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Endurance Exercise as a Countermeasure for Aging

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ABSTRACT

Objective: We determined whether reduced insulin sensitivity, mitochondrial dysfunction and other age-related dysfunctions are inevitable consequences of aging or secondary to physical inactivity.

Research Design and Methods: Insulin sensitivity was measured by hyperinsulinemic-euglycemic clamp and ATP production in mitochondria isolated from vastus lateralis biopsies in 42 healthy sedentary and endurance trained young (18-30 years) and older (59-76 years) humans. Expression of proteins involved in fuel metabolism was measured by mass spectrometry. Citrate synthase activity, mitochondrial DNA abundance (mtDNA), and expression of nuclear-encoded transcription factors for mitochondrial biogenesis were measured. SIRT3, a mitochondrial sirtuin linked to lifespan-enhancing effects of caloric restriction was measured by immunoblot.

Results: Insulin-induced glucose disposal and suppression of endogenous glucose production were higher in the trained young and older people but no age-effect was noted. Age-related decline in mitochondrial oxidative capacity was absent in endurance-trained individuals. Although endurance trained individuals exhibited higher expression of mitochondrial proteins, mtDNA, and mitochondrial transcription factors there were persisting effects of age. SIRT3 expression was lower with age in sedentary but equally elevated in endurance trained individuals.

Conclusions: The results demonstrate that reduced insulin sensitivity is likely related to changes in adiposity and physical inactivity rather than an inevitable consequence of aging. The results also show that regular endurance exercise partly normalizes age-related mitochondrial dysfunction, although there are persisting effects of age at the level of mtDNA abundance, nuclear transcription factors, and mitochondrial protein expression. Furthermore, exercise may promote longevity through pathways common to effects of caloric restriction.

Reduced insulin sensitivity is a common factor in a cluster of clinical conditions currently known as metabolic syndrome (1;2) that increases with age (3). Mitochondrial dysfunction is also prevalent in the elderly (4;5), with reductions in mitochondrial enzyme activities (6), protein synthesis (7), protein expression (5), and DNA (mtDNA) abundance (5;8). A close association between insulin sensitivity and muscle mitochondrial function has been reported in aging (4;5), type 2 diabetes (9), obesity (10), and in offspring of type 2 diabetes (11), prompting a hypothesis that either reduced insulin sensitivity results from muscle mitochondrial dysfunction (4;11) or vice versa (5;12).

Endurance exercise increases insulin sensitivity (13;14) and mitochondrial enzyme activities (15;16). Short-term and longitudinal studies have documented that older populations respond favorably to endurance exercise, but there are persisting age effects that cannot be eliminated by short-term exercise programs (8;17). For practical reasons, most training studies are acute interventions in relation to the chronic effects of aging. This precludes conclusions regarding whether older adults are less adaptable to exercise training than young or if the volume and duration of the training were insufficient to reveal the full potential for adaptation. Furthermore, it remains to be determined whether long term endurance exercise shares some of the same biochemical effects as caloric restriction, which prolongs the lifespan of many species through the DNA-stabilizing actions of sirtuins (18), in particular the mitochondrial-localized SIRT3 (19).

The purpose of this study was to determine if age-related declines in insulin sensitivity and mitochondrial function could be prevented by long-term endurance training. Peripheral and hepatic insulin sensitivity were measured by euglycemic-hyperinsulinemic clamp in sedentary and chronically endurance trained young (18-30 years) and older (59-76 years old) adults. Mitochondrial function was assessed by measuring muscle ATP production rates (MAPR) in isolated mitochondria from vastus lateralis. To examine molecular and cellular mechanisms responsible for group differences in mitochondrial function, citrate synthase (CS) activity, mtDNA, large-scale protein expression using mass spectrometry, and expression of key mitochondrial transcription factors, including nuclear respiratory factor-1 (NRF-1), mitochondrial transcription factor A (TFAM) and their coregulator, PPARγ coactivator 1α (PGC-1α) were measured. We also determined the effects of aging on SIRT3 expression and if chronic endurance training could induce similar effects as caloric restriction by increasing SIRT3 expression. This study was designed as a cross-sectional comparison of sedentary and endurance trained young and old to circumvent the numerous practical limitations that would complicate a prospective study of sufficient duration to satisfy the aim of the study.

RESEARCH DESIGN AND METHODS

Subjects. Twenty two healthy young (18-30yrs) and 20 healthy older (59-76yrs) adults gave written informed consent, as approved by the Mayo Foundation Institutional Review Board. Groups were further divided into young sedentary (YS, 5W, 6M), young trained (YT, 5W, 6M), older sedentary (OS, 4W, 6M), and older trained (OT, 4W, 6M). Sedentary subjects exercised less than 30 min per day, twice per week. Trained subjects performed at least 1 hour of cycling or running 6 days per week over the past 4 years or longer by self-report. Activity levels were confirmed with a Leisure-Time Activity (LTA) questionnaire. Subjects were screened by medical history, physical exam, graded treadmill test, and comprehensive blood tests including blood lipids from standard photometric methods, hormones, and glucose. Magnetic resonance spectroscopy (MRS) was used to measure lipoprotein particle concentrations in plasma (20). Briefly, an EDTA plasma sample diluted 1:1 was injected into the flow probe of a Bruker-Biospin 400MHz ^{1}H-NMR. Lipana software analyzed the data to determine LDL and HDL particle concentration. Exclusion criteria included history of metabolic or cardiovascular disease, plasma glucose > 99mg/dl, BMI > 28 kg/m^2, medications affecting outcome measures, anemia, pregnancy, or substance abuse.

