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Mitochondrial biogenesis and angiogenesis in skeletal muscle of the elderly

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1. Introduction

Ageing has been suggested to be associated with impaired mitochondrial DNA regulation and metabolic dysfunction in skeletal muscle [53]. These unfavourable changes are likely to lead to poor functional abilities in every-day life and sarcopenia [14] in elderly people as well as to enhance the risk of developing age-related metabolic diseases [34]. It has thus been suggested, that an observed age-related ~40% decline in TCA cycle flux and ATP synthesis rate in skeletal muscle ultimately contributes to insulin resistance [38]. Others have however contradicted this hypothesized impaired mitochondrial function in elderly by observing well functioning skeletal muscle mitochondria in elderly human subjects with 90-95% capacity of key oxidative enzymes relative to young subjects, except for β -oxidation which was reported to be 25% reduced [44]. Hence, it is still debated if mitochondrial function is impaired with age. Observed age-related metabolic dysfunction may either be due to a less active lifestyle in the elderly people and/or biological aging per se [4, 15, 46].

Endurance exercise training has many beneficial effects on health in young people, including improved oxidative capacity of skeletal muscle [10]. The molecular mechanisms behind these adaptations in skeletal muscle include an increased content and/or activity of key enzymes in metabolism and angiogenesis, such as citrate synthase (CS), succinate-dehydrogenase (SDH), pyruvate-dehydrogenase (PDH) and vascular endothelial growth factor (VEGF) [12, 16-18, 26, 29, 35]. A key mechanism behind these exercise training induced adaptations seems to be a transient transcriptional response of metabolic and angiogenic proteins to each single exercise bout orchestrated by the transcriptional co-activator peroxisome proliferator activated receptor gamma coactivator 1-alpha (PGC-1 α) [40, 41]. Regularly repeated, these transient gene responses are thought to lead to an accumulation of mRNAs and ultimately proteins involved in oxidative metabolism and angiogenesis [23, 40, 54]. These transient mRNA responses seem partly to be mediated by increased phosphorylation of intracellular signalling molecules with exercise, like AMPK activated protein kinase (AMPK) and P38 MAP kinase [25, 32]. The AMPK activator AICAR has been shown to upregulate PGC-1 α mRNA in mice skeletal muscle [21], and both AMPK and P38 has been reported to activate PGC-1 α by phosphorylation [22, 42]. In addition, AMPK and P38 phosphorylation has been reported to increase in human

skeletal muscle immediately after exercise [24]. Hence, AMPK and P38 may initiate PGC-1 α mediated responses to exercise [37].

However little is known about the ability to prevent the potential age-associated decrease in oxidative enzyme capacity with exercise training, although previous observations indicate that this might be possible [47]. Thus, previous studies reported that elderly orienteer runners had SDH, CS and 3-hydroxy-acyl-CoA-dehydrogenase (HAD) activities at the same level as endurance trained young subjects [9, 48]. Adaptability in other parameters such as maximal oxygen consumption and muscle strength has also been reported in elderly [19, 20] and old people [7] and supports a general maintained ability of aged individuals to adapt to exercise training. However, it is yet not clarified whether lifelong exercise training will enhance the oxidative capacity in skeletal muscle and whether skeletal muscle of elderly people maintains the ability to adapt in metabolic and angiogenic factors to exercise training.

Therefore the aim of this study was to test the hypotheses that 1) skeletal muscles of elderly subjects have the ability to adapt to a single endurance exercise bout and 2) endurance trained elderly people have higher expression/activity of oxidative and angiogenic enzymes in skeletal muscle than untrained elderly subjects. This is evaluated by determining acute exercise induced intracellular signalling and mRNA responses as well as mitochondrial enzyme activities and metabolic and angiogenic protein contents in muscle samples from elderly endurance trained and untrained subjects.

2. Methods

2.1 Subjects

Fourteen healthy elderly men and one healthy elderly woman, aged 67-75 years, participated in the study. Eight subjects were endurance trained (ET) and seven subjects were untrained control subjects (UT). The average age, height, weight and BMI of the ET subjects were: 71.3 years (range: 67.5-76.1), 173 cm (range: 159-183), 73 kg (range: 58.2-87.6) and $24.4 \text{ kg}\cdot\text{m}^{-2}$ (range: 20.6-29.3), respectively. They had been engaged in endurance training (long-distance running, cross-country running, and road cycling) for the last 20-50 years with an average training frequency of 3 times/week (range: 2-6 times/week). Four of the ET subjects participated in National Master Athlete Championships in their respective events and two of them also participated in the Master Athlete World Games and Master Athletes Olympics. The average age, height, weight and BMI of the UT subjects were: 71.3 ± 4 years (range: 67.1-79.2), 176 cm (range: 168-185), 81.9 kg (range: 75.5-89.3) and $26.4 \text{ kg}\cdot\text{m}^{-2}$ (range: 25.1-27.6), respectively. They had not been exercising regularly for the last 30 years. The subjects were fully informed of the risks and discomforts associated with the experimental procedures, and all provided written consent. The study was carried out in accordance with the guidelines in the Declaration of Helsinki and was approved by the Copenhagen and Frederiksberg Ethics Committee.

2.2 Experimental protocol

Each participant visited the laboratory on three occasions. At the first visit, anthropometric measurements were performed, i.e. body weight, height and fat percentage (Durnin & Womersley, 1974). The first visit was also used for familiarization to the bicycle ergometer. On the second visit, the participants performed an incremental bicycle test (Monark 125, Sweden) in order to determine the maximal oxygen uptake ($\text{VO}_{2\text{max}}$) and incremental exercise performance. The test was also used to determine the load eliciting ~75% of individual maximal oxygen uptake ($\text{VO}_{2\text{max}}$). The incremental test consisted of 3 min of exercise at 0 W, after which the load was adjusted upwards by 20 W each minute. Pulmonary gas exchange and ventilation were measured (CPX/D MedGraphics, Saint Paul, Minneapolis, US) and heart rate was recorded throughout the incremental test (Polar Electro Oy, Kempele, Finland). The $\text{VO}_{2\text{max}}$ was defined as the highest 30 s mean value recorded before the subject's volitional termination of the test.

