diabetes *64*, 2104–2115.

Lalia, A.Z., Johnson, M.L., Jensen, M.D., Hames, K.C., Port, J.D., and Lanza, I.R. (2015). Effects of dietary n-3 fatty acids on hepatic and peripheral insulin sensitivity in insulin-resistant humans. Diabetes Care *38*, 1228–1237.

Lanza, I.R., and Nair, K.S. (2009). Functional assessment of isolated mitochondria in vitro. Methods Enzymol. *457*, 349–372.

Lanza, I.R., Short, D.K., Short, K.R., Raghavakaimal, S., Basu, R., Joyner, M.J., McConnell, J.P., and Nair, K.S. (2008). Endurance exercise as a countermeasure for aging. Diabetes *57*, 2933–2942.

Lanza, I.R., Zabielski, P., Klaus, K.A., Morse, D.M., Heppelmann, C.J., Bergen, H.R., 3rd, Dasari, S., Walrand, S., Short, K.R., Johnson, M.L., et al. (2012). Chronic caloric restriction preserves mitochondrial function in senescence without increasing mitochondrial biogenesis. Cell Metab. *16*, 777–788.

Louis, E., Raue, U., Yang, Y., Jemiolo, B., and Trappe, S. (2007). Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle. J. Appl. Physiol. *103*, 1744–1751.

MacInnis, M.J., Zacharewicz, E., Martin, B.J., Haikalis, M.E., Skelly, L.E., Tarnopolsky, M.A., Murphy, R.M., and Gibala, M.J. (2016). Superior mitochondrial adaptations in human skeletal muscle after interval compared to continuous single-leg cycling matched for total work. J. Physiol. Published online July 11, 2016. http://dx.doi.org/10.1113/JP272570.

Martin, D.R., Dutta, P., Mahajan, S., Varma, S., and Stevens, S.M., Jr. (2016). Structural and activity characterization of human PHPT1 after oxidative modification. Sci. Rep. *6*, 23658.

Miller, B.F., Konopka, A.R., and Hamilton, K.L. (2016). The rigorous study of exercise adaptations: why mRNA might not be enough. J. Appl. Physiol. *121*, 594–596.

Myers, J., Prakash, M., Froelicher, V., Do, D., Partington, S., and Atwood, J.E. (2002). Exercise capacity and mortality among men referred for exercise testing. N. Engl. J. Med. *346*, 793–801.

Nair, K.S., Rizza, R.A., O'Brien, P., Dhatariya, K., Short, K.R., Nehra, A., Vittone, J.L., Klee, G.G., Basu, A., Basu, R., et al. (2006). DHEA in elderly women and DHEA or testosterone in elderly men. N. Engl. J. Med. *355*, 1647–1659.

Neufer, P.D., Bamman, M.M., Muoio, D.M., Bouchard, C., Cooper, D.M., Goodpaster, B.H., Booth, F.W., Kohrt, W.M., Gerszten, R.E., Mattson, M.P., et al. (2015). Understanding the cellular and molecular mechanisms of physical activity-induced health benefits. Cell Metab. *22*, 4–11.

Nitert, M.D., Dayeh, T., Volkov, P., Elgzyri, T., Hall, E., Nilsson, E., Yang, B.T., Lang, S., Parikh, H., Wessman, Y., et al. (2012). Impact of an exercise intervention on DNA methylation in skeletal muscle from first-degree relatives of patients with type 2 diabetes. Diabetes *61*, 3322–3332.

Petersen, K.F., Befroy, D., Dufour, S., Dziura, J., Ariyan, C., Rothman, D.L., DiPietro, L., Cline, G.W., and Shulman, G.I. (2003). Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science *300*, 1140–1142.

Rizza, R.A., Mandarino, L.J., and Gerich, J.E. (1981). Dose-response characteristics for effects of insulin on production and utilization of glucose in man. Am. J. Physiol. *240*, E630–E639.

Rooyackers, O.E., Adey, D.B., Ades, P.A., and Nair, K.S. (1996). Effect of age on in vivo rates of mitochondrial protein synthesis in human skeletal muscle. Proc. Natl. Acad. Sci. USA *93*, 15364–15369.

Ross, R., de Lannoy, L., and Stotz, P.J. (2015). Separate effects of intensity and amount of exercise on interindividual cardiorespiratory fitness response. Mayo Clin. Proc. *90*, 1506–1514.

Short, K.R., Vittone, J.L., Bigelow, M.L., Proctor, D.N., Rizza, R.A., Coenen-Schimke, J.M., and Nair, K.S. (2003). Impact of aerobic exercise training on age-related changes in insulin sensitivity and muscle oxidative capacity. Diabetes *52*, 1888–1896.

Short, K.R., Bigelow, M.L., Kahl, J., Singh, R., Coenen-Schimke, J., Raghavakaimal, S., and Nair, K.S. (2005). Decline in skeletal muscle mitochondrial function with aging in humans. Proc. Natl. Acad. Sci. USA *102*, 5618–5623.

Simoneau, J.A., and Kelley, D.E. (1997). Altered glycolytic and oxidative capacities of skeletal muscle contribute to insulin resistance in NIDDM. J. Appl. Physiol. *83*, 166–171.

Cell Metabolism, Volume *25*

Supplemental Information

Enhanced Protein Translation Underlies Improved Metabolic and Physical Adaptations to Different Exercise Training Modes in Young and Old Humans

Matthew M. Robinson, Surendra Dasari, Adam R. Konopka, Matthew L. Johnson, S. Manjunatha, Raul Ruiz Esponda, Rickey E. Carter, Ian R. Lanza, and K. Sreekumaran Nair

Figure S1, related to Figure 2: Plasma and insulin concentrations during clamp. Plasma glucose (Pre: closed circle; Post: open square) and insulin (Pre: closed square; post: open triangle) in young participants before and after high-intensity interval training (HIIT, **A**), resistance training (RT, **B**) combined training (CT, **C**) or sedentary period (SED, **D**) or older participants following HIIT (**E**), RT (**F**), CT (**G**) or SED (**H**). Shaded region represents target glucose at 85-95 mg/dl. Data displayed as mean±SEM.

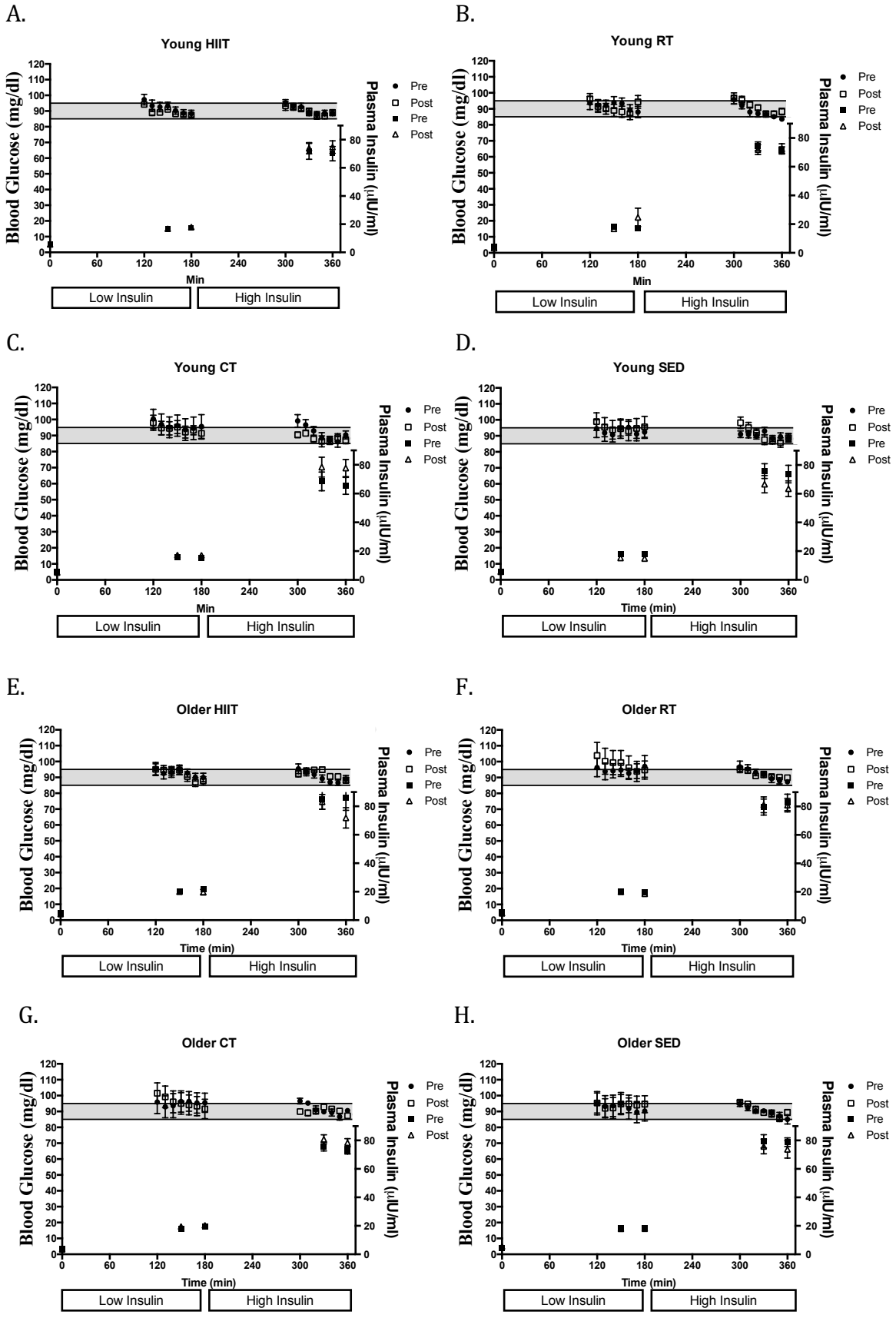


Figure S2, related to Figure 2: Plasma tracer enrichment during clamp. Plasma [6,6]-H_2 glucose enrichment during basal, low and high insulin infusion as baseline or following sedentary (SED) in young (**A**) and older (**B**), high-intensity interval training (HIIT) in young (**C**) or older (**D**), resistance training in young (**E**) or older (**F**) and combined training in young (**G**) or older (**H**). Data displayed as mean±SD.