Study design. Dual x-ray absorptiometry (Lunar DPX-L, Lunar Radiation, Madison, WI) was used to measure fat and fat-free mass. Abdominal and visceral fat area were measured with single-slice CT scans (Imatron C-150, San Francisco, CA), as described previously (21). Whole-body peak oxygen uptake (VO_2 peak) was determined from expired gas analysis during a graded bicycle test. To ensure that subjects achieved VO_2 peak, at least two of the following criteria were confirmed: a plateau in VO_2, respiratory exchange ratio $\geq$ 1.1, and attainment of age-predicted maximal heart rate. At least 7 days after the VO_2 peak test, subjects were given a meat-free weight-maintaining diet for 3 days prior to inpatient testing and refrained from exercise. At 17:00 hours on day 3 of the diet, subjects were admitted to the Mayo Clinic General Clinical Research Center for 48 hours. Muscle mass was estimated from 48 hour urinary creatinine excretion. Subjects were given a standard meal and snack at 18:00 and 22:00 hours of the second day, after which they remained fasted until the end of the study. Following baseline blood sampling at 03:30 the following morning, a primed (2.4 mg/kg FFM), continuous (0.04 mg/kg FFM/min) infusion of [6,6-$^2H_2$]-D-glucose was initiated. After 05:30 hours, one hand was placed in a heated box (120 ºF) with a retrograde IV catheter. At 06:00 hours, arterialized blood samples were taken every 10 minutes until 06:30 for [6,6-$^2H_2$]-D-glucose isotopic enrichment measurements. At 06:30, a percutaneous needle muscle biopsy (350-400 mg) was obtained from the vastus lateralis muscle under local anesthesia (22). Approximately 40 mg of muscle was immediately used for measurement of MAPR, as previously described (5), and the remaining tissue was frozen in liquid nitrogen and stored at -80ºF for other analyses.

At 06:30 hours, following the muscle biopsy, an 8 hour euglycemic-hyperinsulinemic clamp was started with insulin infused at 1.5 mU/kg FFM/min. The infusion rate of [6,6-$^2H_2$]-D-glucose was varied to mimic the anticipated changes in endogenous glucose production, as described previously (23). Plasma glucose was maintained at ~90 mg/dl during insulin infusion with a variable infusion of 40% dextrose containing 2.15% [6,6-$^2H_2$]-D-glucose to minimize the changes in glucose enrichments and maintain constant plasma specific activity (23). The glucose infusion rate was adjusted as needed based on blood samples at 10 minute intervals, which were measured with a Beckman glucose analyzer (Beckman Coulter, Fullerton, CA) (24). [6,6-$^2H_2$]-D-glucose enrichment of blood samples and infusates were measured using mass spectrometry. The steady-state equations of Steele (25) were used to calculate glucose appearance (R_a) and disappearance (R_d), as described previously (23):

$$R_a = R_d = F_{Glu} / \text{SA of } [6,6\text{-}^2H_2] \text{ glucose} \quad (1)$$

Where F_{Glu} is the infusion rate of [6,6-$^2H_2$]-D-glucose and SA of [6,6-$^2H_2$] glucose is the plasma specific activity of [6,6-$^2H_2$] glucose. Endogenous glucose production (EGP) was calculated as the difference between total glucose rate of appearance and exogenous glucose infusion rate (23;26). Peripheral insulin sensitivity was assessed from the rate of glucose infusion required to maintain euglycemia during the clamp, while hepatic insulin sensitivity was assessed by the extent to which EGP was suppressed during hyperinsulinemia.

Mitochondrial ATP production. MAPR was measured using a bioluminescent technique, as described previously (5). Briefly, ~40 mg of fresh muscle was homogenized and centrifuged to isolate mitochondria. The mitochondria were suspended in buffer containing 35 μM ADP and a luciferin-luciferase ATP-monitoring reagent (BioThelma, Haninge, Sweden). A BioOrbit 1251 luminometer (BioOrbit Oy, Turku, Finland) was used to monitor bioluminescence in response to the addition of glutamate plus malate, palmitoyl-L-carnitine plus malate, and succinate plus rotenone. All reactions were monitored for 25 minutes at 25ºC, followed by calibration with an ATP standard. CS activity was measured using spectrophotometric analyses in the mitochondrial pellet (7).

Mitochondrial DNA abundance. DNA were extracted from frozen muscle samples using a QIAamp DNA mini kit (QIAGEN, Chatworth, CA). The abundance of mtDNA-encoded NADH dehydrogenase 1 (ND1) and 4 (ND4) genes were measured using a real-time quantitative PCR system (PE Biosystems, Foster City, CA). Samples were run in duplicate and normalized to S28 ribosomal DNA, as described previously (5).

Westen blotting. Muscle samples were homogenized in cold lysing buffer with protease inhibitors. Following centrifugation for 15 minutes at 1,000*g*, the supernatants were diluted to 2 μg/μL based on protein concentrations determined based on the Lowry procedure (DC Protein Assay, BioRad). 10 μL quantities were loaded and separated on SDS-PAGE (Invitrogen) and transferred onto polyvinylidene difluouride membranes (BioRad). Membranes were blocked with Tris-buffered saline with 5% nonfat milk and then washed with primary antibodies for PGC-1α (516557; Calbiochem, San Diego, CA), TFAM (PA1-24435; Affinity Bioreagents, Golden, CO), NRF-1 (33771; Santa Cruz Biotechnology, Santa Cruz, CA), and SIRT3 (ARP32389; Aviva Systems Biology, San Diego, CA). Following incubation with secondary antibody conjugated to horseradish peroxidase, membranes were exposed to enhanced chemiluminescence reagents for 5 minutes. Densitometric analyses were conducted using a Kodak Image Station 1000.

Proteomics. To determine the abundance of several individual proteins relevant to cellular ATP production, muscle samples from each subject were prepared as previously described, and the relative abundance of single peptides was determined using iTRAQ approach (27). Samples from YT, YS, OT, OS were labeled with iTRAQ reagents 114, 115, 116, and 117, separated, and identified using liquid chromatography / tandem mass spectrometry.

Statistical analyses. Data are presented as means ± SEM throughout. Two-way (age, activity level) ANOVA was used to examine the effects of age, activity level, and the interaction on all outcome variables. When significant interactions were found, post-hoc pairwise comparisons were conducted using Tukey's procedure. Individual t-tests were used for *a priori* planned comparisons between OS and YS, and OT and YT. Regression analyses were conducted to explore the relationships between insulin sensitivity and predictors such as age, fat mass, activity score, BMI, and abdominal adiposity. Statistical analyses were conducted using SAS software (SAS, Cary, NC).