At the third visit to the laboratory, at least 48 hours after their last training session, the subjects arrived at 9 a.m. after having consumed a light standardized breakfast consisting of cereals, bread and milk at 8 a.m. A catheter was placed in an antecubital vein and a venous blood sample was collected. In preparation for biopsies from m. vastus lateralis two incisions were made under local anesthesia (20 mg/ml lidocain) and a muscle biopsy was obtained from one of the incisions using the needle biopsy technique [1] with suction. This was followed by a continuous bicycle exercise test at 75% $\text{VO}_{2\text{max}}$ to exhaustion, where a muscle biopsy was obtained from the other incision 15-60 seconds after cessation of exercise. An additional muscle biopsy was taken two hours into recovery through a new incision. All biopsies were obtained with the subjects lying in the supine position on a bed standing next to the bicycle. Additional blood samples were taken from the arm vein at exhaustion and 2 hours into the recovery period.

2.3 RNA isolation and reverse transcription

Total RNA was isolated from ~20 mg muscle tissue by a modified guanidinium thiocyanate-phenol-chloroform extraction method as previously described [5, 40].

Reverse transcription (RT) was performed using the Superscript II RNase H⁻ system (Invitrogen, Carlsbad, CA) as previously described [13, 40]. RT products (cDNA) were diluted in sterile filtered water (Millipore, Billerica, MA) to 60 $\mu\text{l}/\mu\text{g}$ cDNA.

2.4 PCR

The cDNA (mRNA) content of a given gene was determined by real time PCR using Taqman[®]-assay as previously described [33]. Primers and TaqMan probes were designed using human database Ensembl.org and Primer Express (Applied Biosystems). Primers and TaqMan Probes with 5' FAM and 3' TAMRA labelling were purchased from TAG Copenhagen (Copenhagen, Denmark) and are listed in Table 1.

Triplicates of 10 μl reaction volume were performed using Universal Mastermix (Applied Biosystem, Foster City, CA). The number of cycles until a sample reach a given fluorescence level is defined as the cycle threshold (Ct). A serial dilution of a pooled representative sample was run on each plate and used to construct a standard curve, from which the Ct values of the samples were transformed to a relative mRNA amount. For each sample, the specific target

mRNA content was normalised to GAPDH mRNA content in the given sample. The GAPDH mRNA content was not affected by training state or the acute exercise.

2.5 Immunoblotting

To determine the basal protein levels and exercise-induced intracellular signalling, muscle samples of ~20mg wet weight were homogenized as previously described [26]. After centrifugating the samples for 30 min at 17500g, the supernatant was collected. Total protein content was determined by the bicinchoninic acid assay (PIERCE REAGENTS, Rockford, IL). The protein amount was calculated based on a serial dilution of a BSA standard with known protein concentrations, which was run with the samples.

The samples were then diluted in sample buffer containing 0.5 M Tris Base (pH 6.8), DTT, SDS, Glycerol, Bromphenol blue to obtain 2 µg total protein per µl. Proteins were separated on 10% or 15% Tris-HCL gels (BioRad, Stockholm, Sweden) by SDS-PAGE followed by semi-dry transfer to PVDF membranes (Millipore, Copenhagen, Denmark). Membranes were blocked for 1 hour at 4 °C (either TBST + 2 or 3% skim milk or 3 or 5% BSA), incubated with primary antibodies in a solution of skim milk/BSA and TBST overnight and lastly incubated with horse radish peroxidase (HRP) conjugated secondary antibody in a solution of skim milk/BSA and TBST for 1 hour. HRP substrate was added (ECL-Plus reagent, GE Healthcare) to the membranes and band intensity of each sample was detected using Kodak Image Station E440CF (Kodak, Ballerup, Denmark). Protein content was quantified using Kodak Imaging Software (Kodak, Ballerup, Denmark). Protein content is given in arbitrary units relative to a human skeletal muscle standard sample run on each gel. Primary antibodies used were: Anti-GLUT4 (#998101, Affinity BioReagents, Rockford, IL), anti-hexokinase II (#28675, Santa Cruz, CA), anti-Cytocrome c (#29573, BD, Pharmingen, CA), anti-VEGF (#L1008, Santa Cruz, CA), anti-phospho-AMPK (#2535S, Cell Signalling, MA), anti-AMPKα2 (Graham Hardie), anti-phospho-P38 (#4511, Cell Signaling, MA) and anti-P38 (#9212, Cell Signalling, MA).

2.6 Mitochondrial preparation and enzyme activity

The preparation method and the measurements of mitochondrial respiratory activities have previously been described in detail [43-45]. Less than 100mg muscle tissue was used and the

preparative operations were conducted so that the measured activities could be related to tissue mass. The mitochondrial fraction contained about 40% of the tissue mitochondria.

All reported activities, except two, were measured in so-called functional assays, i.e. the enzyme was located in its normal environment, physiological substrates were used, the mitochondrial respiration was limited by the enzyme in question, and the substrate/oxygen stoichiometry was known. More detailed description of these assays is given in e.g. [43, 44].

The COX and citrate synthase assays were not functional assays. The former used non-physiological substrates, but it was useful for comparisons. Citrate synthase was assayed by the photometric method of Shepherd and Garland after treatment of the mitochondria with Triton X-100 [51].