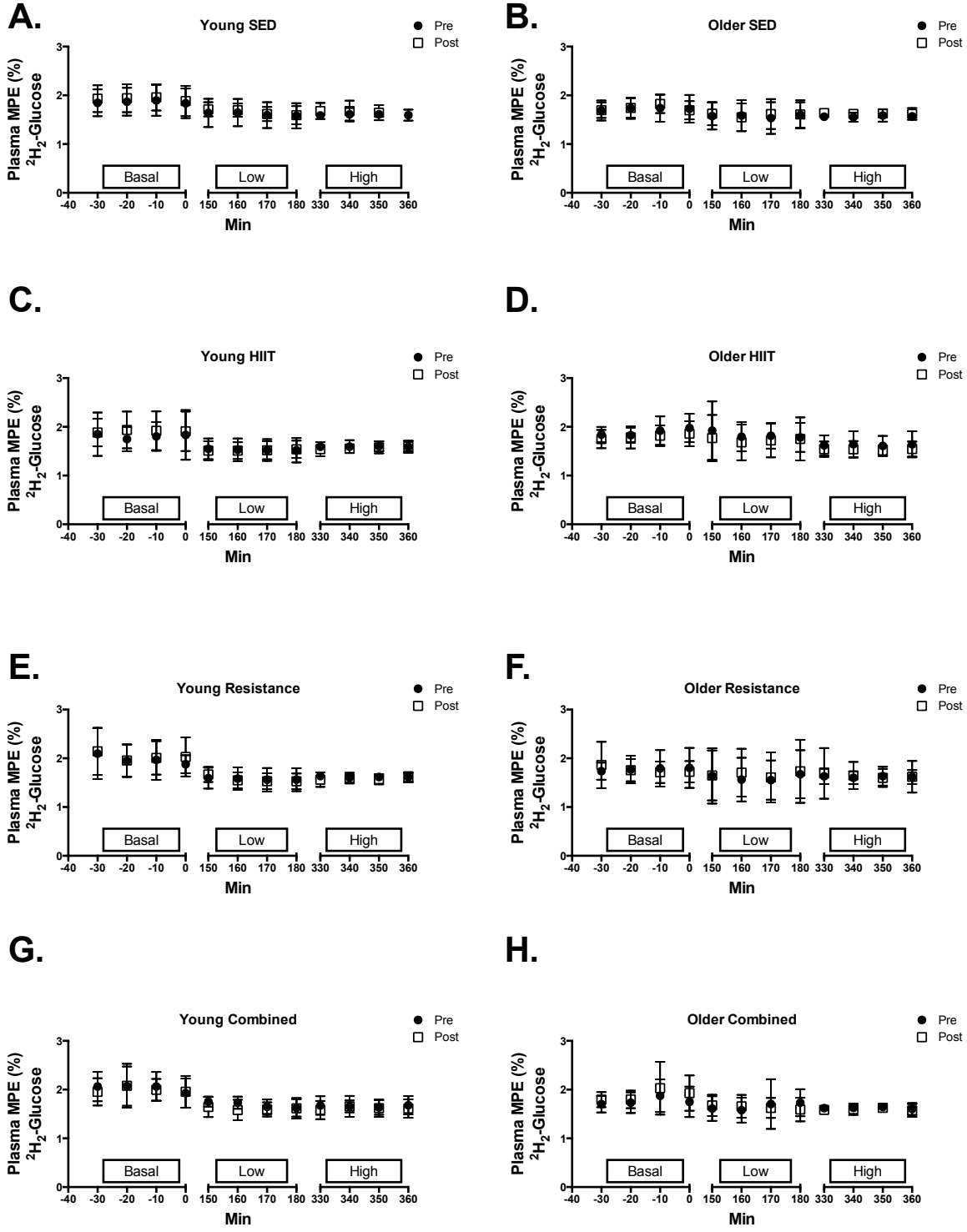


Figure S3, related to Figure 2: Endogenous glucose production (EGP) per kg lean mass during Basal, Low and High insulin euglycemic clamp stages in young (**A**) and older (**B**). Carbohydrate metabolism was measured using $^{2}H_2$-glucose and indirect calorimetry revealing similar rates of glucose disappearance (R_d) through oxidative and non-oxidative pathways at baseline (**C**). The increased glucose R_d with training occurred predominantly through increases in non-oxidative glucose disposal, primarily representing glycogen storage (**D**). Data are displayed as mean±SD. Measurements were performed before and after 12 weeks of high-intensity interval training (HIIT), resistance training (RT) combined training (CT) or sedentary period (SED).

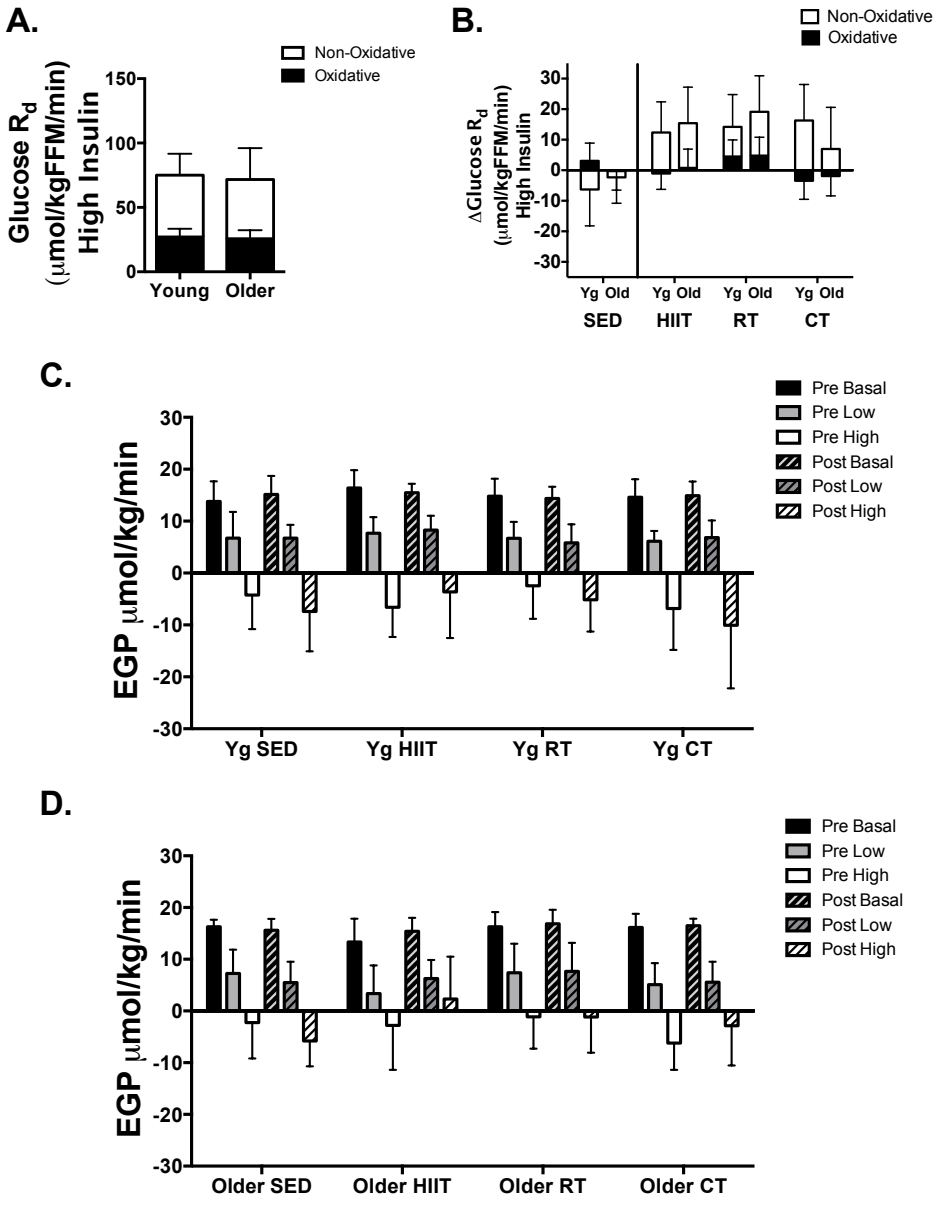


Figure S4, related to Figure 3: Respiratory control ratio, H_2O_2 production and mtDNA content.
Mitochondrial respiratory control ratio (RCR) measured using high-resolution respirometry during State 3 respiration of substrates for CI and II (Glutamate+Malate+Succinate+ADP) and leak state induced by oligomycin ($Leak_{Oligo}$) at baseline (**A**) and following 12 weeks of training (**B**). Mitochondrial H_2O_2 production normalized to mitochondrial protein content was similar at baseline (**C**) and did not change with 12 weeks of exercise training (**D**). The ratio of mitochondrial DNA (mtDNA) to nuclear DNA was measured by qPCR and was lower at baseline in older adults (**E**), then increased in older adults (**F**) following 12 weeks of high-intensity interval training (HIIT), resistance training (RT) and combined training (CT). There was a high correlation between Pre and Post testing for peak aerobic capacity ($VO_{2\ peak}$) in the sedentary control group (**G**). Changes during SED were analyzed separately and included in graphs for comparison. Data from baseline comparisons are displayed as Mean±SD with p-values for un-paired t-test. Changes with training are presented as least square adjusted mean with Tukey honest significant difference 95% confidence intervals. Non-overlapping confidence intervals are different at the corrected α level. Dotted line is set at zero (no change from baseline). *p<0.05 for old versus young.

