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ORIGINAL ARTICLE

Effects of acute aerobic and concurrent exercise on skeletal muscle metabolic enzymes in untrained men

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Abstract

Background Acute exercise can increase skeletal muscle citrate synthase (CS) enzyme activity and resting skeletal muscle mitochondrial enzyme activity has been linked to maximal oxygen consumption ($\dot{V}O_{2max}$). We investigated: (1) if acute aerobic exercise (AEx) increases muscle metabolic enzyme activities other than CS; (2) if the addition of acute resistance exercise (REx) enhances the response to AEx (A + REx); and (3) if post-exercise muscle metabolic enzyme activity was related to $\dot{V}O_{2max}$.

Methods Twelve young, sedentary men completed 45 min of two-legged cycle ergometry at 55% of $\dot{V}O_{2max}$ and 3 sets of 8–12 repetitions of one-leg knee extensor at 55% of 1 repetition maximum (1-RM). Vastus lateralis biopsies were taken prior to and 1 h post AEx and A + REx for the measurement of phosphofructokinase (PFK), 3-L-hydroxyacyl CoA dehydrogenase (β-HAD), succinate dehydrogenase (SDH) and CS.

Results As a group, there was no effect of acute AEx or A + REx on muscle PFK, β-HAD, CS, and SDH activities. Post exercise muscle PFK, β-HAD, CS, and SDH activities were related to higher $\dot{V}O_{2max}$ ($r = 0.62$–0.74). With participants grouped by $\dot{V}O_{2max}$ (LOW, < 30th %; NORM, > 50th %), acute exercise-induced changes in muscle PFK, β-HAD, CS, and SDH were greater in NORM compared to LOW.

Conclusion These findings suggest acute exercise muscle metabolic enzyme activities are predictive of $\dot{V}O_{2max}$ and possibly supportive of higher $\dot{V}O_{2max}$. Also, low $\dot{V}O_{2max}$ (below 30th percentile) appears to impair skeletal muscle metabolic enzyme responses to acute exercise.

Keywords Aerobic exercise capacity · Metabolic enzyme activity · Acute exercise · Skeletal muscle

Abbreviations

AEx	Aerobic exercise
A + REx	Aerobic + resistance exercise
β-HAD	3-L-Hydroxyacyl CoA dehydrogenase
BMI	Body mass index
CS	Citrate synthase
FPG	Fasting plasma glucose
HDL	High density lipoprotein
HOMA-β	Homeostasis model assessment-β cell function
HOMA-IR	Homeostasis model assessment-insulin resistance
KE	Maximal knee extensor strength
LDL	Low-density lipoprotein
PFK	Phosphofructokinase
PRE	Prior to exercise
SDH	Succinate dehydrogenase
TC	Total cholesterol
$\dot{V}O_{2max}$	Maximal oxygen consumption

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Introduction

Moderate intensity, chronic aerobic exercise (AEx) training is well known to increase the maximal activity of several skeletal muscle mitochondrial enzymes including 3-L-hydroxyacyl-CoA dehydrogenase (β-HAD), citrate synthase (CS), and succinate dehydrogenase (SDH) [1]. The activity of phosphofructokinase (PFK), the rate limiting enzyme of glycolysis, is increased with intense AEx training [2]. In addition, chronic REx training can increase skeletal muscle β-HAD [3], CS [3], and SDH [4] activities. Coordinated upregulation of muscle metabolic enzymes would be important to minimize individual enzyme rate limits on metabolic flux.

Interestingly, a single 30-min bout of acute, aerobic knee extension (KE) exercise at 50% of maximum work rate increased CS activity 50% at 1 h post-exercise in men [5]. A single bout of cycling (75% of peak oxygen consumption ($\dot{V}O_{2peak}$) until exhaustion (mean 71 min)) increased the activity of skeletal muscle citrate synthase (CS) by 20% immediately post-exercise, but not β-HAD or PFK activity [6]. In rodents, acute exercise appears to either decrease (CS, SDH, PFK, β-HAD) or not change (β-HAD) muscle enzyme activities accessed immediately post-exercise [7–10]. Whether skeletal muscle enzyme activity is increased in response to more traditional (mode, duration, intensity) acute exercise in humans is unknown.