All activities were measured at 25°C and extrapolated to 37 °C with a factor of 2.29, corresponding to a doubling of the rates from 25 to 35 °C.

2.7 Statistics

All results are given as means \pm SE. A student t-test was used to test the effect of life-long endurance exercise training on VO_2max , time to exhaustion, enzyme activity, protein content, muscle glycogen and capillarization. The hypothesis was that training increased each of these parameters and the p-values were obtained with a one-tailed test. A two way ANOVA with repeated measures was used to test the effect of training status and acute exercise on mRNA levels and on signalling. A one way ANOVA with repeated measures was used to test the effect of acute exercise on mRNA levels separately within each group. Student Newman Keuls post hoc test was used to locate differences. A significance level of $p < 0.05$ was chosen and a tendency is reported when $0.05 \leq p < 0.1$. In addition, VEGF protein and capillarization was tested for correlation using a Pearson correlation coefficient.

3. Results

3.1 Maximal whole-body oxygen uptake and endurance

VO₂max, (l·min⁻¹·kg⁻¹bodyweight) was 46% higher (P<0.05) in ET than in UT (Figure 1a), and when given as l·min⁻¹ ~28% higher (p<0.05) in ET than in UT.

ET bicycled continuously for 86.6±7.9 min at 75% VO₂max before exhaustion, which was 35% longer (p<0.05) than UT (Figure 1b).

3.2 *M. vastus lateralis* capillarization

The number of capillaries per fibre was 27% higher (p<0.05) in ET than UT, 1.77 and 1.39, respectively. Capillarization and VEGF protein content in the vastus lateralis muscle was positively correlated, with a Pearson Correlation coefficient ~0.87.

3.3 Mitochondrial enzyme activities

Mitochondrial protein concentration relative to muscle weight was 19% higher (p<0.05) in ET than in UT (Figure 2a).

PDH activity and β-oxidation were ~43% and 42% higher (p<0.05), respectively, in ET than UT (Figure 2b-c).

Furthermore CS, SDH and α-KG-dehydrogenase activities were 28, 36 and 54% higher (p<0.05) in ET than in UT, respectively (Figure 3a-c).

ATP synthase activity and NADH-oxidase activity (reflecting the respiratory chain activity) were 46% and 35% higher (p<0.05) in ET than UT, respectively, while COX activity tended to be 23% higher (0.05≤p<0.1) in ET than UT (Figure 3d-f).

3.4 Protein expression

Cyt c, GLUT 4 and HKII protein content was not significantly different between the groups, but the HKII protein level appeared ~45% higher (p>0.1) in ET than in UT (Figure 4). VEGF protein content was ~230% higher (p<0.05) in ET than in UT (Figure 5).

3.5 Muscle glycogen content

Resting muscle glycogen was 447 ± 49 mmol/kg in ET and 429 ± 19 mmol/kg in UT and the absolute glycogen utilization during exercise was 229 mmol/kg and 172 mmol/kg, respectively, with no significant difference between the groups. However, glycogen utilization/KJ work was 42% lower ($p < 0.05$) in ET than in UT.

3.6 mRNA expression

Relative to Pre the PGC-1 α mRNA content increased ($p < 0.05$) ~11 fold in UT and 5 fold in ET two hours after the acute exercise bout (Figure 6a).

Exercise did not affect the HKII mRNA content significantly in any of the groups when tested with a two way or one way ANOVA. The HKII mRNA content in UT, however, tended to be ~ 4 fold higher ($0.05 \leq p < 0.1$) in UT than ET immediately after the exercise bout and 2h into recovery, when tested by a paired t-test (Figure 6b).

VEGF mRNA increased ($p < 0.05$) ~4 fold in UT immediately after the cycling bout and remained elevated 2h into recovery, while there were no changes in the VEGF mRNA content with exercise in the ET group (Figure 6c).

A tendency to an overall difference ($0.05 \leq p < 0.1$) in TFAM mRNA between the ET and UT group was present (Figure 6d). The UT group had an apparent increase ($p > 0.1$) in TFAM mRNA content after the cycling bout.

3.7 Intracellular signalling

AMPK phosphorylation increased ($p < 0.05$) 4.6 and 2.5 fold in UT and ET, respectively, immediately after the exercise bout (Figure 7a), while P38 phosphorylation increased 3-4 fold in both groups after the exercise bout (Figure 7b).

4. Discussion

The main findings of the present study are that endurance trained elderly subjects had higher oxidative enzyme activities and higher capillarization than untrained elderly subjects indicating an improved oxidative capacity and maintained mitochondrial function with lifelong endurance exercise training. Furthermore, untrained elderly subjects had marked exercise-induced AMPK and P38 phosphorylation as well as mRNA responses of PGC-1 α and VEGF, implying that skeletal muscle of elderly subjects maintains the ability to adapt and induce mitochondrial biogenesis and angiogenesis.

The present observation that skeletal muscle β -oxidation, PDH, CS, SDH, α -KG-DH, NADH-oxidase and ATPase activities as well as VEGF protein content and capillarization were higher in the elderly endurance trained than in the untrained subjects is in accordance with well known endurance training induced effects in young subjects [3, 10, 17, 18, 31, 50]. The higher enzyme activities can partly be explained by the higher mitochondrial protein content (~19%) in the endurance trained subjects, implying increased mitochondrial biogenesis in skeletal muscle of this group. The lack of a corresponding difference in Cyt c protein content is surprising, but may be related to the sensitivity of western blotting relative to enzyme activity assays combined with potential small changes in Cyt c protein content with endurance training as previously reported in mice [31]. In addition, the ~40% higher oxidative enzyme activities and the 27% higher capillarization in the elderly endurance trained than untrained subjects, in the present study, are the same degree of improvements previously reported in young individuals [12, 27, 29] suggesting that the trainability of oxidative enzymes and capillarization in skeletal muscle is similar in elderly and young subjects. This indicates that elderly subjects who have been physically active throughout life maintain skeletal muscle metabolic adaptations which are likely to delay or prevent the development of metabolically related diseases as well as maintaining the functional abilities in daily activities.