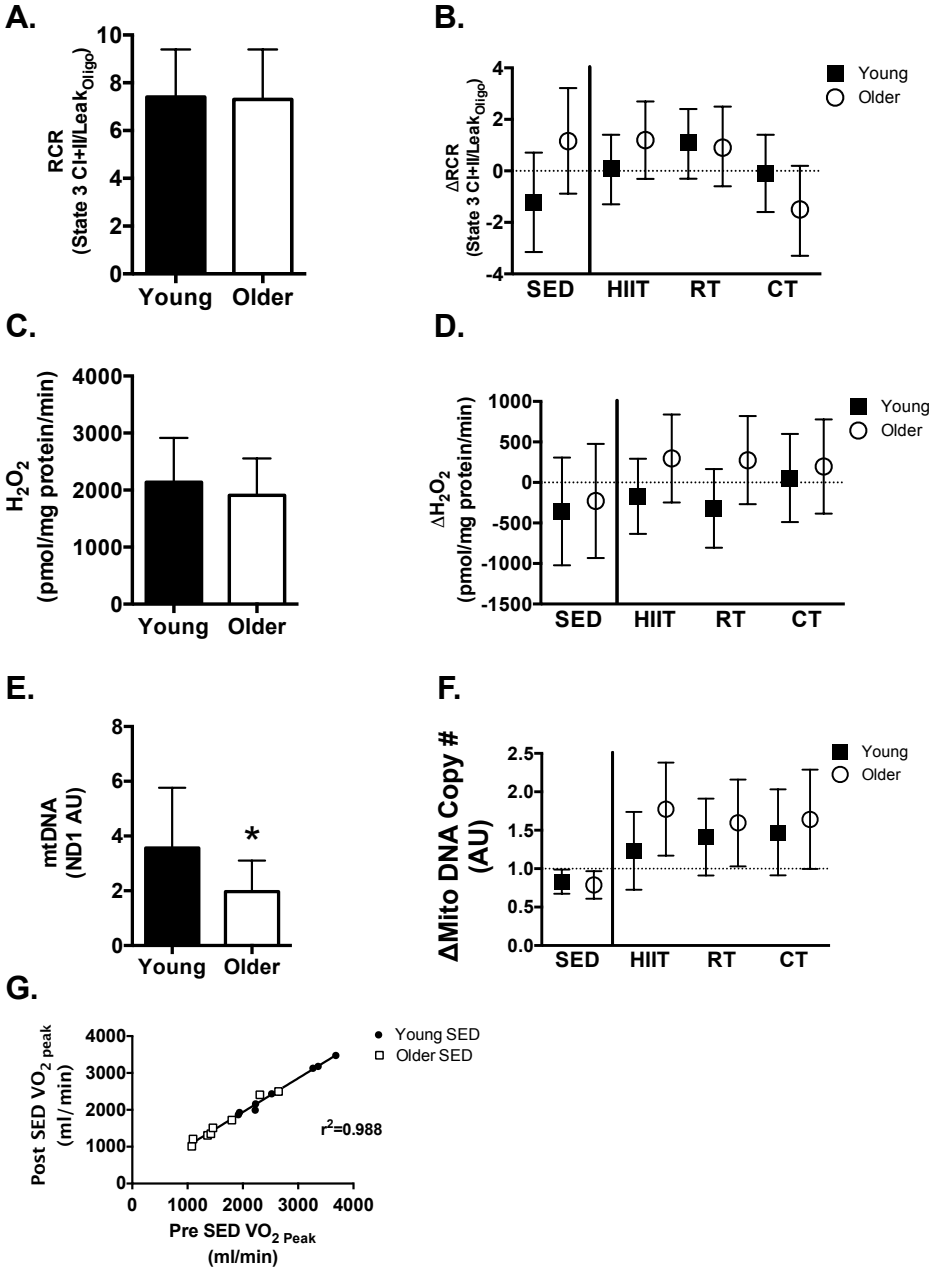
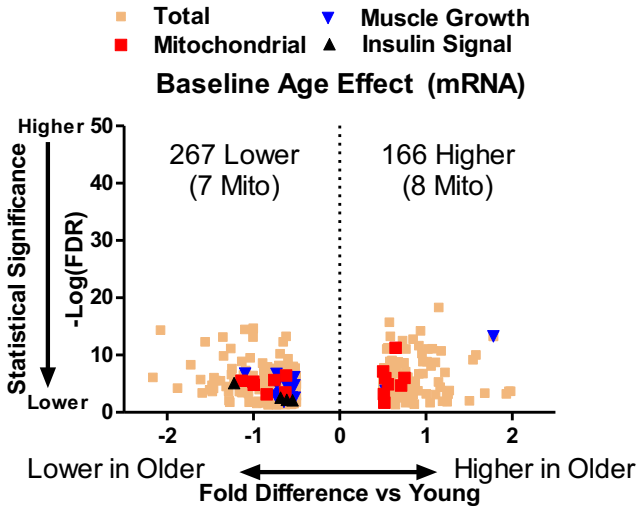


Figure S5, related to Figure 4: Gene expression between age groups and after training. (**A**) Genes that were differentially expressed between young and old, at baseline, were detected by using an adjusted p-value of ≤ 0.05 and an absolute fold change of ≥ 0.5. Genes were annotated according to their mitochondrial specificity (using MitoCarta) and molecular function (using KEGG). Mito stands for mitochondrial. (**B**) Gene set enrichment analysis of baseline gene expression differences between young and old subjects against genes that were up regulated with HIIT in younger subjects with indicated false discovery rate (FDR). Genes that increased expression with age are more likely increase their expression with HIIT in younger subjects.

A.



B.

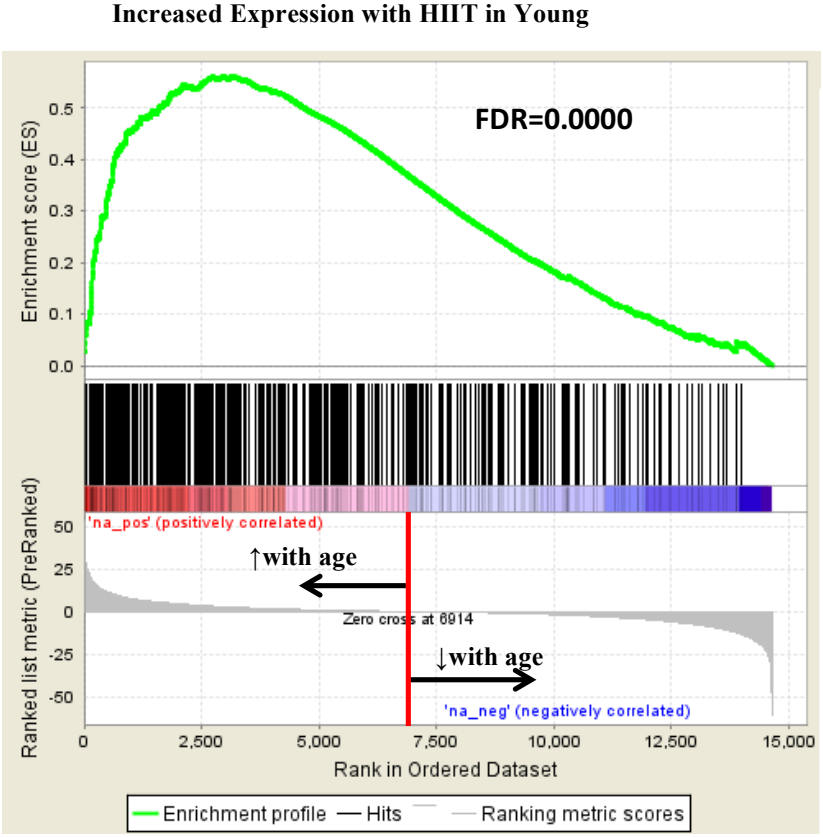
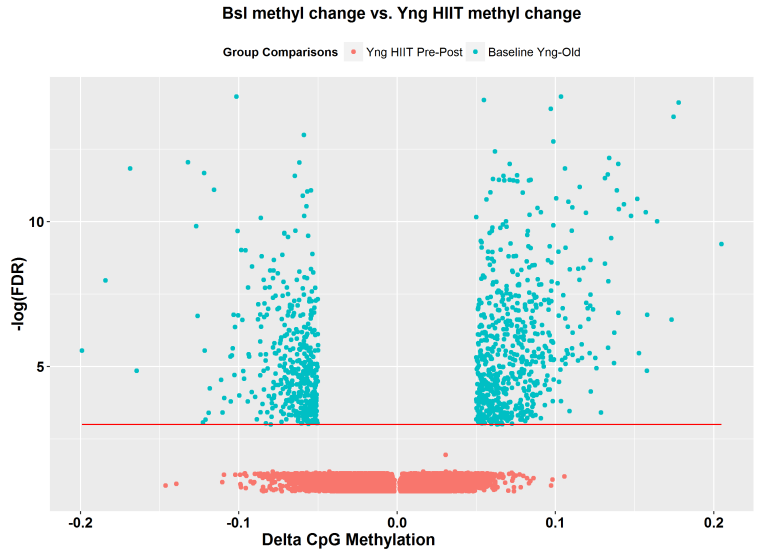


Figure S6, related to Figure 4: Promoter CpG methylation is not affected by exercise training. Skeletal muscle biopsies were analyzed using Illumina 450K methylation array. GenomeStudio software processed the data to remove background and normalize signal to account of technical variation. Partek software analyzed the processed CpG data. Site-level CpG methylation (β) values were used to compute differential methylation p-values and delta methylation change between two groups. Computed p-values were corrected using Benjamini-Hochberg method. CpG sites that were within promoter regions (defined as 2000bp≤TSS≤500bp) were considered for further analysis. Panels show the FDR and Δ values of promoter CpGs that were differentially expressed (defined as FDR≤0.05 and absolute Δβ≥0.05 between young vs. old (in blue color) at baseline. For exercise training, Δβ was computed as Pre-exercise training minus Post-exercise training. Example data from young (**A**) and older HIIT (**B**) show the exercise training induced Δβ and FDR values associated with promoter CpGs (in red). Red horizontal line in the panels correspond to FDR≤0.05 threshold. We did not observe significant changes in methylation of promoter CpGs with any type of exercise modality.

A.



B.

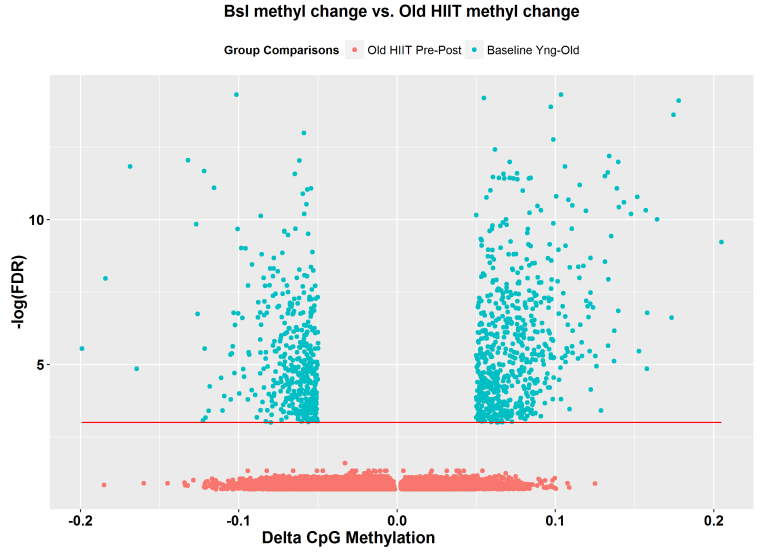


Table S1, related to Figure 4: This table shows genes that had a statistically significant decrease in expression with age as well as statistically significant increase in expression with HIIT in older subjects. Gene expression data of young and old subjects at baseline was used to derive a set of genes that changed expression with age. Gene expression data of post- and pre-HIIT samples collected from older subjects was used to detect genes that changed expression with HIIT. Both lists were filtered to retain only genes with an adjusted p-value ≤ 0.05 and at least an absolute fold change of ≥ 0.5. The method is described in greater detail in the methods section of the study. Genes that went up with age also had a higher change to go up with physical activity in older subjects. * "up-up" means that the gene's expression increased with age and HIIT. "down-up" means that the gene's expression decreased with age and increased with HIIT.