RESULTSs

Subject characteristics. Age was associated with decreased muscle mass, increased adiposity, and decreased VO_2 peak (Table 1).

In exercise trained young and old people VO_2 peak was higher but percent fat and abdominal and visceral fat content were lower than in the respective sedentary young and old groups. Total cholesterol and LDL were elevated in older compared to young sedentary adults, and HDL was elevated in endurance trained individuals in both age groups. Although LDL cholesterol did not differ between sedentary and endurance trained individuals, both total and small LDL particle concentrations measured by NMR were significantly lower in exercising individuals.

Insulin sensitivity. Average plasma insulin during the clamp were similarly high in YS (44.7 ± 1.9 μU/ml), OS (43.1 ± 3.2 μU/ml), YT (45.7 ± 2.1 μU/ml), and OT (47.3 ± 2.3 μU/ml). The glucose infusion rate (GIR) required to maintain euglycemia during the clamp was similar in young and old, but higher in trained compared to sedentary (Figure 1A). The area under the GIR curve (AUC, Figure 1A insets) during minutes 0 through 480 and minutes 240 through 480 (plateau region) indicate greater glucose infusion per unit fat free mass in trained compared to sedentary subjects with no effects of age in either activity group. Likewise, total R_d during hyperinsulinemia was higher in trained compared to sedentary individuals with no effects of age (Figure 1B). Endogenous glucose production (EGP) was lower in trained compared to sedentary individuals at baseline (Figure 1B). During the clamp, EGP was suppressed in all groups (YS=69±7%, YT=100±11%, OS=78±7%, OT=97±10%), although the magnitude of EGP suppression by insulin was greater in trained compared to sedentary subjects (Figure 1B). No effects of age on EGP or relative suppression of EGP were noted. The relationship between R_d and age was non-significant ($R = 0.11$, $P = 0.53$, data not shown). However R_d during the clamp was inversely related to BMI ($R = -0.37$, $P = 0.026$) fat mass ($R = -0.53$, $P = 0.001$), and abdominal fat ($R = -0.37$, $P = 0.027$, data not shown).

Mitochondrial ATP production capacity. MAPR was reduced in older versus younger people using substrates glutamate+malate, succinate+rotenone, and palmotoyl-L-carnitine+malate, but was higher in endurance trained compared to sedentary subjects ($P_{age} \leq 0.004$, Figure 2). Furthermore, paired comparisons revealed age-related declines in MAPR in sedentary ($P \leq 0.006$) but not trained subjects. Similar results were found for citrate synthase activity (Figure 3A).

Mitochondrial DNA abundance. The abundance of mtDNA using both the ND1 and ND4 gene probes was lower in older compared to young subjects ($P_{age} \leq 0.0004$, Figure 3B, C). Although mtDNA abundance was significantly higher in trained compared to sedentary subjects ($P_{training} < 0.05$), mtDNA remained significantly lower in OT compared to YT.

Protein expression of mitochondrial biogenesis genes. Protein expression of PGC-1α, NRF-1 and TFAM were similar in YS and OS and higher in trained compared to sedentary ($P_{training} < 0.001$, Figure 4). PGC-1α and TFAM protein content was significantly lower in OY compared to YT ($P < 0.0001$), however, NRF-1 expression was similar in YT and OT.

Proteomic data. Figure 5A-D depicts the relative abundance of several proteins involved in key metabolic processes in skeletal muscle. Comparison between OS and YS revealed 27 oxidative and glycolytic proteins with significantly lower relative concentrations in older adults (Figure 5A). Endurance trained young and older individuals exhibited higher expression of numerous proteins involved in oxidative ATP production relative to their sedentary counterparts (Figure 5B,C). A comparison of YT and OT revealed that, with the exception of 3 subunits of cytochrome c oxidase and an aminotransferase enzyme, the age differences in protein abundance that were observed in sedentary individuals were no longer apparent in endurance trained individuals (Figure 5D).

SIRT3 expression. Protein expression of SIRT3 was lower with age in sedentary adults and significantly higher in trained compared to sedentary subjects with no effect of age in trained adults (Figure 6).

DISCUSSION

The current study examined the effects of aging and endurance exercise on insulin sensitivity and mitochondrial function to further explore whether age-related mitochondrial dysfunction and lowering of insulin sensitivity are inevitable consequences of chronological age. The main findings of the present study are first, endurance exercise-trained young and older people have substantially higher insulin sensitivity than the sedentary groups and no differences between young and older people were observed in either sedentary or exercise trained groups. Secondly, in contrast, we found age-related declines in various markers of mitochondrial function in sedentary group but these age-related differences were partly, but not completely, abolished in people who practice regular endurance exercise. Finally, we show that endurance exercise may exert similar potentially lifespan-enhancing effects as caloric restriction through elevated SIRT3 expression in both young and older adults.

In agreement with previous reports (4;5), the capacity for mitochondrial ATP production declined with age in sedentary adults. As expected, MAPR and CS activity were higher in endurance trained compared to sedentary individuals. Although direct comparisons revealed ~10-15% lower CS activity and MAPR in OT compared to YT, these differences were not statistically significant. Thus, our data underscore the effectiveness of regular endurance exercise in largely preventing age-related declines in mitochondrial oxidative capacity. Similarly, histochemical and enzymatic measurements in young (27±3 yrs) and older (63±6 yrs) endurance runners showed that despite 11% lower VO_2 peak compared to young runners, master's level runners exhibited no signs of decreased mitochondrial enzyme activities or capillary density (28). *In vitro* measures of oxidative capacity, such as employed in the present study, provide distinct information regarding mitochondrial function compared to *in vivo* measurements of resting mitochondrial ATP production, as measured using magnetic resonance spectroscopy (4). Given that skeletal muscle is relatively metabolically inert in the postabsorptive resting state, and that endurance training increases VO_2peak with minimal effects on resting VO_2, it is our belief that the maximal capacity for mitochondrial ATP synthesis is more relevant to the study of endurance exercise training and aging.