In contrast to previously reported mitochondrial dysfunction in elderly [28, 39, 53], the present findings are in line with previous studies showing that mitochondria from skeletal muscle of elderly subjects are well functioning [44, 47]. The finding that both the endurance trained and untrained elderly subjects in the present study had ~2 fold lower α KG-DH/CS activity ratio than previously observed in young subjects [2], is apparently due to a higher CS activity rather than a

lower α KG-DH in the elderly subjects than the young [2]. It may be speculated that this upregulation of CS activity is caused by an increased requirement of biosynthesis of triglycerides from citrate in elderly subjects for a yet unknown reason. A potential impairment of mitochondrial function in elderly and old subjects may be related to their training status, as the elderly [44] and old [47] subjects with well functioning mitochondria in previous studies were endurance trained at different levels, while the elderly with impaired mitochondrial function were sedentary [39]. In addition, it is likely that mitochondrial dysfunction may be more pronounced after the age of ~75 and only slightly detectable at a younger age, as it has been suggested for the hypertrophic response to resistance exercise training [52].

The finding that the enhanced oxidative capacity in the elderly endurance trained subjects was associated with markedly longer time to exhaustion during cycling than the untrained subjects provides evidence for functional effects of the adaptations in skeletal muscle. This is further supported by additional findings from the same subjects showing that muscle glycogen utilization/KJ and the RER value during the first 10 minutes of the exercise were lower in the endurance trained than the untrained elderly subjects (Krustrup et al 2011). This glycogen sparing and higher fat oxidation in the endurance trained subjects are likely due to the improved skeletal muscle oxidative capacity shown in the present study.

The exercise induced AMPK phosphorylation as well as PGC-1 α and VEGF mRNA responses, and tendency to increased HKII mRNA in the untrained subjects, are in agreement with previous findings in young subjects [8, 16, 40, 41], and support that skeletal muscle of elderly subjects maintains the ability to adapt metabolically and angiogenically to exercise training. Because the two groups in the current study exercised at 75% VO_2max , and because a previous study in young subjects has shown that the relative workload seems to determine the acute PGC-1 α mRNA response [36], similar acute mRNA responses were anticipated in the untrained and the endurance trained elderly subjects. The present observations of relatively lower or complete lack of exercise induced AMPK phosphorylation, PGC-1 α , HKII and VEGF mRNA responses in the elderly endurance trained subjects may therefore indicate an age-related loss of adaptability to acute exercise, which means a possibility of an age-associated decline in the ability to further adapt to endurance training, when already trained. There may thus exist a critical age for gaining full endurance exercise induced mRNA responses, in line with the reported decrease in hypertrophic response to resistance exercise training of same relative

intensity from the age of 60-80 [52]. The intensity dependency observed in young subjects may therefore be shifted upwards with age and therefore require a higher exercise intensity in elderly trained to obtain a given acute mRNA response in skeletal muscle. However based on the longer time to exhaustion, it seems that the relative workload determined from whole body $\text{VO}_{2\text{max}}$ does not correspond well with the relative workload based on metabolic fitness/endurance in these elderly subjects [49]. Therefore it is possible that the acute mRNA responses would be present/more marked in the endurance trained elderly subjects, if the relative workload was based on leg muscle endurance or leg muscle $\text{VO}_{2\text{max}}$ instead of whole body $\text{VO}_{2\text{max}}$. The difference in mRNA responses between the two groups may be due to the different exercise induced AMPK phosphorylation, as the AMPK activator AICAR has been shown to increase the mRNA content of PGC-1 α , VEGF and HKII in skeletal muscle [21, 30]. Furthermore the more marked exercise induced AMPK phosphorylation in UT than ET may imply a higher level of intracellular metabolic disturbance in the untrained subjects during cycling, although working at the same % $\text{VO}_{2\text{max}}$ as the endurance trained. On the contrary, the similar exercise induced P38 phosphorylation response in skeletal muscle of the untrained and endurance trained subjects may be explained by the same degree of mechanical stress during cycling in the two groups, as P38 phosphorylation has been reported to be enhanced by mechanical stress [6, 11].

In conclusion, the observed higher skeletal muscle oxidative enzyme activities and capillarization in lifelong endurance trained elderly subjects than in untrained elderly subjects provides evidence for beneficial effects of lifelong endurance exercise training on skeletal muscle oxidative capacity, which may improve daily functional abilities and delay sarcopenia and development of metabolically related diseases. The clear exercise induced AMPK phosphorylation as well as mRNA responses of metabolically and angiogenically related proteins in the untrained elderly subjects indicate that elderly subjects maintain the ability to adapt to an acute endurance exercise bout and thus potentially to repeated bouts of exercise. Together the present findings suggest that metabolic and angiogenic adaptability is sustained in elderly subjects at least up to the age of 75.

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Table and figure legends

Table 1.

Forward Primers (FP), Reverse Primers (RP) and TaqMan Probes sequences used for real time PCR to detect peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α), hexokinase II (HKII), vascular endothelial growth factor (VEGF) and Transcription factor A, mitochondrial (TFAM) mRNA.

Figure 1.