	Baseline Old vs. Young		Old HIIT Pre vs. Post		
Gene	Log2(Fold Change)	FDR	Log2(Fold Change)	FDR	Change Interpretation*
IGF2	0.573225577	2.73E-06	0.860981461	2.6105E-11	up-up
ELN	0.845097465	0.000700318	0.859524056	1.53209E-07	up-up
PTPRC	0.612853682	0.004924745	0.830512358	0.00126463	up-up
AC132217.4	0.504441162	6.16E-05	0.816010314	7.83098E-10	up-up
INS-IGF2	0.663745361	3.56E-07	0.802602631	5.21645E-10	up-up
LAPTM5	0.536464347	0.002708274	0.781841327	0.000121204	up-up
LYZ	0.642874452	0.016727124	0.765799265	0.018607754	up-up
ITGB2	0.686089398	0.001140919	0.746669634	2.7138E-05	up-up
POSTN	1.139298138	0.000262533	0.743662601	0.003101744	up-up
CTSS	0.529063288	0.001447605	0.729192133	0.005030352	up-up
COL14A1	0.628259085	0.005614664	0.719181437	0.002396621	up-up
PLVAP	0.584887234	0.000170106	0.704998152	2.29907E-07	up-up
CCL15-CCL14	0.760680639	0.000191943	0.645393409	7.84269E-06	up-up
LUM	0.707076636	6.00E-06	0.63843606	4.69192E-05	up-up
CCL14	0.778072652	0.000146586	0.635797524	2.89738E-05	up-up
C1QA	0.728285516	0.000297163	0.622780551	0.000525376	up-up
RP11-475J5.5	0.846631514	0.007335166	0.607245373	0.00024757	up-up
MS4A6A	0.604126451	0.000938859	0.568254916	0.003459616	up-up
SULF1	0.756460344	0.00020251	0.532929432	0.003041846	up-up
SVEP1	0.516246704	7.20E-05	0.528584541	1.21371E-06	up-up
CD68	0.585070646	7.45E-05	0.522973162	0.005329399	up-up
STAB1	0.601861305	2.07E-05	0.515764604	5.10974E-06	up-up
METTL21EP	-1.544862436	6.80E-10	1.149941483	5.42431E-20	down-up
MYLK4	-1.7816154	5.35E-14	0.987422649	6.26132E-06	down-up
SCN4B	-0.728624604	1.63E-06	0.832109883	8.82236E-12	down-up
PKP1	-0.505637978	0.016918581	0.825261225	9.81716E-08	down-up
MT-TR	-0.711199107	2.02E-05	0.809985436	2.71365E-24	down-up
RP11-296E23.1	-0.637033435	0.01146624	0.724176323	1.41583E-12	down-up
MTRNR2L6	-0.5874597	0.007294797	0.66503461	2.03776E-08	down-up
AC009234.1	-0.765398424	0.020402689	0.620189476	1.94449E-09	down-up
KAZALD1	-0.675924682	0.001985578	0.602525638	0.00957209	down-up
RP11-61I13.3	-0.966345784	1.74E-07	0.530722984	0.000671561	down-up
CKMT2	-0.530069962	1.03E-06	0.527590589	8.13471E-06	down-up

Table S2, related to Figure 4: Genes that were universally upregulated with exercise training regardless of age and modality are shown here. FC stands for fold change. FDR stands for false discovery rate which was calculated with Benjamini-Hochberg adjusted p-values of mRNA differential expression. A log2(FC) of ≥ 0.3 and an FDR ≤ 0.05 were applied to generate the list. Log2(FC) was relaxed from 0.5 in order to increase the likelihood to detect genes whose expression changed by a small degree and yet consistently across all age groups and modalities. These genes were used for pathway analysis by Ingenuity Pathways (IPA, Qiagen, Germany).

	Young Combined		Young Resistance		Young HIIT		Old Combined		Old Resistance		Old HIIT	
Gene Symbol	**log2 (FC)**	**FDR**	**log2 (FC)**	**FDR**	**log2 (FC)**	**FDR**	**log2 (FC)**	**FDR**	**log2 (FC)**	**FDR**	**log2 (FC)**	**FDR**
CD34	0.508	2.96E-02	0.411	4.19E-04	0.329	3.43E-02	0.437	3.66E-04	0.326	6.10E-03	0.570	2.51E-09
EFNA1	0.731	3.08E-03	0.556	8.06E-04	0.426	4.05E-02	0.549	4.45E-02	0.687	1.88E-03	0.925	2.62E-17
NID1	0.637	8.19E-04	0.605	2.14E-11	0.686	2.00E-05	0.637	1.34E-06	0.655	4.01E-10	1.035	3.57E-38
NPR1	0.689	8.39E-05	0.726	3.49E-06	0.617	3.60E-03	0.625	2.57E-03	0.574	5.10E-03	1.033	5.77E-12
TIE1	0.854	3.97E-07	0.699	3.47E-05	0.406	3.60E-02	0.455	6.23E-03	0.593	7.35E-04	0.961	6.41E-16
UNC5B	0.626	2.79E-02	0.881	1.53E-06	0.668	1.94E-03	0.749	5.91E-05	1.005	1.36E-07	1.330	5.44E-19
AC132217.4	0.635	3.83E-03	0.814	7.19E-08	0.499	7.75E-03	0.474	4.84E-02	0.629	3.01E-05	0.816	7.83E-10
ETS1	0.592	3.30E-03	0.607	1.06E-06	0.399	9.82E-03	0.565	9.54E-06	0.390	1.92E-03	0.721	1.03E-15
A2M	0.661	6.47E-04	0.506	1.46E-06	0.360	4.34E-02	0.399	8.04E-03	0.398	3.84E-03	0.680	1.73E-09
ELK3	0.629	8.97E-03	0.572	5.75E-07	0.470	2.53E-03	0.469	9.63E-04	0.508	2.62E-04	0.724	4.69E-13
KCNJ8	0.716	1.44E-03	0.542	1.76E-03	0.413	3.51E-02	0.543	5.34E-03	0.533	5.31E-06	0.992	1.33E-17
KITLG	0.584	1.09E-03	0.554	3.24E-05	0.320	4.26E-02	0.443	3.37E-02	0.363	1.01E-02	0.386	6.57E-04
OAS2	0.704	5.49E-03	0.782	2.04E-07	0.879	9.18E-03	0.670	1.60E-03	0.653	3.25E-04	1.343	1.34E-06
COL4A1	0.887	7.39E-05	1.418	3.56E-25	1.118	2.28E-06	0.975	1.34E-06	1.237	1.59E-31	1.855	5.17E-43
COL4A2	0.779	2.57E-03	1.132	6.87E-19	0.912	8.48E-05	0.861	2.82E-05	1.013	3.33E-21	1.601	9.12E-42
EDNRB	1.086	5.15E-05	1.168	1.28E-18	0.947	4.69E-05	1.187	5.80E-12	0.965	3.11E-04	1.704	1.37E-19
LPAR6	0.588	4.51E-02	0.519	5.54E-05	0.581	1.85E-03	0.612	5.34E-03	0.367	1.51E-02	0.839	1.34E-12
RNASE1	0.482	2.27E-03	0.537	2.33E-06	0.389	9.94E-03	0.398	8.92E-04	0.527	7.18E-06	0.635	2.17E-13
CDH5	0.686	5.08E-05	0.657	5.10E-09	0.478	5.79E-03	0.393	3.29E-03	0.551	6.92E-06	0.949	8.23E-22
ACE	0.534	1.21E-02	0.598	3.90E-05	0.430	3.14E-02	0.550	1.29E-03	0.484	3.00E-03	0.707	4.10E-10
BCL6B	0.592	1.89E-02	0.829	1.87E-07	0.582	1.94E-02	0.740	2.72E-04	0.843	1.29E-06	1.071	1.54E-16
CD300LG	1.044	3.98E-12	0.822	7.19E-08	0.638	1.18E-02	0.918	1.75E-11	0.809	1.97E-07	1.364	1.29E-18
RNF152	0.718	3.44E-02	0.578	2.12E-06	0.604	1.30E-02	0.660	2.25E-03	0.777	6.40E-07	1.282	1.17E-31
HECW2	0.834	3.72E-03	0.468	3.50E-04	0.563	6.55E-03	0.892	1.29E-05	0.710	9.15E-03	1.008	6.19E-08
ID2	0.693	3.02E-02	0.390	2.96E-02	1.102	5.35E-05	0.722	3.09E-03	0.935	5.33E-04	0.795	3.07E-07
MYO1B	0.617	3.77E-03	0.599	2.72E-07	0.425	5.04E-03	0.543	2.06E-03	0.564	3.67E-06	0.907	7.40E-12

PXDN	0.692	8.45E-04	1.227	3.94E-23	0.971	2.99E-06	0.734	3.75E-03	1.064	6.31E-20	1.413	2.99E-36
CD93	0.678	5.82E-05	0.800	5.69E-10	0.528	3.05E-03	0.559	1.37E-03	0.596	2.13E-07	0.957	5.44E-19
EPB41L1	0.590	7.98E-04	0.485	4.19E-06	0.390	1.37E-02	0.381	7.10E-03	0.327	2.79E-02	0.562	1.42E-09
JAM2	0.684	3.09E-03	0.442	1.12E-03	0.467	1.20E-02	0.551	2.01E-03	0.580	1.05E-05	0.899	5.75E-11
ARHGAP31	0.597	4.77E-04	0.522	1.88E-04	0.396	1.66E-02	0.406	1.86E-02	0.426	1.57E-02	0.776	1.15E-11
MECOM	0.763	2.91E-06	0.785	3.59E-07	0.518	2.94E-02	0.512	2.03E-02	0.566	5.95E-03	1.053	8.23E-22
PLXND1	0.424	1.02E-02	0.581	1.26E-05	0.456	3.53E-03	0.345	2.13E-02	0.369	6.43E-03	0.693	4.88E-13
TM4SF18	0.778	3.59E-03	0.824	1.24E-08	0.824	8.97E-07	0.684	3.09E-03	0.575	1.34E-03	0.993	1.34E-18
GUCY1A3	0.585	7.22E-03	0.508	4.62E-05	0.377	1.26E-02	0.377	3.00E-02	0.455	1.93E-03	0.630	5.33E-07
BTNL9	1.085	2.79E-07	0.632	6.09E-06	0.626	9.28E-03	0.843	4.58E-18	0.574	1.31E-02	1.116	9.38E-21
F2R	0.868	6.24E-03	0.817	7.15E-07	0.946	2.33E-05	0.568	2.93E-02	0.865	3.00E-07	1.133	2.49E-16
PCDH12	0.791	1.24E-03	0.772	1.87E-06	0.557	8.36E-03	0.643	5.34E-03	0.817	1.37E-04	1.061	7.19E-13
PDGFRB	0.493	2.71E-02	0.511	2.05E-08	0.451	1.71E-03	0.379	1.52E-02	0.476	1.34E-06	0.722	1.24E-14
SPARC	0.486	2.99E-02	0.769	1.05E-11	0.907	1.85E-07	0.719	3.66E-04	0.841	1.24E-08	1.162	2.57E-34
GJA1	0.646	3.59E-03	0.861	1.35E-15	0.674	6.45E-04	0.418	2.33E-02	0.407	1.49E-02	0.799	5.76E-12
NOTCH4	0.644	1.38E-04	0.696	5.82E-06	0.474	1.62E-02	0.499	2.18E-02	0.419	4.90E-02	0.890	4.87E-18
SH3BGRL2	0.809	8.61E-05	0.451	4.42E-03	0.514	5.31E-03	0.489	2.17E-02	0.377	2.16E-02	0.777	1.10E-09
CAV1	0.652	4.63E-04	0.535	2.27E-05	0.352	2.60E-02	0.393	1.66E-02	0.403	2.40E-03	0.742	7.88E-13
CDK6	0.435	4.11E-02	0.746	5.10E-09	0.581	1.91E-03	0.506	1.54E-02	0.578	2.38E-04	0.831	1.52E-10
GNG11	0.754	1.49E-05	0.625	5.23E-09	0.419	3.64E-02	0.599	3.23E-04	0.390	7.15E-04	0.868	3.27E-18
LAMB1	0.576	2.65E-03	0.624	1.07E-11	0.772	7.14E-08	0.679	1.67E-09	0.440	2.06E-03	0.763	5.20E-11
RAPGEF5	0.816	6.75E-04	0.875	4.47E-09	0.587	3.21E-03	0.705	1.06E-04	0.470	4.28E-03	0.808	2.37E-09
C8orf4	0.923	2.00E-06	0.608	6.35E-06	0.540	3.01E-02	0.892	2.55E-06	0.770	3.28E-06	1.119	1.68E-13
ASPN	0.656	7.47E-03	0.902	4.30E-06	0.801	1.19E-03	0.822	1.30E-03	1.281	3.66E-11	1.214	7.27E-19
ECM2	0.497	1.28E-02	0.689	3.20E-05	0.805	6.75E-06	0.534	5.93E-03	0.793	1.29E-06	0.844	1.12E-10
S1PR3	1.082	4.60E-04	1.102	7.39E-08	0.794	1.86E-04	0.786	9.63E-04	0.679	1.81E-04	1.086	9.29E-07
ITM2A	0.373	3.15E-02	0.386	4.67E-06	0.398	2.96E-03	0.433	7.71E-03	0.447	5.19E-04	0.577	5.47E-08
PLS3	0.545	4.51E-03	0.421	6.35E-06	0.405	2.34E-03	0.465	3.85E-04	0.515	2.98E-06	0.664	1.98E-09
TMSB4X	0.545	4.67E-04	0.541	5.07E-07	0.406	5.02E-03	0.404	1.49E-02	0.379	5.88E-04	0.780	5.43E-16