Several additional mitochondrial markers were also assessed in an effort to ascribe molecular and cellular mechanisms to explain our MAPR findings. Using mass spectrometry methods, we demonstrate that numerous proteins involved in the citric acid cycle (isocitrate dehydrogenase, aspartate aminotransferase, malate dehydrogenase, oxoglutarate dehydrogenase) and electron transport (cytochrome c oxidase, ubiquinol-cytochrome c reductase, NADH dehydrogenase, ATP synthase) were expressed at lower levels in OS compared to YS. Regardless of age, endurance trained individuals exhibited higher expression of these proteins. Furthermore, the age-differences in protein expression evident in sedentary individuals were absent in endurance trained individuals, with the exception of 3 subunits of cytochrome c oxidase and aspartate aminotransferase, which remained significantly lower in OT compared to YT. Our proteomic data closely parallel our MAPR data, suggesting that the expression level of key mitochondrial proteins may be a primary determinant of age-related declines in oxidative capacity and the beneficial effects of regular endurance exercise. An important point is that regular endurance exercise could not entirely eliminate the effects of aging on protein expression, consistent with the ~15% lower ATP production capacity in OT versus YT.

The abundance of mtDNA plays a role in the expression level of several proteins that were measured using mass spectrometry, specifically subunits of cytochrome c oxidase and NADH dehydrogenase. Consistent with previous reports (5;8), we found that mtDNA abundance was significantly reduced with age in sedentary individuals and that endurance trained individuals in both age groups exhibited higher mtDNA abundance. Of interest, the regular endurance exercise did not completely normalize age-related declines in mtDNA abundance, which is a tempting mechanism to explain persisting age-related deficits in the protein expression of mitochondrial-encoded cytochrome c oxidase subunits in endurance trained individuals.

The replication, maintenance, and transcription of mitochondrial DNA are controlled by mitochondrial transcription factor A (TFAM). TFAM expression is controlled by upstream nuclear transcription factors (NRF1, NRF2), which also regulate the expression of nuclear genes encoding mitochondrial proteins. PGC-1α plays a key role in activating these downstream transcription factors and, therefore, acts as a control point regulating mitochondrial biogenesis (29). The protein expression of PGC-1α, NRF1, and TFAM was similar in young and older sedentary individuals and cannot explain our observations of decreased mtDNA abundance in sedentary older adults. Age-related accumulation of DNA oxidative damage (5) could explain reduced mtDNA in sedentary older adults in spite of similar expression of mitochondrial transcription factors. PGC-1α, NRF1 and TFAM were all expressed at higher levels in endurance trained individuals; however, a key finding is that significant effects of age were evident for PGC-1α and TFAM, but not NRF1. Thus, the persisting effects of age on mtDNA abundance despite endurance exercise training may be explained by blunted expression of upstream transcription factors involved in mitochondrial biogenesis.

A key finding of the current study is related to the effects of aging and endurance exercise on insulin sensitivity. Although decreased insulin sensitivity is reported to occur with age, we find that both hepatic and peripheral insulin sensitivity were similar in sedentary young and older adults. While the absence of age-related declines in insulin sensitivity is in contrast with numerous previous reports (4;21;30;31), our results are consistent with a growing body of literature indicating that adiposity and physical activity levels, rather than chronological age, are primary determinants of age-related declines in insulin sensitivity (32-36). In support of this notion, we find that fat mass, abdominal adiposity, and BMI were significant predictors of R_d during the clamp. The sedentary older adults in our cohort were healthy, relatively lean people with substantially less visceral and abdominal adiposity than what has been reported from older adults with decreased insulin sensitivity (21). Our findings are in keeping with earlier studies that also revealed similarities in insulin sensitivity in relatively lean sedentary young and older adults (34;37). Peripheral and hepatic insulin sensitivity were significantly higher in endurance trained compared to sedentary individuals, and these effects were independent of age. These data are supported by previous investigators who have demonstrated that endurance exercise confers substantial improvements in peripheral and hepatic insulin sensitivity, regardless of age (14;38;39). The results from the current study strongly support the notion that decreased insulin sensitivity may not be an inevitable consequence of aging, and age-related changes in body composition is a key factor contributing to the previously reported decline in insulin sensitivity with aging. Furthermore, in addition to maintenance of normal body weight, endurance exercise should be considered as a viable, effective intervention to reverse, delay, or prevent the onset of age-related declines in insulin sensitivity that is likely to occur at more advanced age.

Mitochondrial dysfunction and decreased sensitivity to insulin are often observed concurrently in type 2 diabetic (9), elderly (4;5), and obese individuals (10), prompting 2 opposing hypotheses concerning this relationship. It has been proposed that mitochondrial dysfunction plays an etiological role in the development of insulin resistance (11;40), while some evidence supports an alternative hypothesis that mitochondrial dysfunction may result from insulin resistance (41;42). Still some other studies find a dissociation between mitochondrial capacity and insulin sensitivity (21;43;44). In the present study, sedentary older adults were as insulin sensitive as young sedentary people despite significantly reduced MAPR, casting further doubt on the causal role of mitochondrial dysfunction in decreasing insulin sensitivity. However, we observe significant elevations in both oxidative capacity and insulin sensitivity in endurance trained individuals regardless of age. These data underscore the fact that, although mitochondrial function has been extensively studied in insulin-resistant populations, our understanding of its role in the development of insulin-resistance is far from complete.

We assessed other outcomes relevant to age-related co-morbidities that may impact insulin sensitivity and mitochondrial function. Percent body fat, abdominal fat, and visceral fat were elevated in older adults, and although these measures were lower in endurance trained individuals, the effects of age on body composition were not completely normalized in endurance trained adults. Similarly, the age-related decline in muscle mass observed in sedentary individuals was also apparent in endurance trained individuals, suggesting that endurance exercise can partially prevent age-related changes in adiposity but has limited utility in preventing sarcopenia as would resistance training. Both the traditional photometric-based clinical lipid panel and NMR-based method of measuring lipoprotein particle concentrations revealed the expected elevations in total cholesterol and LDL in older compared to young sedentary adults as well as beneficial effects of endurance exercise on increasing HDL in both age groups. However, while exercise was not related to total LDL cholesterol measured photometrically, both total and small LDL particle concentrations measured by NMR were significantly lower in exercising individuals.