Maximal oxygen uptake, $\text{VO}_{2\text{max}}$ ($\text{ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$) and time to exhaustion (min) at 75% $\text{VO}_{2\text{max}}$ in endurance trained (ET; n=8) and untrained (UT; n=7) elderly subjects. Values are mean \pm SE. α : significantly different from UT, $p<0.05$

Figure 2.

a) Mitochondrial protein concentration ($\text{mg}\cdot\text{g}^{-1}$ muscle), b) β -oxidation and c) pyruvate dehydrogenase (PDH) activity in isolated mitochondria obtained from vastus lateralis muscle biopsies from endurance trained (ET; n=8) and untrained (UT; n=7) elderly subjects at rest. Values are mean \pm SE. α : significantly different from UT, $p<0.05$.

Figure 3.

a) Citrate synthase (CS), b) succinate dehydrogenase (SDH), c) α -ketoglutarat-dehydrogenase (α -KG-DH), d) NADH oxidase, e) cytochrome c oxidase (COX) and f) ATP synthase activity in isolated mitochondria obtained from vastus lateralis muscle biopsies from endurance trained (ET; n=8) and untrained (UT; n=7) elderly subjects at rest. Values are mean \pm SE. α : significantly different from UT, $p<0.05$, #: Tends to be significantly different from UT, $0.05\leq p<0.1$.

Figure 4.

a) Glucose transporter type 4 (GLUT4), b) hexokinase II (HKII) and c) cytochrome c (Cyt c) protein content in vastus lateralis muscle biopsies from endurance trained (ET; n=7) and untrained (UT; n=6) elderly subjects at rest. Values are mean \pm SE.

Figure 5.

Vascular endothelial growth factor (VEGF) protein content in vastus lateralis muscle biopsies from endurance trained (ET; n=5) and untrained (UT; n=5) elderly subjects at rest. Values are mean \pm SE. α : significantly different from UT, $p<0.05$.

Figure 6.

a) mRNA content of peroxisome proliferator activated receptor gamma coactivator 1-alpha (PGC-1 α), b) hexokinase II (HKII), c) vascular endothelial growth factor (VEGF) and d) transcription factor A, mitochondrial (TFAM) in vastus lateralis muscle biopsies obtained from endurance trained (ET; n=7) and untrained (UT; n=6) elderly subjects before (Pre) and immediately after (0') an acute exercise bout and at 2h of recovery (2h). The target mRNA was normalized to GAPDH mRNA content. Values are mean \pm SE. *: significantly different from Pre, $p<0.05$, ‡ tendency to overall difference between the two groups, $0.05\leq p<0.1$.

Figure 7.

a) mRNA content in skeletal muscle of phosphorylated AMP activated protein kinase (p-AMPK) and b) phosphorylated P38 MAP kinase (p-P38) in lifelong endurance trained (n=7) and untrained (UT) (n=6) elderly subjects before (Pre) and immediately after (0h) an acute exercise bout. The target protein was normalized to total protein content. Values are mean \pm SE. *: significantly different from Pre, $p < 0.05$, §: Tends to be significantly different from Pre, $0.05 \leq p < 0.1$.

Table 1. Primer and TaqMan probe sequences used for real time PCR

PGC-1 α	FP	5'CAAGCCAAACCAACAACCTTTATCTCT3'
	RP	5'CACACTTAAGGTGCGTTCAATAGTC3'
	Probe	5'AGTCACCAAATGACCCCAAGGGTTCC3'
HKII	FP	5' TTGTCCGTAACATTCTCATCGATT 3'
	RP	5' TGTCTTGAGCCGCTCTGAGAT 3'
	Probe	5' ACCAAGCGTGGACTGCTCTTCCGA 3'
VEGF	FP	5' CTTGCTGCTCTACCTCCACCAT 3'
	RP	5' ATGATTCTGCCCTCCTCCTTCT 3'
	Probe	5' AAGTGGTCCCAGGCTGCACCCA 3'
TFAM	FP	5' AGATTCCAAGAAGCTAAGGGTGATT 3'
	RP	5' TTTCAGAGTCAGACAGATTTTCCA 3'
	Probe	5' CCGCAGGAAAAGCTGAAGACTGTAAAGGA 3'