Table S3, related to Figure 4: This table shows the top two Gene Ontology Processes that were enriched when using the 55 genes that were universally upregulated with exercise training, regardless of age and training modality (listed in **Supplemental Table S4**). MetaCore software (Thomson Reuters, New York, NY) was used for the analysis. FDR stands for false discovery rate.

Name	FDR	In Data	Target molecules in the data set
angiogenesis	4.158E-15	20	ETS, Ephrin-A, ACE1, NOTCH4, Ephrin-A1, PDGF-R-beta, UNC5B, Plexin D1, Notch, ETS1, PDGF receptor, Galpha(q)-specific peptide GPCRs, COL4A2, Caveolin-1, TIE, COL4A1, Galpha(i)-specific EDG GPCRs, Collagen IV, Elk-3, CD34
regulation of angiogenesis	6.360E-13	16	Guanylate cyclase A (NPR1), ETS, Ephrin-A, NOTCH4, Ephrin-A1, Galpha(s)-specific nucleotide-like GPCRs, Plexin D1, Notch, ETS1, Guanylate cyclase, Galpha(q)-specific peptide GPCRs, COL4A2, Osteonectin, TIE, Collagen IV, CD34

Table S4, related to Figure 4: This table shows the top up-stream regulators of the 55 genes that were universally up-regulated with exercise training, regardless of age and training modality. Ingenuity Pathway Analysis software (Qiagen, Germany) was used for the analysis. A p-value threshold of ≤ 0.05 was used to derive the list.

Upstream Regulator	Molecule Type	Predicted Activation State	Activation z-score	p-value of overlap	Target molecules in the dataset
VEGFA	growth factor	Activated	2.069	1.15E-11	ACE,BCL6B,CAV1,CD34,CDH5,ETS1,GJA1,NOTCH4, SPARC,TMSB10/TMSB4X,UNC5B
Vegf	group	Activated	3.095	1.76E-07	A2M,ACE,CD93,CDH5,EFNA1,ETS1,JAM2,KITLG,LP AR6,NOTCH4
AGT	growth factor	Activated	2.419	7.20E-07	ACE,CAV1,COL4A1,EDNRB,ETS1,GJA1,KITLG,LAM B1,NOTCH4
FGFR2	kinase	Activated	2	9.66E-06	COL4A1,COL4A2,GJA1,ID2,LAMB1
CTNNB1	transcription regulator	Activated	2.607	2.33E-05	CD34,COL4A1,COL4A2,F2R,GJA1,ID2,LAMB1,MECO M,PLS3
SP1	transcription regulator	Activated	2.392	3.01E-05	CAV1,CDK6,F2R,GJA1,JAM2,NPR1,PDGFRB,SPARC
IL10RA	transmembrane receptor	Activated	2.449	8.01E-05	ASPN,CD34,EDNRB,KITLG,MECOM,SPARC

Table S5, related to Figure 5: This table shows the top pathways that were enriched for proteins that were up-regulated with HIIT and resistance training regimens in old cohort. Up-regulated proteins that are associated with each enriched pathway is also listed. MetaCore software (Thomson Reuters, New York, NY) and WEBGESTALT software were used for the analysis. FDR stands for false discovery rate.

Pathway	FDR	# Genes	Genes
			Older HIIT
Electron transport chain	1.16E-47	34	ATP5B, ATP5C1, ATP5D, ATP5F1, ATP5H, ATP5J, ATP5S, COX17, COX4I1, COX5A, COX5B, COX6B1, COX6C, NDUFA10, NDUFA2, NDUFA5, NDUFA9, NDUFB10, NDUFB3, NDUFB4, NDUFB6, NDUFB9, NDUFS2, NDUFS4, NDUFS5, NDUFS8, NDUFV2, SLC25A4, SURF1, UCP3, UQCRC1, UQCRC2, UQCRFS1, UQCRH
Oxidative phosphorylation	2.79E-26	19	ATP5B, ATP5D, ATP5F1, ATP5H, ATP5J, ATP5S, NDUFA10, NDUFA2, NDUFA5, NDUFA9, NDUFB10, NDUFB4, NDUFB6, NDUFB9, NDUFS2, NDUFS4, NDUFS5, NDUFS8, NDUFV2,
TCA cycle	3.40E-12	8	DLST, IDH2, IDH3A, IDH3B, IDH3G, MDH2, SUCLA2, SUCLG1
Glycolysis and Gluconeogenesis	2.00E-04	5	GOT1, GOT2, MDH2, MPC2, PDHA1
Branched-chain amino acid catabolism	1.83E-08	6	ACAD8, AUH, HIBADH, HIBCH, HSD17B10, IVD
tRNA aminoacylation	1.93E-07	7	IARS, IARS2, LARS, LARS2, PPA2, WARS2, YARS2
			Older Resistance Training
tRNA Aminoacylation	1.08E-16	12	AARS, AIMP2, DARS, GARS, HARS, IARS, LARS, LARS2, NARS, QARS, SARS, SARS2
Insulin Pathway	2.77E-06	21	CTNNA1, CTNNB1, CTTN, CUL1, DYNLRB1, EXOC1, EXOC4, HK1, HSPA4, HSPA8, HSPD1, INPPL1, NDUFAF2, PPP2CB, PPP5C, RUVBL2, SERPINB5, SLC2A1, TMEM126A, TRIM28, USP7
TCA cycle	1.44E-02	2	IDH3A, IDH3B
Valine, leucine, and isoleucine biosynthesis	1.00E-04	3	LARS, IARS, LARS2

Table S6, related to Figure 6: This table shows GSEA enrichment results of HIIT-induced muscle proteome changes in older individuals. Differential expression p-value and fold change (FC) between post-HIIT and pre-HIIT samples in older individuals were computed as described in the methods. Protein accessions were converted to gene names. Genes were ranked based on the decreasing order of -1*sign(FC)*log2(FDR adjusted differential P-value). GSEA software was used to configure to search the MSigDB for enriched gene sets using the ranked protein list. Gene sets with an adjusted q-value of ≤ 0.05 were listed above. ES stands for enrichment score. NES stands for normalized enrichment score. The false discovery rate (FDR) q-value was computed by using 1000 random permutations of the gene set.

MSigDB NAME	SIZE	ES	NES	FDR q-val
REACTOME_TCA_CYCLE_AND_RESPIRATORY_ELECTRON_TRANSPORT	98	0.80670726	1.6402091	0.015359001
MITOCHONDRIAL_MEMBRANE_PART	37	0.8358765	1.643043	0.015876135
MITOCHONDRIAL_PART	98	0.8013573	1.6335788	0.016824273
MITOCHONDRIAL_INNER_MEMBRANE	44	0.82584995	1.6446171	0.01856849
MITOCHONDRIAL_ENVELOPE	64	0.8213732	1.6453375	0.023904275
REACTOME_BRANCHED_CHAIN_AMINO_ACID_CATABOLISM	16	0.84954023	1.6063995	0.024836736
KEGG_OXIDATIVE_PHOSPHORYLATION	74	0.7972609	1.6082413	0.02569602
REACTOME_RESPIRATORY_ELECTRON_TRANSPORT_ATP_SYNTHESIS_BY_CHEMIOSMOTIC_COUPLING_AND_HEAT_PRODUCTION_BY_UNCOUPLING_PROTEINS_	65	0.80456537	1.614258	0.028167063
ORGANELLE_INNER_MEMBRANE	46	0.8238658	1.650944	0.030729769
REACTOME_RESPIRATORY_ELECTRON_TRANSPORT	53	0.79198813	1.5957903	0.031386584
ENVELOPE	94	0.7735709	1.5799711	0.036652796
MITOCHONDRION_ORGANIZATION_AND_BIOGENESIS	26	0.8183807	1.5822403	0.037440244
ORGANELLE_ENVELOPE	94	0.7735709	1.574672	0.039162777
REACTOME_PYRUVATE_METABOLISM_AND_CITRIC_ACID_TCA_CYCLE	37	0.7918554	1.5834688	0.03953332
MITOCHONDRION	217	0.77007204	1.5699617	0.041331954
MITOCHONDRIAL_MEMBRANE	57	0.82512456	1.6592821	0.042696852
MITOCHONDRIAL_RESPIRATORY_CHAIN	19	0.82958716	1.5651703	0.0432108
REACTOME_CITRIC_ACID_CYCLE_TCA_CYCLE	19	0.8183239	1.5543818	0.049609795

Table S7, related to Figure 6: Ribosomal proteins were up regulated with high-intensity interval training (HIIT) and resistance training (RT) in older subjects. Differential expression p-value and fold change (FC) between post-HIIT and pre-HIIT samples in older individuals were computed as described in the methods. Protein accessions were converted to gene names. FDR stands for false discovery rate, which was computed using Benjamini-Hochberg method. Mitochondrial ribosomal proteins that play a role in mitochondrial protein synthesis were up regulated with HIIT and RT in older subjects.