Current physical activity guidelines recommend regular participation in both aerobic (AEx) and resistance (REx) exercise [11] and there is the possibility of altered effects of concurrent (A + REx) exercise training on skeletal muscle. In healthy individuals, A + REx training increases $\dot{V}O_{2max}$ and muscle CS activity to a similar extent as AEx alone [12], while in T2D patients A + REx training increases more than either AEx or REx alone [13, 14]. Also in T2D patients, both REx alone and A + REx, but not AEx training alone, increase skeletal muscle β-HAD and CS activities supporting potential additive benefits of REx to AEx [15]. Thus, the addition of resistance exercise to aerobic exercise appears be additive on the skeletal muscle enzyme responses to training. Whether this is true with acute exercise is unknown.

Low aerobic exercise capacity is an important predictor of mortality [16] and is closely associated with $\dot{V}O_{2max}$ [17]. Theoretically, $\dot{V}O_{2max}$ can be limited at different points along the O_2 cascade from ambient air down to the mitochondrial electron transport chain. In adults, skeletal muscle accounts for approximately 40% of total body weight and is the consumer of 75–85% of the O_2 used during cycling exercise [18]. There is a positive linear relationship between $\dot{V}O_{2max}$ and skeletal muscle mitochondrial volume suggesting an important role for muscle metabolism in setting aerobic exercise capacity [19]. However, increases in CS activity with acute exercise have not been linked to $\dot{V}O_{2max}$ [6].

Metabolic flexibility is the ability of an individual to shift metabolism in response to energy demand, such as occurs with exercise [20]. During moderate-intensity exercise, obesity impairs the ability to shift toward greater reliance on carbohydrate utilization and this metabolic inflexibility is linked to insulin resistance [21, 22]. Skeletal muscle accounts for 70–80% of insulin stimulated glucose disposal [23].

Physiologically, the benefit to acute exercise-induced increases in enzyme activity could be to support greater aerobic exercise capacity and could lead to a better understanding of inherent limits in exercise tolerance and $\dot{V}O_{2max}$. Metabolically, the flexibility to acutely increase muscle enzyme activity in response to increases in metabolic demand could provide insight into individual metabolic flexibility as it relates to insulin sensitivity. In the current report, we hypothesized that traditional, systemic aerobic exercise increases the activity of skeletal muscle PFK, β-HAD, and SDH as well as CS and the addition of acute resistance exercise enhances the response to aerobic exercise. Given the importance of skeletal muscle in aerobic exercise capacity and glucose disposal, we hypothesized that muscle enzyme activity after exercise would be related to $\dot{V}O_{2max}$ and insulin sensitivity.

Materials and methods

Participants

Twelve sedentary, young (range 19–30 years of age) men volunteered and consented to participate in the study after receiving written and verbal explanations of the content and intent of the study as approved by the University Institutional Review Board and in accordance with the Helsinki Committee for Human Rights. All participants were healthy non-smokers, with no history of cardiopulmonary disease. Sedentary participants were defined as participating in less than 1 h of strenuous physical activity per week as assessed by questionnaire.

Determination of $\dot{V}O_{2max}$ and 1-RM

Maximal oxygen consumption ($\dot{V}O_{2max}$) was measured on an electronically braked cycle ergometer (Lode, Excaliber Sport, Groningen, The Netherlands) as previously described [24]. Minute ventilation ($\dot{V}_E$), oxygen uptake ($\dot{V}O_2$), and carbon dioxide production ($\dot{V}CO_2$) were continuously monitored via open circuit spirometry (True Max 2400, Parvo Medics, Salt Lake City, UT). Heart rate (model T31, Polar

Electro Inc., Woodbury, NY) and rating of perceived exertion (RPE) were measured continuously. The test began with a 5-min warm-up at 50W. After the warm-up, the workload was increased 25W every min until volitional fatigue. Participants were verbally encouraged to continue for as long as possible. The criterion used to assess $\dot{V}O_{2max}$ included the following: (1) heart rate in excess of 90% of age-predicted max (220—age); (2) a respiratory exchange ratio greater than or equal to 1.10; and (3) identification of a plateau ($\leq$150 ml increase) in $\dot{V}O_2$ despite a further increase in workload. In all tests, at least two of three criteria were met.