We also provide data to link endurance exercise with the lifespan-enhancing effects of caloric restriction (45). Mitochondrial-localized SIRT3 is linked to longevity, possibly by interfering with the release of apoptosis-inducing factor (19; 46). In a similar manner to which nutrient restriction increases lifespan (19), we found that SIRT3 expression was higher in endurance trained compared to sedentary individuals. Furthermore, SIRT3 expression was lower with age in sedentary, but not older endurance trained individuals. Thus, our results support a hypothesis that exercise may confer similar lifespan-extending effects as caloric restriction through the actions of mitochondrial SIRT3.

It is important to consider that many detrimental effects of old age are compounded after the 8th decade of life. It is unclear if similar effects of regular endurance exercise would be evident in individuals at more advanced ages than were studied in the present study. It is theoretically possible that the cross-sectional design of the study may introduce a sampling bias whereby individuals who exercise across their lifespan do so because their mitochondrial function is inherently higher than their more sedentary peers. Notwithstanding, it is becoming increasingly apparent that waning physical activity levels are a primary determinant of age-related mitochondrial dysfunction and may accelerate the aging process through adverse effects on many biochemical and molecular factors that require ATP.

In conclusion, we find that age-related declines in oxidative capacity can be largely ameliorated by regular endurance exercise, highlighting the fact that physical inactivity plays an important role in age-related dysfunctions and underscores the need for better control of this variable in the literature. However, certain mitochondrial markers, specifically mtDNA, transcription factors, and mitochondrial-encoded proteins, remain depressed with age despite endurance exercise. Neither hepatic nor peripheral insulin sensitivity was impaired with age in these healthy, relatively lean individuals, in keeping with the notion that adiposity is a primary determinant of age-related reductions in insulin sensitivity. Notwithstanding, we illustrate that endurance trained compared to sedentary individuals exhibit elevated insulin sensitivity in a manner independent of age. Finally, we observed an age-related decline in muscle expression of SIRT3 in sedentary, but not endurance trained individuals, suggesting that endurance exercise may exert similar potentially life-span enhancing effects as demonstrated with caloric restriction in other organisms.

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Table 1. Subject Characteristics

	YS (n = 11)	YT (n = 11)	OS (n = 10)	OT (n = 10)	P age	P training	P interaction
Physical characteristics							
Age (years)	22.7±0.7	26.0±1.0	65.1±1.5*	65.4±1.8*	<0.01	0.17	0.26
Height (cm)	172.6±2.7	174.4±2.9	170.8±3.0	167.8±3.0	0.16	0.85	0.41
Weight (kg)	69.4±3.6	69.2±3.8	71.5±4.0	69.1±4.0	0.80	0.73	0.79
BMI (kg/m^2)	23.1±0.9	22.7±0.7	24.4±0.7	24.4±1.0	0.09	0.81	0.75
Fat-free mass (kg)	48.5±3.1	53.5±3.0	45.5±3.8	50.4±3.1	0.35	0.14	0.98
Body fat (%)	25.8±1.9	17.4±2.2	32.2±2.9	20.8±3.2	0.06	<0.001	0.55
Muscle mass (kg)	33.7+4.5	33.6±2.3	25.4±2.3*	27.1±1.7	0.02	0.80	0.77
Abdominal fat (cm^2)	185±23	101±20	264±31	186±33*	0.005	0.08	0.57
Visceral fat (cm^2)	59.2±11.0	32.1±4.2	127±16.3*	91.9±19.3*	0.001	0.03	0.98
VO_2peak (ml/kg FFM/min)	51.2±2.3	66.7±1.7	36.5±1.6*	54.0±3.6*	<0.001	<0.001	0.67
LTA score (min/wk)	198±59	990±110	217±77	1114±206	0.56	<0.001	0.67
Lipids							
Cholesterol (mg/dl)	132±6	135±8	182±9*	167±11*	<0.01	0.45	0.27
HDL (mg/dl)	32±2	43±3	47±5*	51±3	<0.01	0.04	0.33
LDL (mg/dl)	73±5	78±6	116±9*	97±10	<0.01	0.37	0.13
TG (mg/dl)	134±17	74±7†	98±8*	91±11	-	-	0.03
Total HDL particles (µmol/L)	30.4±1.3	29.4±1.8	35.3±1.6*	31.9±1.1	0.02	0.12	0.45
Large HDL particles (µmol/L)	4.67±0.81	8.25±0.40	6.62±1.27	7.66±0.75	0.42	0.01	0.14
Total LDL particles (nmol/L)	966±79	727±63	1178±74*	1013±70*	0.001	0.008	0.65

Small LDL particles (nmol/L)	582±112	191±48	487±78	363±79	0.64	0.004	0.11
Glucose and hormones							
Glucose (mg/dl)	84.8±1.8	83.2±1.2	89.5±2.0*	92.0±1.7*	<0.01	0.78	0.24
Glucagon (pg/ml)	151±14	142±8	138±6	121±10	0.11	0.23	0.68
Insulin (µU/ml)	6.0±0.5	5.0±0.4	5.1±0.5	5.1±2.0	0.70	0.61	0.60

Data are given as mean ± SEM. * denotes where unpaired t-tests indicated values significantly ($P < 0.05$) different from young subjects within the same physical activity group. † denotes where Tukey's procedure indicated values significantly ($P < 0.05$) different from untrained subjects within the same age group in the event of a significant interaction term.