Accession	Symbol	Entrez Gene Name	Log FC [Post-Pre]	P-value	FDR
		Older HIIT			
NP_001186780.1	MRPS29	28S ribosomal protein S29	2.133979418	2.24E-10	1.26E-07
NP_612455.1	MALSU1	mitochondrial assembly of ribosomal large subunit 1	20.84197352	0.0017	0.027768
NP_660298.2	MRPL10	mitochondrial ribosomal protein L10	2.125749708	0.000167	0.004961
NP_002940.2	MRPL12	mitochondrial ribosomal protein L12	1.246930861	2.05E-05	0.001034
NP_115487.2	MRPL14	mitochondrial ribosomal protein L14	0.78721493	0.000975	0.018312
NP_060310.1	MRPL16	mitochondrial ribosomal protein L16	1.040303677	0.000394	0.009306
NP_071344.1	MRPL17	mitochondrial ribosomal protein L17	1.177362424	2.78E-11	1.88E-08
NP_055578.2	MRPL19	mitochondrial ribosomal protein L19	1.952533129	0.003567	0.045504
NP_054899.2	MRPL22	mitochondrial ribosomal protein L22	1.625204792	1.05E-05	0.000604
NP_057575.2	MRPL37	mitochondrial ribosomal protein L37	1.639537175	3.09E-07	3.37E-05
NP_115867.2	MRPL38	mitochondrial ribosomal protein L38	1.860911054	1.39E-06	0.000117
NP_059142.2	MRPL39	mitochondrial ribosomal protein L39	1.141660382	0.000595	0.012804
NP_666499.1	MRPL4	mitochondrial ribosomal protein L4	1.58667978	0.003386	0.044718
NP_003767.2	MRPL40	mitochondrial ribosomal protein L40	1.685635314	0.000148	0.00456
NP_115866.1	MRPL41	mitochondrial ribosomal protein L41	1.507623245	9.46E-05	0.003107
NP_115488.2	MRPL43	mitochondrial ribosomal protein L43	1.313233802	6.03E-05	0.002215
NP_075066.1	MRPL44	mitochondrial ribosomal protein L44	1.201727649	0.000829	0.016203
NP_071446.2	MRPL46	mitochondrial ribosomal protein L46	1.253316938	0.000444	0.010149
NP_065142.2	MRPL47	mitochondrial ribosomal protein L47	1.314489494	5.83E-06	0.000358
NP_057139.1	MRPL48	mitochondrial ribosomal protein L48	1.091745841	0.000306	0.007671
NP_004918.1	MRPL49	mitochondrial ribosomal protein L49	1.207735868	0.004113	0.049489
NP_060611.2	MRPS10	mitochondrial ribosomal protein S10	1.311540717	1.16E-08	2.81E-06
NP_071383.1	MRPS14	mitochondrial ribosomal protein S14	1.108393289	1E-05	0.000583

NP_057118.1	MRPS2	mitochondrial ribosomal protein S2	1.721281062	0.00376	0.04744
NP_064576.1	MRPS22	mitochondrial ribosomal protein S22	1.667368136	1.1E-06	9.77E-05
NP_057154.2	MRPS23	mitochondrial ribosomal protein S23	1.686166276	0.000228	0.006358
NP_071942.1	MRPS25	mitochondrial ribosomal protein S25	1.102409689	0.001547	0.025817
NP_110438.1	MRPS26	mitochondrial ribosomal protein S26	1.031468386	0.000584	0.012649
NP_055899.2	MRPS27	mitochondrial ribosomal protein S27	1.510058968	1.64E-12	1.85E-09
NP_054737.1	MRPS28	mitochondrial ribosomal protein S28	1.255229473	2.63E-08	4.68E-06
NP_005821.2	MRPS31	mitochondrial ribosomal protein S31	1.392781781	0.002226	0.033299
NP_076425.1	MRPS34	mitochondrial ribosomal protein S34	1.17542536	2.24E-05	0.001099
NP_068593.2	MRPS35	mitochondrial ribosomal protein S35	1.413581473	2.78E-05	0.001324
NP_114108.1	MRPS5	mitochondrial ribosomal protein S5	2.212230307	7.14E-05	0.002485
NP_057055.2	MRPS7	mitochondrial ribosomal protein S7	1.10874506	0.000725	0.014769
NP_872578.1	MRPS9	mitochondrial ribosomal protein S9	1.981006242	4.51E-06	0.000293
NP_620132.1	MRRF	mitochondrial ribosome recycling factor	0.921605867	0.003923	0.048402
		Older Resistance Training			
NP_004578.2	RRBP1	ribosome-binding protein 1	20.8031769	0.002159	0.044038
NP_733934.1	MRPL11	39S ribosomal protein L11, mitochondrial isoform b	2.345803022	9.60E-05	0.008532
NP_115867.2	MRPL38	39S ribosomal protein L38, mitochondrial	1.697080014	0.00015	0.009858
NP_003767.2	MRPL40	39S ribosomal protein L40, mitochondrial	1.522891707	0.000404	0.018395
NP_057118.1	MRPS2	28S ribosomal protein S2, mitochondrial	1.497722004	0.00035	0.017447
NP_071942.1	MRPS25	28S ribosomal protein S25, mitochondrial	1.466613292	0.000146	0.009855
NP_001186780.1	MRPS29	28S ribosomal protein S29, mitochondrial isoform 3	1.024101229	0.002623	0.04697
NP_001030178.1	RPL17	60S ribosomal protein L17 isoform a	0.951976294	0.001219	0.033703

Table S8, related to Figures 2 and 3: Exact p-values from 2-way ANOVA using Type 3 Estimates for change with training (ΔTraining= post – pre) comparing main effects of Age, Group and the Age x Group interaction. The p-values for ΔTraining are adjusted for multiple comparisons using Tukey honest significant difference and compared against 0. P-values of less than or equal to 0.05 were considered statistically different than 0. HIIT: high-intensity interval training; RT: resistance training; CT: combined training; $VO_{2\ peak}$: Peak aerobic capacity; BW: body weight; FFM: Fat free mass; 1RM: 1-repitition maximum leg press; Mito FSR: Mitochondrial Fractional Synthesis Rate; Glucose R_d: Glucose Rate of Disappearance; GMS: Glutamate-Malate-Succinate stimulated respiration; RCR: Respiratory Control Ratio; mtDNA: mitochondrial DNA.

	ΔTraining								
Variable	Age	Group	Age x Group	Young HIIT	Young RT	Young CT	Older HIIT	Older RT	Older CT
$VO_{2\ peak}$ (ml/min)	0.0569	**0.0285**	0.1527	**<0.0001**	**0.0488**	**0.0001**	**0.0091**	0.0528	**0.0096**
$VO_{2\ peak}$ (ml/kgBW/min)	0.1082	**0.0015**	**0.0374**	**<0.0001**	0.2458	**<0.0001**	**0.0042**	0.0596	**0.0011**
FFM (kg)	0.2767	0.0535	0.2453	**0.0361**	**<0.0001**	**0.0058**	**0.0270**	**0.0042**	**0.0238**
1RM/Leg FFM	0.8507	**0.0015**	0.8871	0.1601	**<0.0001**	**0.0016**	0.2737	**<0.0001**	**0.0004**
Mito FSR (%/hr)	**0.0064**	0.2681	0.1664	**0.0244**	0.2785	0.5691	**0.0457**	**0.0206**	**0.0011**
Glucose R_d (μmol/kgFFM/min)	0.945	0.4303	0.5412	**0.0226**	**0.0127**	**0.0423**	**0.0156**	**0.0032**	0.5357
Mitochondrial State 3 GMS (pmol O_2/μg mito/sec)	0.2732	0.5667	0.6929	0.5626	0.6981	0.7954	0.0571	0.0871	0.9102
Mitochondrial State 3 GMS (pmol O_2/ml/sec)	0.8149	0.093	0.7263	**0.0003**	0.3933	**0.0061**	**0.005**	0.1462	0.0715
RCR (State 3/State 4)	0.8102	0.0614	0.2476	0.9253	0.1167	0.9244	0.1149	0.2233	0.0884
Mitochondrial H_2O_2 emission (pmol H_2O_2/mg mito/min)	0.0673	0.8562	0.6898	0.4593	0.1920	0.8384	0.2784	0.3130	0.4987
mtDNA copy (ND1/B2M)	0.2630	0.9780	0.7728	0.3942	0.0731	0.2031	0.0544	0.1251	0.0675

Supplemental Experimental Procedures

Overall study design

The study design was approved by the Mayo Clinic Institutional Review Board and registered under Clinical Trials #NCT01477164 and # NCT01738568 (clinicaltrials.gov). All participants were informed of study procedures and provided written consent. Eligible participants were randomized to either aerobic, resistance or combined groups. All groups had baseline testing that included an out-patient visit (for DEXA and $VO_{2\ peak}$) and two in-patient studies (insulin clamp and biopsy). Participants then completed 12 weeks of training or sedentary period followed by repeated testing days. The combined group then performed 12 weeks of combined aerobic and resistance training followed by repeated testing days.

Screening

The study was advertised through television, radio, posted flyers, newspaper and word of mouth. Potential participants contacted the study team and completed a phone-screening questionnaire then eligible people were scheduled for an in-person screening visit. The screening visit including meeting with a study team member who described all procedures, then participants provided written consent. A fasting blood sample for complete blood count and urine sample for urinalysis were collected then general medical examination was performed. Participants met with a registered dietician to discuss food preferences for weighed-meals.

Inclusion/Exclusion Criteria

Participants were 18-30 years old or 65-80 years old. Exclusionary criteria included regular exercise routine (>20 minutes more than twice per week), cardiovascular disease, metabolic diseases (type 2 diabetes, fasting blood glucose >110 mg/dl and untreated hypo- or hyperthyroidism), renal disease, increased body mass index (>32 kg/m^2), implanted metal devices (including joint replacements, stents, pacemaker, neurostimulators), pregnancy, smoking, history of blood clotting. Exclusionary medication included anticoagulants, insulin, corticosteroids, sulfonylureas, barbituates, PPAR γ agonists, β-blockers, sulfonylureas, insulin sensitizers, opiates and tricyclic antidepressants.

Out-patient visit

Approximately one week after screening, eligible participants reported to the Clinical Research Unit following an overnight fast (>10 hours) for resting energy expenditure (REE), body composition by dual-energy x-ray absorptiometry (DEXA) and peak aerobic capacity ($VO_{2\ peak}$). First, participants lay in a quiet room with low lights for 30 minutes then REE was measured for 20 minutes using a ventilated hood (Parvo, Sandy, UT). Next, participants were analyzed for fat mass and fat free mass (FFM) by DEXA (Lunar). $VO_{2\ peak}$ was determined on an electronically braked cycle ergometer using indirect calorimeter. Heart rate was measured with 12-lead electrocardiogram (Quintin) and manual blood pressures were collected. The incremental exercise test was 2-minute stages with the following workloads: Young Male 50W + 30W stages; Young Female 50W+ 20W stages; Older Male 50W+20W stages; Older Female 25W+20W stages. Participants were verbally encouraged throughout the test. The test was terminated when participants could not maintain a cadence of at least 60 revolutions per minute. $VO_{2\ peak}$ was defined as reaching a rating of perceived exertion >17 according to Borg scale with respiratory exchange ratio >1.1 and within 10% of age predicted maximal heart rate.