Figure 1

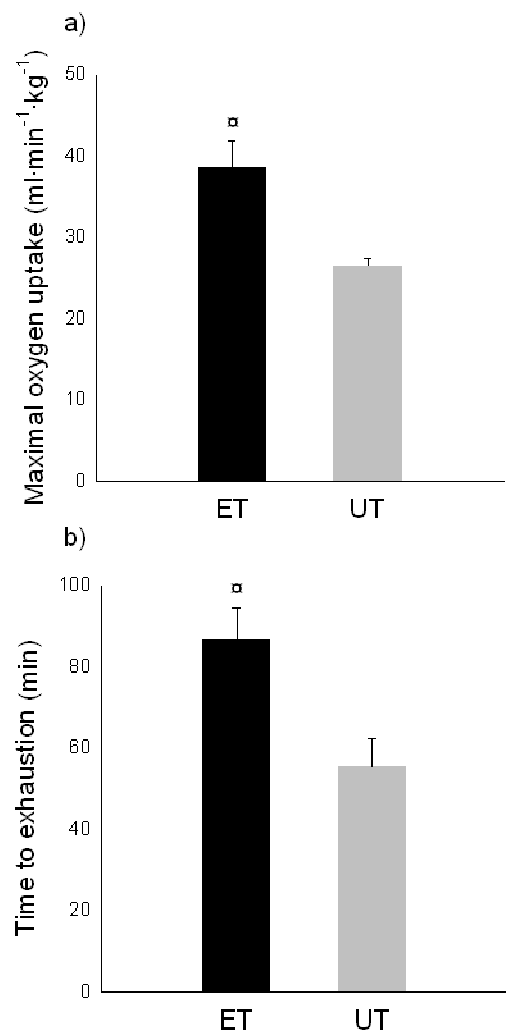


Figure 2

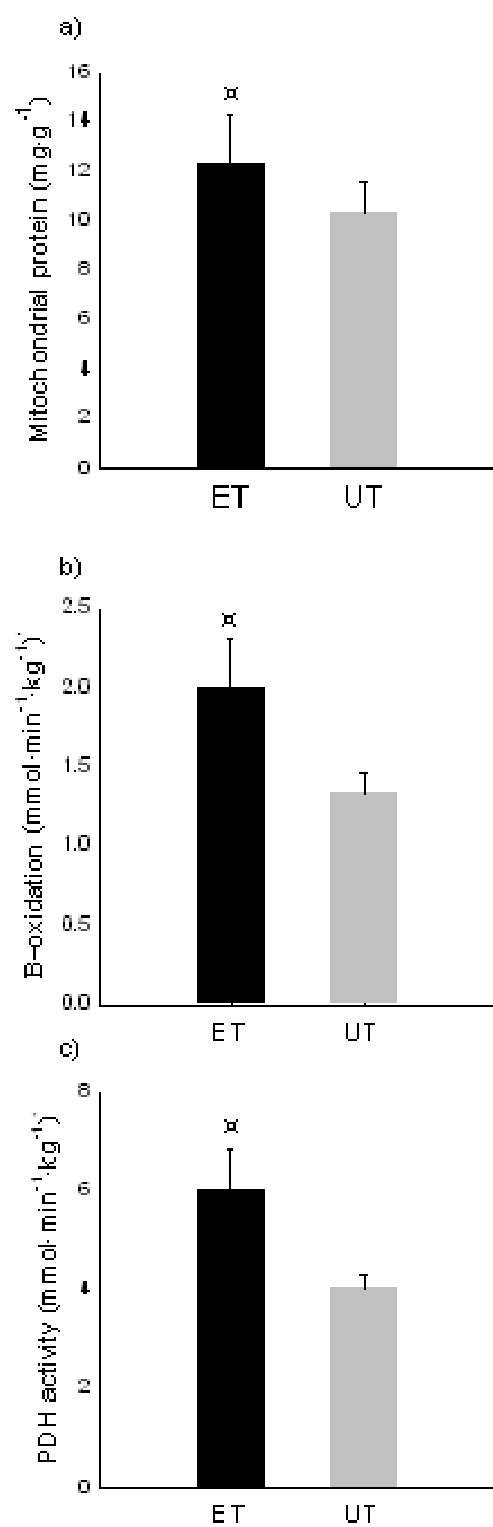


Figure 3

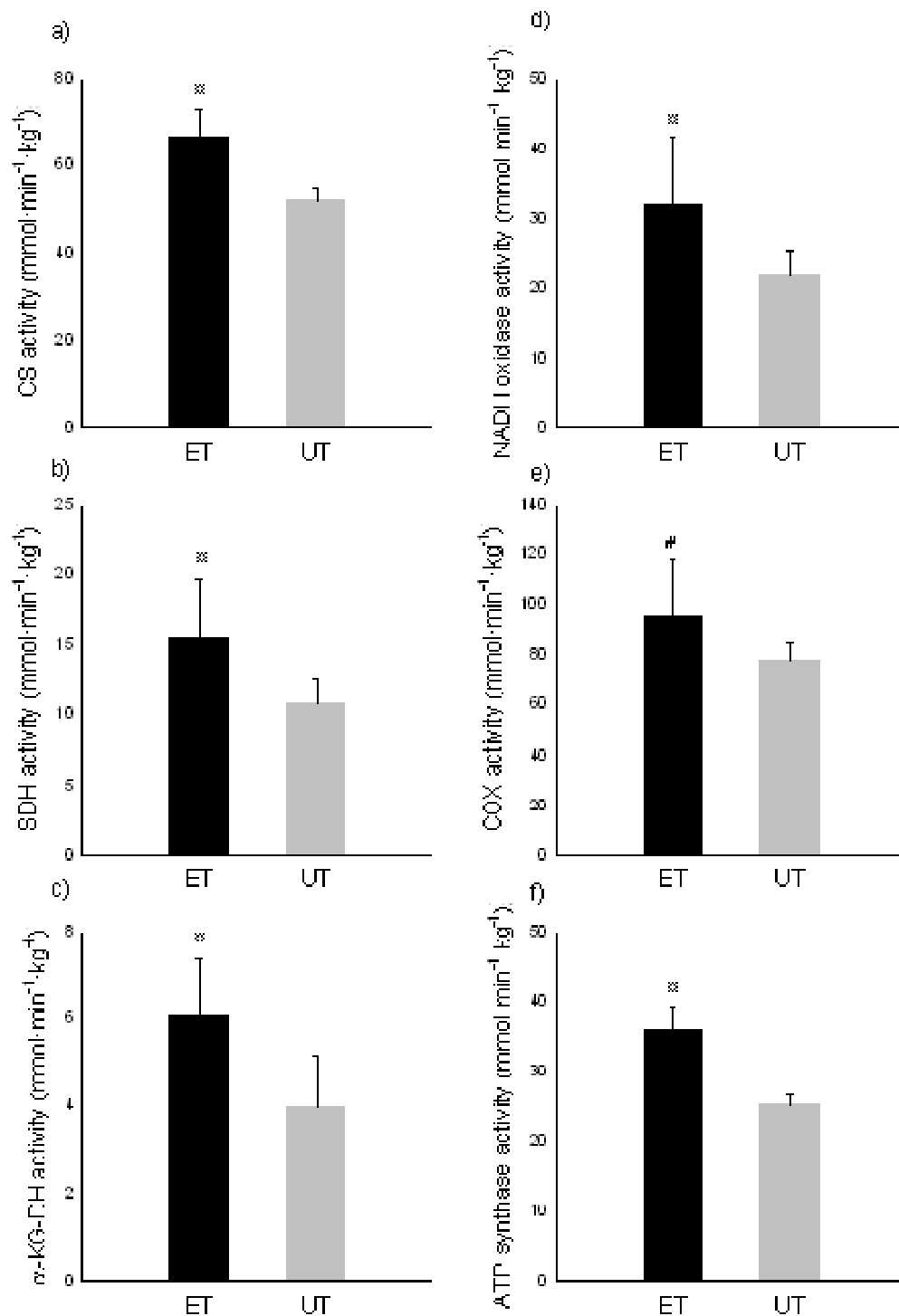


Figure 4

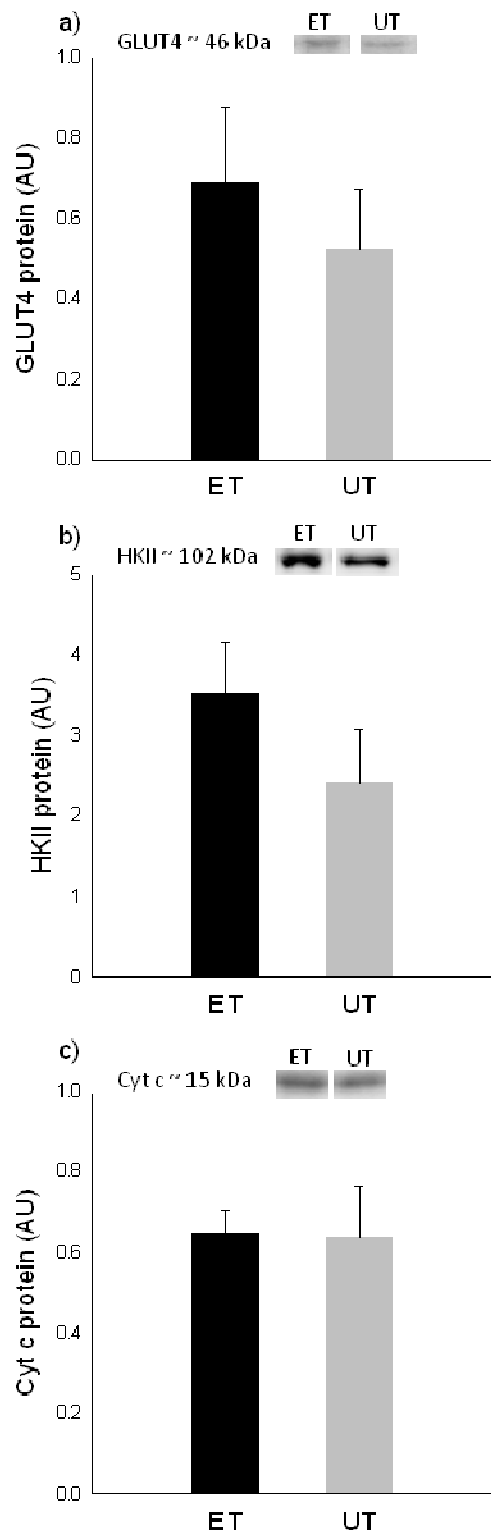


Figure 5

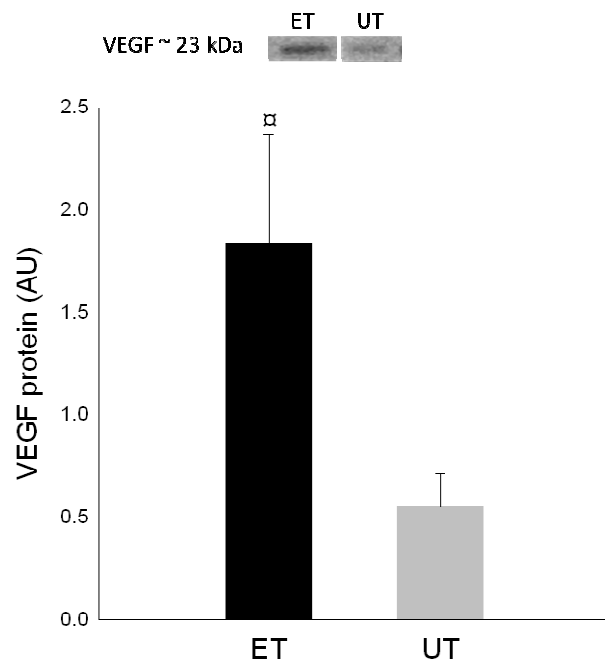


Figure 6