FIGURE LEGENDS

Figure 1. Hyperinsulinemic-euglycemic clamp results. The rates of glucose infusion (GIR) required to maintain euglycemia during the 8 hour clamp were higher in trained compared to sedentary subjects with no effects of age (A). Insets in panel A show the area under the curve (AUC) during minutes 240-480, corresponding to a plateau in GIR, and during the entire clamp (0-480). Glucose rate of disappearance (Rd) during hyperinsulinemia was higher in trained than sedentary subjects (B). No effects of age on Rd were observed. Endogenous glucose production (EGP) was suppressed during the clamp in all groups (YS = 69 ± 7 %, YT = 100 ± 11 %, OS = 78 ± 7 %, OT = 97 ± 10 %). The extent of suppression did not differ by age, but was greater in trained compared to sedentary individuals (P = 0.01). EGP was not significantly different from zero in trained individuals. Data are presented as mean ± SEM. ** denotes where significant (P<0.05) differences in relative suppression were detected.

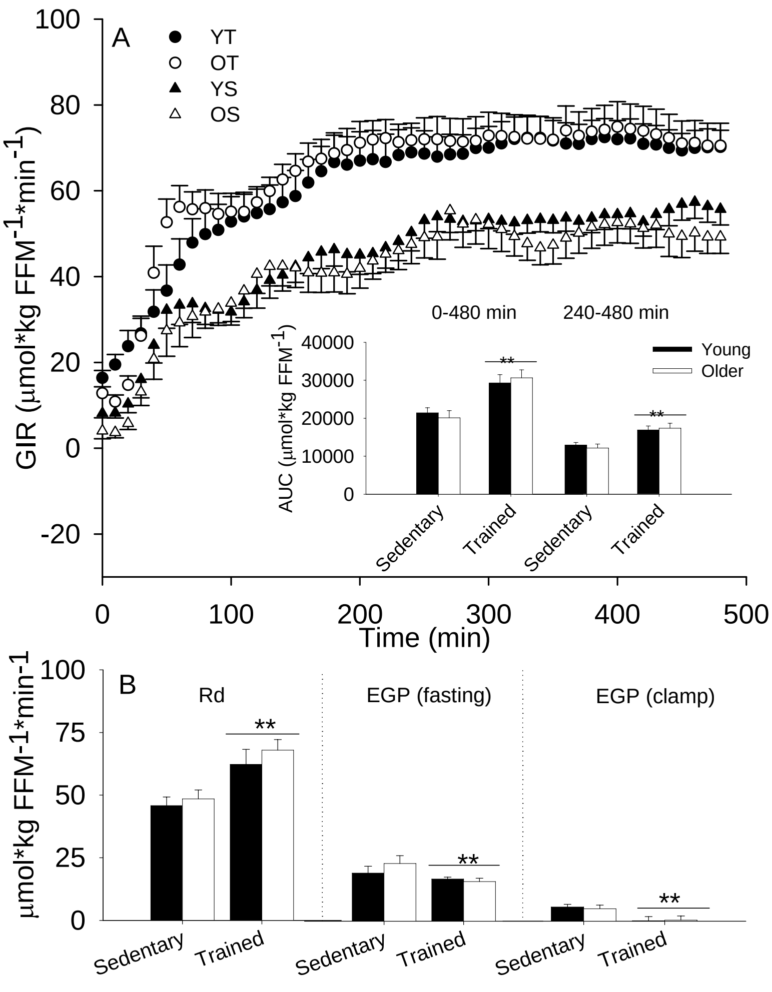

Figure 2. Mitochondrial ATP production rates. An age-related decline in mitochondrial ATP production was observed using substrates glutamate plus malate (GM, panel A), succinate plus rotenone (SR, panel B) and palmitoyl-L-carnitine plus malate (PCM, panel C) in sedentary subjects. These effects of age were not apparent in endurance trained subjects. Data are presented as means ± SEM. ** denotes where significant (P<0.05) main effects of training were detected. * denotes where pairwise comparisons revealed significant (P < 0.05) effects of age within activity groups.