Insulin Clamp

Insulin sensitivity was determined using a two-stage euglycemic clamp. Participants consumed 3-days of weighed meals (20% protein, 50% carbohydrates and 30% fat) to maintain body weight based on caloric requirement using Harris-Benedict equations. On the evening of the third day, participants reported to the Clinical Research Unit at 1730, consumed an evening meal at 1830 then were fasted overnight and throughout the insulin clamp. A catheter was inserted into an antecubital vein and maintained patent by saline infusion (30 ml/min). REE was measured for 20 minutes beginning at 0700 then a second catheter was inserted into an arm vein and a third into a dorsal hand vein on the contralateral arm. The hand was heated (120-131F) for arterialized blood sampling. Endogenous glucose production was traced using intravenous infusion of [6,6]-$^2H_2$-glucose with a prime at 0500 (6 mg/kg FFM) followed by titrated infusion [3.6 (0500-0800), 2.52 (0800-0900), 1.8 (0900-1000), 1.368 (1000-1100) and 0.9 (1200-1400) mg/kg FFM/hour]. At 0800, regular insulin (Humulin) was infused (0.62 mU/kgFFM/min) along with somatostatin (0.093 mcg/kgFFM/min), glucagon (0.001 mcg/kgFFM/min) and human growth hormone (0.0047 mcg/kgFFM/min). Euglycemia (85-95mg/dl) was maintained by titrated infusion of 40% dextrose enriched (2%) with [6,6]-$^2H_2$-glucose and saline was co-infused at an equal or greater rate to minimize phlebitis. At 1100, a second insulin infusion (1.68 mU/kg FFM/min) was added create a combined insulin infusion of 2.3 mU/kg FFM/min. Blood was sampled every 10 minutes and analyzed for glucose concentration in duplicate (±3mg/dl for replicates).

Larger blood volumes were drawn hourly then every 15 minutes during the final steady state hour of low and high insulin infusions. The clamp was completed at 1400 then hormone infusions were tapered and discontinued at 1430. Participants consumed a meal and were discharged.

Muscle Biopsy
A muscle biopsy was collected 7-days after the insulin clamp. Participants repeated the 3-days of weighed meals and were admitted to the Clinical Research Unit of the evening of the third day. A light snack was provided at 2100 then participants remained fasting overnight. At 0700, 1000 and 1500 hours, biopsies (~350 mg) were collected from the vastus lateralis with analgesia by 2% lidocaine with sodium bicarbonate buffer. The 0700 sample was used for mitochondrial respiration and the 1000 hour sample for RNA sequencing.

Exercise training
Exercise training was performed for 12-weeks at the Dan Abraham Healthy Living Center at Mayo Clinic under the supervision of an exercise physiologist. Training intensity ranges were determined as heart rate at a percentage of $VO_{2\ peak}$ and maintained ±5 beat per minute using wireless monitors (Polar). Energy expenditure was determined for cycling and treadmill training from indirect calorimetry during the $VO_{2\ peak}$ test and for resistance training from previously reported values (Phillips and Ziuraitis, 2003, 2004). Maximal strength was determined on leg press, lat pull down and chest press machines at baseline and following training for all groups. Participants warmed up for 10 minutes on bike, treadmill or elliptical machine, then were fitted to the machine and selected a weight to perform 10 repetitions. The weight was then increased and participants completed sets of up to 5 repetitions separated by a 1-minute rest. The highest weight that the participant could perform 1 repetition maximum (1RM) with good form was recorded.

High-intensity interval training consisted of 3 days per week of intervals on an electronically braked cycle ergometer (Monday, Wednesday and Friday) and 2 days per week on motorized treadmill walking (Tuesday and Thursday). The interval protocol was a 10-minute warm-up followed by 4 cycles of 4-minute high intervals (> 90%) with 3-minute rest (pedaling at no load) then a 5-minute cool down. The time per session at high intensity was 16 minutes. The treadmill protocol was a self-selected walking pace (2-4 mph) with a 10-minute warm-up, 45 minutes at incline at 70% $VO_{2\ peak}$ then a 5-minute cool down.

The resistance protocol was weight training for 60 minutes on 4-days per week of lower (Monday and Thursday) and upper body exercises (Tuesday and Friday). Participants were instructed on proper lifting technique and performed 8-12 repetitions per exercise with one-minute rest between sets. Participants completed two sets of each exercise for week 1, three sets for week 2, and four sets for weeks 3-12. Weights were increased when participants could perform 12 repetitions while maintaining good form. Lower body exercises were leg press, toe raise, lunge, abdominal crunch, leg extension and leg curl. Upper body exercises were chest press, lat pull down, incline chest press, seated row, lateral raise, biceps curl and triceps push down.

The combined protocol was 30 minutes of cycling 5-days per week (Monday through Friday) followed by 30 minutes of weight lifting. The cycling protocol was a five-minute warm-up, 20 minutes at 70% $VO_{2\ peak}$, then 5 minutes of cool down. The weight lifting was a 4-day program with lower body (Monday and Thursday) and upper body exercises (Tuesday and Friday). Lower body exercises were leg press, abdominal crunch, leg extension and leg curls. Upper body exercises were chest press, lat pull down, triceps extensions and biceps curls.

Post-testing
The follow-up outpatient visits and maximal strength testing were performed on week 12 then participants continued training until beginning the metabolic meals. We were interested in determining the longer lasting effects of exercise training on insulin sensitivity and muscle mitochondrial function therefore follow-up in-patient studies were performed 72 hours after final training session (clamp performed 7 days after $VO_{2\ peak}$). To avoid prolonged inactivity, participants performed 3 days of exercise training between in-patient study days followed by 3 days of inactivity during the metabolic meals.

Mitochondrial respiration
High-resolution respirometry was performed on mitochondria freshly isolated from the 0700 biopsy sample as previously described (Lanza and Nair, 2009). Approximately 100 mg of tissue was homogenized and mitochondria were separated using differential centrifugation. Mitochondria were added to a 2 ml chamber (Oxygraph-2K,

Oroboros) and allowed to equilibrate. Glutamate (10 mM) and malate (2 mM) were added to stimulate State 2 respiration specific to Complex I then ADP added at saturating concentrations (2.5 mM) to induce State 3 respiration of Complex I. Cytochrome-c was added to verify mitochondrial membrane integrity. Succinate (10 mM) was added to stimulate State 3 respiration through Complex I+II then rotenone (0.5 μM) added to inhibit complex I for State 3 respiration specific to Complex II. State 4 respiration (Leak) was induced by addition of oligomycin (2 μg/μl) then the proton gradient was dissipated by sequential titration of 0.05 mM carbonylcyanide-4-(trifluoromethoxy)-phenylhydrazone (FCCP) to induce uncoupled respiration. Protein content of isolated mitochondrial was determined using a commercially available kit (DC Protein Assay, Bio-Rad). Mitochondrial respiration was normalized to tissue wet weight (reflective of mitochondrial content) and mitochondrial protein (reflective of mitochondrial protein quality).

Muscle Tissue mRNA Sequencing

We used RNA-Seq technology to assess the muscle gene expression differences between various training groups. Total RNA was isolated from ~20 mg of the 1000 hour biopsy sample using a commercially available kit according to manufacturer's instructions (RNeasy Fibrous Tissue, Qiagen). Total RNA was eluted in 100 μl of PCR grade water and concentration adjusted to 50 ng/μl using a spectrophotometer (Nanodrop, Thermo Scientific, Waltham, MA).

RNA libraries were prepared for sequencing using the TruSeq RNA Sample Prep Kit v2 (Illumina, San Diego, CA), following the manufacturer's recommendations. Briefly, poly-A mRNA was purified from 100 ng total RNA using oligo dT magnetic beads. Purified mRNA was fragmented at 95°C for 8 minutes and eluted from the beads. Double stranded cDNA was made using SuperScript III reverse transcriptase, random primers (Invitrogen, Carlsbad, CA) and DNA polymerase I and RNase H. The cDNA ends were repaired and an "A" base was added to the 3' ends. TruSeq paired end index DNA adaptors (Illumina, San Diego, CA) with a single "T" base overhang at the 3' end were ligated and the resulting constructs were purified using Agencourt AMPure SPRI beads (Beckman Coulter, Chaska, MN). Adapter-modified DNA fragments were enriched by 12 cycles of PCR using TruSeq PCR primers (Illumina, San Diego, CA). The concentration and size distribution of the libraries was determined on an Agilent Bioanalyzer DNA 1000 chip and Qubit fluorometry (Invitrogen, Carlsbad, CA).

Samples were divided into batches of eight and their indexed libraries were pooled at equimolar concentrations. Pooled library was loaded onto paired end flow cells at a concentration of 8.5pM to generate cluster densities of 700,000/mm^2 following the standard protocol for the Illumina cBot and cBot Paired-end cluster kit version 3. The flow cells were sequenced as 51X2 paired end reads on a HiSeq 2000 sequencer (Illumina, San Diego, CA) using TruSeq SBS sequencing kit version 3 (Illumina, San Diego, CA) and HCS version 2.0.12.0 data collection software. Base-calling is performed using Illumina's RTA version 1.17.21.3. On average, 56 million reads were generated for each sample. The RNA-Seq data were analyzed using the MAPRSeq (version 1.2.1) system for RNA-Sequencing data analysis.(Kalari et al., 2014)

Gene Expression Analysis

The total number of reads mapping to a gene was considered as a semi-quantitative measure of its abundance. We utilized edgeR software to perform differential expression analysis (Robinson et al., 2010). Expression values of the genes present in each sample were normalized using its library depth. For each (age, exercise type) group, edgeR used generalized linear models, configured to take into account the paired design model, to detect differentially expressed genes. Genes with a FDR corrected p-value of ≤ 0.05 and an absolute log2 fold change of ≥ 0.5 (where 0.0 signifies no change) were considered for further analysis.

mtDNA copy number

Total DNA was extracted using a kit (DNA mini, Qiagen) from the 1000 skeletal muscle biopsy sample and analyzed for mtDNA to nuclear DNA ratio as previously described (Lanza et al., 2012). qPCR was performed in 384 well clear plates with 20 μl reaction volume. Amplification conditions were 10 minutes at 60°C followed by 40 cycles of denaturing (95°C for 15 s) and annealing (60°C for 60 s) using a ViiA7 thermocycler (Applied Biosystems). Samples were amplified with multiplex conditions for mtDNA and nDNA targets in triplicate. Each plate included a repeated control repeated on the plate (intra-assay control) and between plates (inter-assay control) along a no template control, and 7-point relative standard curve spanning 4 log dilutions. Primers and probes were commercially produced (Applied Biosystems) for ND1 (F: CCC GCC ACA TCT ACC ATC A, R: GAA GAG CGA TGG TGA GAG CTA AG, FAM® Probe: CCT CTA CAT CAC CGC CCC GA) and ND4 (F: CCC CAC CTT

GGC TAT CAT CA R: TAG GAA GTA TGT GCC TGC GTT C, FAM® Probe: CGA TGA GGC AAC CAG CCA GAA C) as mtDNA targets and β-2 microglobulin for nDNA (F: GTG CCT GAT ATA GCT TGA CAC AA, R: TCG GGA AAA GAC ACA TTA ATA TTG CCA, VIC® Probe: CCC CAA GTG AAA TAC C).