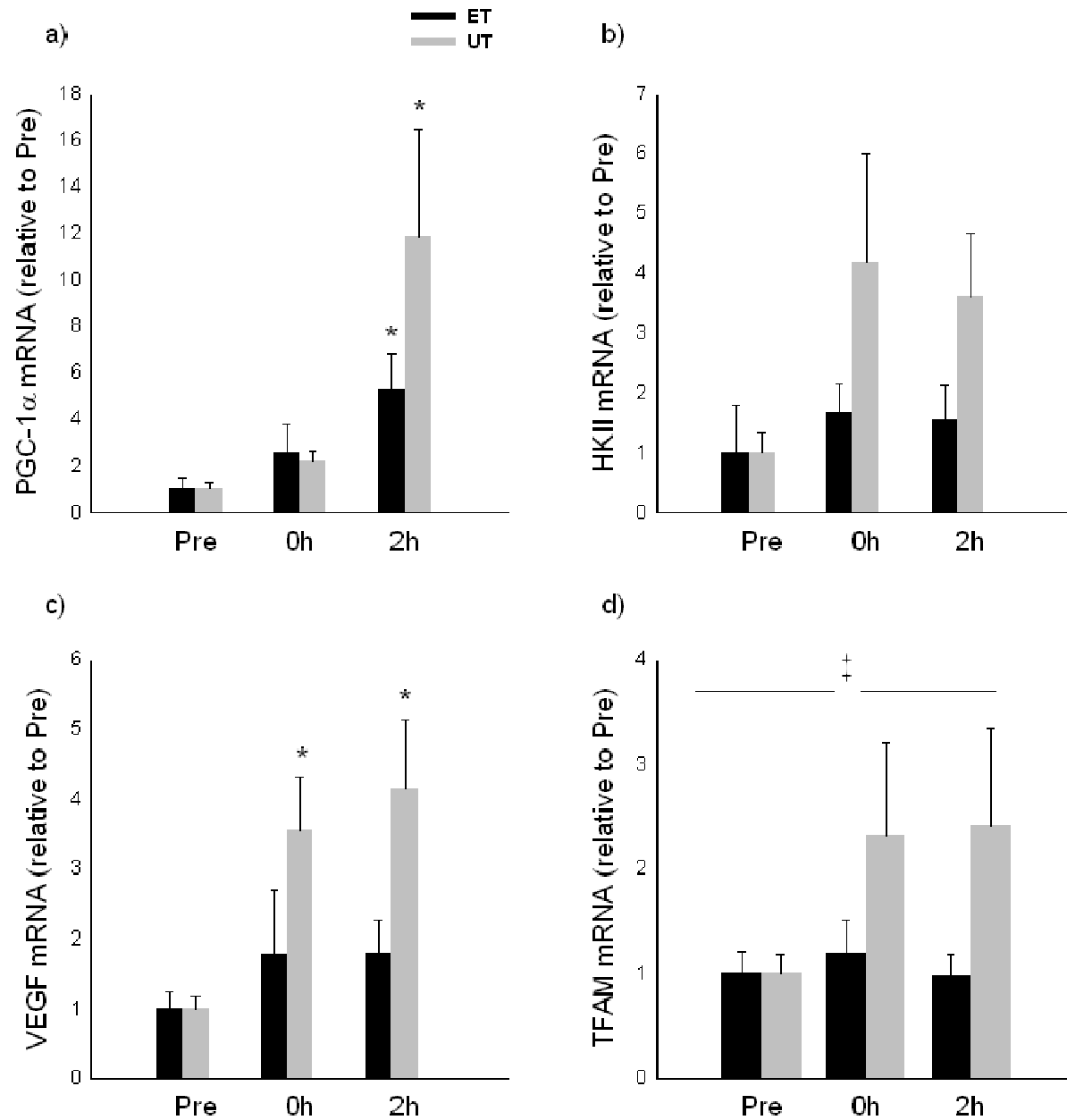
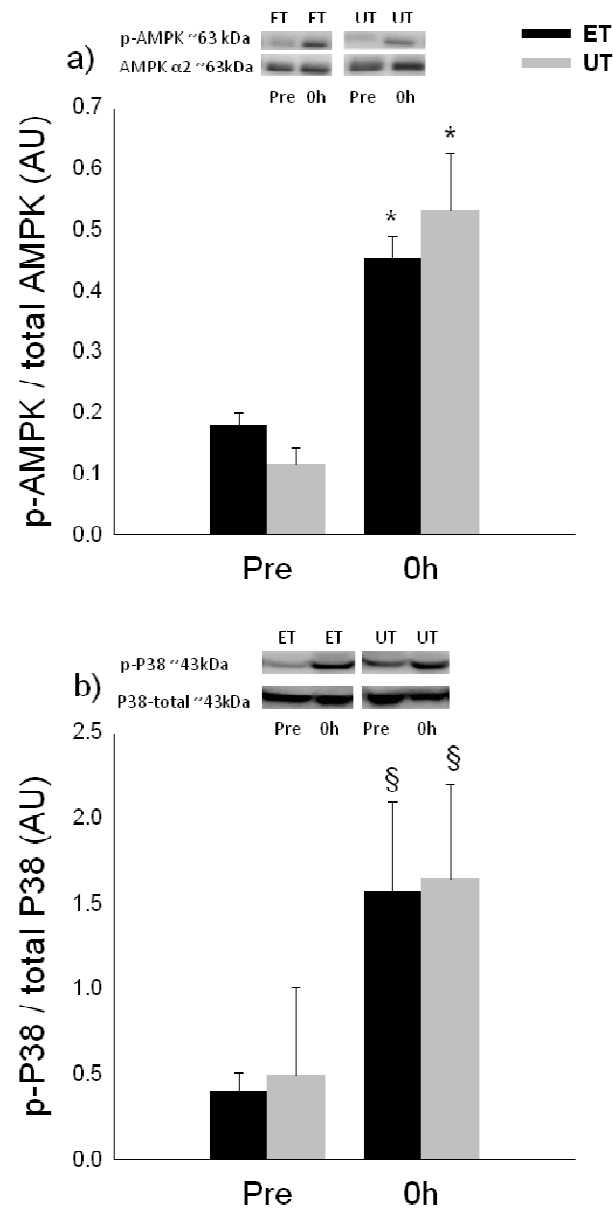


Figure 7



Research highlights

The main findings of the present study are that endurance trained elderly subjects had higher oxidative enzyme activities and higher capillarization than untrained elderly subjects indicating an improved oxidative capacity and maintained mitochondrial function. Furthermore, untrained elderly subjects had marked exercise-induced mRNA responses of PGC-1 α and VEGF, implying that skeletal muscle of elderly subjects maintains the ability to induce mitochondrial biogenesis and angiogenesis.