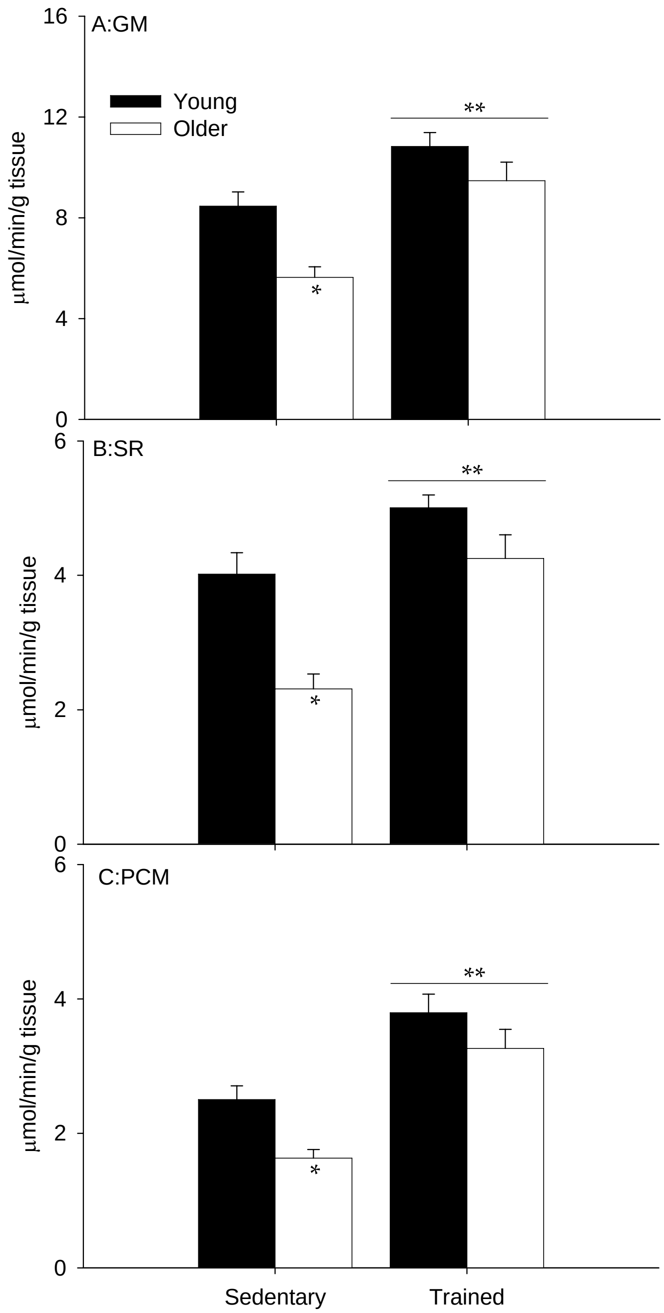

Figure 3 Citrate synthase activity and mitochondrial DNA abundance. An age-related decline in citrate synthase activity was observed in sedentary but not endurance trained subjects (CS, panel A). Mitochondrial DNA copy number for subunits 1 (ND1, panel B) and 4 (ND4, panel C) of NADH dehydrogenase were lower with age in sedentary and trained subjects. Training was associated with higher mitochondrial DNA abundance in both age groups, but the effects of age remained apparent. Data are presented as means ± SEM. ** denotes where significant ($P < 0.05$) main effects of training were detected. * denotes where pairwise comparisons revealed significant ($P < 0.05$) effects of age within activity groups.

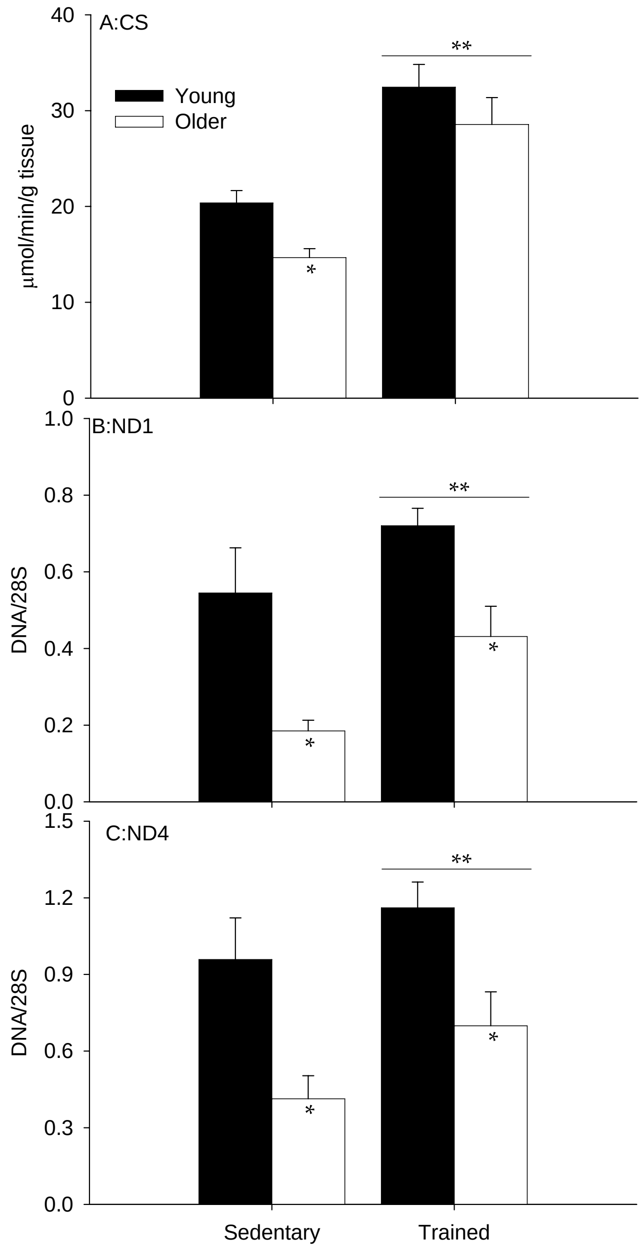

Figure 4. Expression of proteins involved in mitochondrial biogenesis. Protein expression of peroxisomal proliferator activator receptor gamma co-activator 1 alpha (PGC-1α, panel B), nuclear respiratory factor 1 (NRF-1, panel C), and mitochondrial transcription factor A (TFAM, panel D) were similar in young and old sedentary and higher in trained subjects. Representative blots of each protein are showed in panel A. Data are presented as means ± SEM. ** denotes where significant ($P < 0.05$) main effects of training were detected. * denotes where pairwise comparisons revealed significant ($P < 0.05$) effects of age within activity groups.