Infinium Methylation protocol

For the Infinium methylation protocol (Bibikova et al., 2009), 250ng of bisulfite modified DNA (1000ng input) is required the HumanMethylation450 BeadArray. Each probe is 75 bases long; 25 bases at the 5' end and are used for decoding (Gunderson et al., 2004) and 50 bases are locus-specific. The oligonucleotides are immobilized on activated beads using a 5' amino group. The protocol involves isothermal whole genome amplification, followed by fragmentation and precipitation. These steps yield 50 μg/ reaction and the average size after digestion to ~100-200 bases. Denatured products are hybridized to the BeadChip. The allele specific extension reaction, washing and staining are carried out in a TECAN Te-Flow Chamber. Stained BeadChips are then dried and imaged on an Illumina iScan reader. Following scanning on a BeadArray or iScan reader, intensity data are loaded into the GenomeStudio Methylation Module for analysis. Analysis includes control probes for assessing sample-independent and -dependent performance. The methylation status of the target CpG sites is determined by comparing the ratio of fluorescent signal from the methylated allele to the sum from the fluorescent signal from both methylated and unmethylated alleles. These values range from 0 (unmethylated) to 1 (methylated). Sample dependent controls include those for bisulfite conversion, allowing identification of samples with incomplete conversion. Positive (*Sss*I treated DNA) and negative (WGA DNA) controls are included to determine whether there are any probes that should be excluded.

Raw methylation chip data was processed using GenomeStudio software (version 2011.1; Illumina, San Diego, CA). The software was configured to remove background using control probe data and normalize the spot intensities to account for chip-to-chip variation. GenomeStudio computed the methylation ratios (β) of all CpGs in each sample and exported them for processing in Partek Genomics Suite (version 6.13.0621; Partek Inc., St. Louis, MO). Samples were grouped in Partek according to age and exercise training regimen and any two groups of interest were compared using ANOVA models. Computed p-values were corrected using Benjamini-Hochberg method. For each CpG site, the software also computed the average beta values observed between the two groups. CpG sites that had a corrected p-value ≤ 0.05 and an absolute $\Delta\beta$ value of $\geq 0.05\%$ were considered as significant. CpG sites were filtered to retain candidates that were present with in the known gene promoter regions (defined as $2000bp \leq TSS \leq 500bp$; TSS stands for transcription start site). The following pairwise group comparisons were performed: young vs. old at baseline, young pre-HIIT vs. post-HIIT, young pre-RT vs. post-RT, young pre-CT vs. post-CT, old pre-HIIT vs. post-HIIT, old pre-RT vs. post-RT, and old pre-CT vs. post-CT. A total of 3,874 promoter CpG sites were differentially methylated at baseline between young and old subjects. We did not observe any promoter CpG sites passing the above significant thresholds in any of the exercise training group comparisons.

Proteomics

A 25 mg portion of the 1000 hour biopsy was powdered and homogenized at 1:10 dilution in buffer (50 mM Tris-HCl (pH 7.5), 5 mM MgCl2, 250 mM sucrose, 0.5 mM EDTA). Homogenization was in a cooled bead mill (Beadruptor, Omni International) with 3x15 second disruption protocol. Homogenates were then incubated on ice for ~10 minutes then spun at 720g for 10 minutes at 4°C, then supernatants transferred to a new tube and centrifuged at 16,600g for 20 minutes. Supernatant were collected for analysis. Protein estimation is performed using the Pierce 660 protein assay. Samples were diluted down to same concentration, and 40 μg of muscle protein homogenate was loaded on to a 4-12% Bis-Tris Criterion Gel (345-0123, Bio-Rad). The gel was run at 80V constant with MES running buffer until the dye front reaches the bottom of the gel. The gel was then fixed and stained using coomassie. Each gel lane was cut into 8 segments that were incubated in 200 mM Tris 30 min prior to destaining with 50 mM Tris/40% acetonitrile for 1-2 hours. Segments were then dehydrated with 100% acetonitrile until gel pieces appeared opaque. Destaining was repeated three times followed by reduction with 50 mM TCEP for an hour, and alkylation with 25 mM IAA for an hour, dehydration with acetonitrile, hydration with 25 mM Tris for 10 minutes, and another dehydration prior to trypsin digest with 0.15 to 0.20 μg trypsin in 20 mM Tris/0.0002% zwittergent 3-16 overnight at 37°C. Trypsin was inactivated and peptides were extracted by adding 3% trifluoroacetic acid to the digest for 20 minutes followed by the addition of acetonitrile for an additional 30 minutes. The supernatant was saved. Acetonitrile was added a second time to the gel segments which was added to the already saved supernatant. These peptide containing fractions were dried to completeness under vacuum and stored at -20°C.

Dried tryptic digests were reconstituted in 80 µL sample buffer containing 0.2% formic acid, 0.1% TFA, 0.002% zwittergent 3-16, 0.2 fmol/µL Pierce retention time standards. A total of 10 µl of the sample was used for analysis by nano-scale liquid chromatography interfaced to tandem mass spectrometry (nLC-MS/MS) using a Q-Exactive high resolution, accurate mass hybrid mass spectrometer. The Q-Exactive measured peptide molecular weights using 70,000 resolving power (FWHM, m/z 200) survey scans (MS1) followed by automated MS/MS experiments of the top 15 abundant precursor masses (charge states 2-4, inclusive). The peptides were separated by reversed phase nano-scale chromatography on a 100 µm i.d. fused silica column packed in-house with 32 cm of Agilent Poroshell C18 stationary phase using a gradient of 2-45% B over 60 minutes with a mobile phase flow rate of 400 nL/min where mobile phase A was water, acetonitrile, and formic acid (98/2/0.2 by volume), and mobile phase B was composed of acetonitrile, isopropanol, water, and formic acid (80/10/10/0.2 by volume).

We utilized a peptide intensity-based label-free method for detecting protein and posttranslational modification differences between experimental groups. For this, MS/MS data were processed with MaxQuant software (version 1.5.1.2) configured to match the spectra against a composite protein sequence containing RefSeq human proteome (v58) and common contaminants. Reversed protein sequences were appended to the database for estimating peptide identification false discovery rates (FDR). MaxQuant used 20ppm peptide mass tolerance for first search and 4.5 ppm peptide mass tolerance for second search. The software was configured to derive semitryptic peptides from the database and identify the following PTMs as variable modifications: carbamidomethylation of cysteine, oxidation of methionine, oxidation of phenylalanine, oxidation of tryptophan, deamidation of asparagine, deamidation of glutamine, and n-terminal pyroglutamic acid. Peptide and protein identification results were filtered at 1% FDR. Non-redundant protein groups with at least two distinct peptide identifications were considered as present in the sample.

MaxQuant software reported protein group and peptide intensities for each sample. For protein differential expression, an in house R script processed the protein group intensity data. This script started by normalizing the sample level protein group intensities with the total ion current (TIC) detected for that sample. For each protein group, the normalized intensities observed in two groups of samples were modeled using a Gaussian-linked generalized linear model. An ANOVA test was used to detect the differentially expressed protein groups between pairs of experimental groups. Differential expression p-values were FDR corrected using Benjamini-Hochberg-Yekutieli procedure. Protein groups with an FDR ≤ 0.05 and an absolute log2 fold change of ≥ 0.5 were considered as significantly differentially expressed. This process was previously described (Ayers-Ringler et al., 2016).

Peptide intensities were used to assess differential expression of specific PTMs between groups. For this, we obtained the ratio of modified to unmodified intensities (Mod/UnMod) for each peptide as previously described (Martin et al., 2016). These ratios were grouped by the PTM type (oxidation or deamidation) and experimental group. For each PTM type and experimental group, the difference between the post-exercise training and pre-exercise training Mod/UnMod ratios were computed for each peptide. The distribution of these delta Mod/UnMod ratios observed in each exercise training type was compared with those obtained from the sedentary group (SED) using a Mann-Whitney rank sum test. The resulting PTM results were summarized as average delta [Post-Pre] Mod/UnMod ratios observed for each exercise training type and a p-value comparing the observed distribution to that of SED group.

Supplemental References

Ayers-Ringler, J.R., Oliveros, A., Qiu, Y., Lindberg, D.M., Hinton, D.J., Moore, R.M., Dasari, S., and Choi, D.S. (2016). Label-Free Proteomic Analysis of Protein Changes in the Striatum during Chronic Ethanol Use and Early Withdrawal. Front Behav Neurosci *10*, 46.

Bibikova, M., Le, J., Barnes, B., Saedinia-Melnyk, S., Zhou, L., Shen, R., and Gunderson, K.L. (2009). Genome-wide DNA methylation profiling using Infinium(R) assay. Epigenomics *1*, 177-200.

Gunderson, K.L., Kruglyak, S., Graige, M.S., Garcia, F., Kermani, B.G., Zhao, C., Che, D., Dickinson, T., Wickham, E., Bierle, J., et al. (2004). Decoding randomly ordered DNA arrays. Genome Res *14*, 870-877.

Kalari, K.R., Nair, A.A., Bhavsar, J.D., O'Brien, D.R., Davila, J.I., Bockol, M.A., Nie, J., Tang, X., Baheti, S., Doughty, J.B., et al. (2014). MAP-RSeq: Mayo Analysis Pipeline for RNA sequencing. BMC Bioinformatics *15*, 224.

Lanza, I.R., and Nair, K.S. (2009). Functional assessment of isolated mitochondria in vitro. Methods in enzymology *457*, 349-372.

Lanza, I.R., Zabielski, P., Klaus, K.A., Morse, D.M., Heppelmann, C.J., Bergen, H.R., 3rd, Dasari, S., Walrand, S., Short, K.R., Johnson, M.L., et al. (2012). Chronic caloric restriction preserves mitochondrial function in senescence without increasing mitochondrial biogenesis. Cell metabolism *16*, 777-788.
Martin, D.R., Dutta, P., Mahajan, S., Varma, S., and Stevens, S.M., Jr. (2016). Structural and activity characterization of human PHPT1 after oxidative modification. Sci Rep *6*, 23658.
Phillips, W.T., and Ziuraitis, J.R. (2003). Energy cost of the ACSM single-set resistance training protocol. J Strength Cond Res *17*, 350-355.
Phillips, W.T., and Ziuraitis, J.R. (2004). Energy cost of single-set resistance training in older adults. J Strength Cond Res *18*, 606-609.
Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics *26*, 139-140.