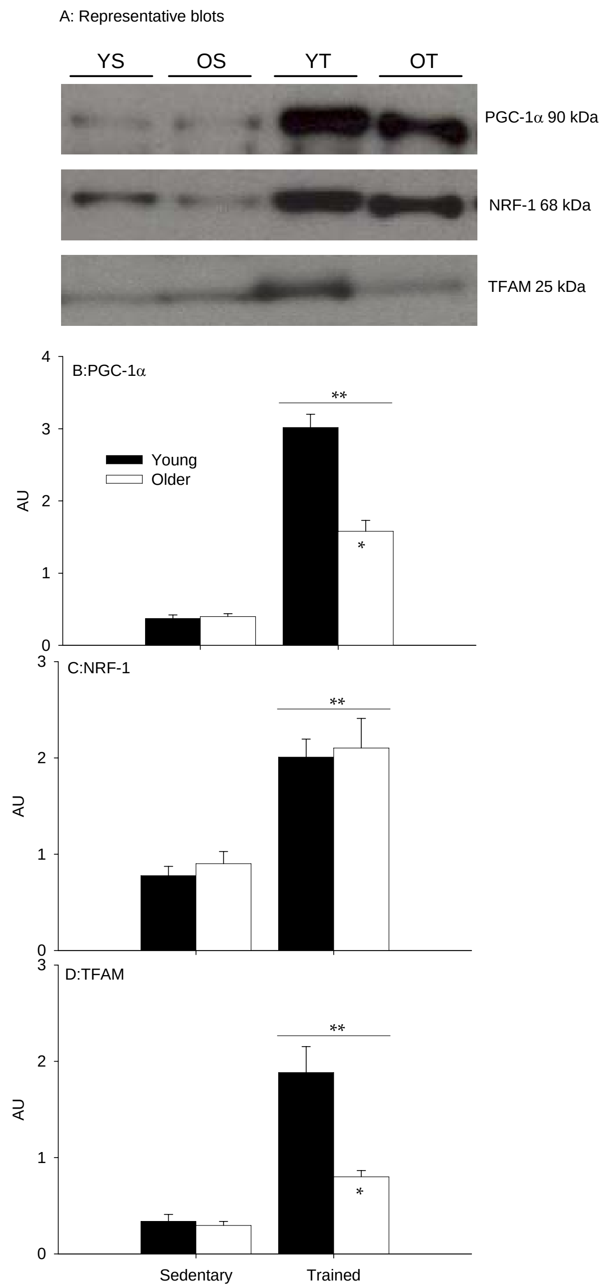

Figure 5. Relative abundance of proteins involved in oxidative and glycolytic ATP production in skeletal muscle Horizontal bars indicate the percent difference in relative abundance of proteins involved in oxidative (red) and glycolytic (blue) energy metabolism. Panel A shows that numerous proteins involved oxidative and glycolytic ATP synthesis are reduced in older sedentary (OS) compared to young sedentary (YS) individuals. Panels B and C indicate that endurance training is associated with elevated expression of proteins involved in oxidative ATP synthesis in young (Panel B) and older (Panel C) subjects. Panel D indicates that long-term endurance exercise normalized the effects of age on the expression of all but 4 mitochondrial proteins. Data are presented as means ± SEM. * denotes where significant (P < 0.05) differences in relative expression were detected.

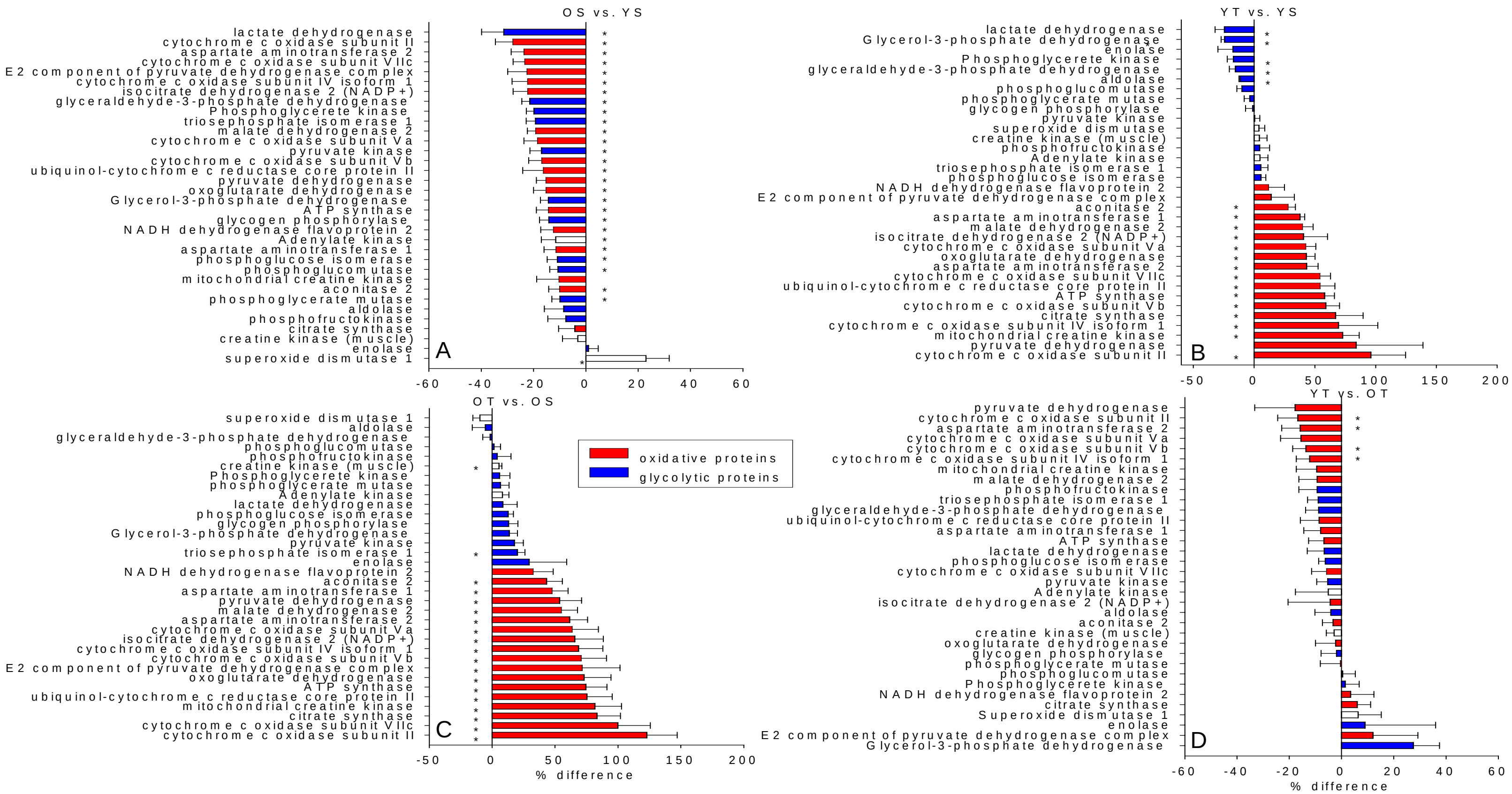

Figure 6. Protein expression of mitochondrial sirtuin 3 (SIRT3) Protein expression of SIRT3 was lower with age in sedentary adults, with no effect of age in trained adults. Data are presented as means ± SEM. ** denotes where significant ($P < 0.05$) main effects of training were detected. * denotes where pairwise comparisons revealed significant ($P < 0.05$) effects of age within activity groups.