After a 15-min rest period following the $\dot{V}O_{2max}$ test, one leg resistance KE 1-Repetition Maximum (1-RM) was determined by having participants lift a progressively greater weight until they were unable to continue. Participants were given 1 min rest between each attempt. After an initial weight of 13.6 kg, the weight was increased by 4.5 kg before the next attempt. The highest successfully lifted weight was designated as 1-RM.

Experimental design

At least 2 weeks after the determination of $\dot{V}O_{2max}$ and 1-RM, participants reported to the laboratory between 0600 and 0700 after a 12-h fast. A blood sample was drawn from a catheter inserted in an antecubital vein for the measurement of insulin, glucose, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TG). The homeostasis model assessment for insulin resistance (HOMA-IR) and β cell function (HOMA-β) were subsequently calculated [25].

Participants performed 45 min of two-legged cycle ergometry at a workload corresponding to 55% of $\dot{V}O_{2max}$. This duration and intensity of AEx was selected in an effort to replicate the 30 min of 50% of single leg, aerobic KE maximum employed when a 50% increase in CS activity was reported [5]. Directly following AEx, participants completed three sets of 8–12 repetitions of resistance KE at 55% of the 1-RM workload with a 2-min rest interval between sets (Fig. 1). The aerobic and resistance bouts chosen align with current exercise recommendations [11] and preliminary analysis found this procedure to be fatiguing without requiring outside assistance to complete the KE sets. All participants reached fatigue by the end of the prescribed resistance exercise work bout.

Prior to and 1 h post exercise, vastus lateralis biopsies were obtained. The initial, resting biopsy was alternated between AEx and A + REx legs across participants. Biopsies for AEx and A + REx were alternated between being the 2nd and 3rd biopsy across participants to minimize potential time bias post-exercise. Biopsies (rest and acute exercise) when taken from the same leg were separated by at least 3 cm as previously described [24]. The 1-h post-exercise time point was chosen in an effort to replicate the post-exercise biopsy time previously employed when a 50% increase in CS activity was reported [5]. Samples were stored at − 80 °C until analysis.

Muscle metabolic enzyme analysis

Frozen muscle was homogenized on ice in isolation buffer (20 mM Tris, 40 mM KCl, 2 mM EGTA, 50 mM sucrose; 0.2 mM Na_3VO_4; 50 mM NaF, pH 7.4) in the presence of 20 µl proteinase inhibitor cocktail per ml buffer (Sigma, St. Louis, MO) [26]. Protein concentration was determined by BCA (Thermo Fisher Scientific, Waltham, MA). Following total protein determination, homogenized samples were frozen and kept at − 80 °C until enzyme analysis. Analysis of enzyme activity was performed according to previously reported procedures for PFK [27], β-HAD [28], CS [26], and SDH [26]. There was a strong correlation between PRE CS and SDH activities suggesting an internal consistency in mitochondrial enzyme activity measurement ($r = 0.95$).

Quantitative real-time PCR

Total muscle RNA was extracted by using a Trizol reagent (Thermo Fisher Scientific) as previously described [29]. For mRNA reverse transcription, first-strand cDNA was generated by random hexamer primers with MMLV Reverse Transcriptase (Thermo Fisher Scientific). Real-time PCR detection was performed using SYBR green-based chemistry on a CFX Connect (BioRad, Hercules, CA, USA). Primers for mRNA are listed in Table 1. Gene expression was determined with the $2^{-\Delta\Delta Ct}$ relative quantification method and normalized to 18 s for mRNA. Housekeeping genes were validated to ensure their expression was not influenced by the experimental procedure.

Fig. 1 Time line of the activities on Day 2. Pre-exercise biopsies were alternated between legs and timing for post-exercise biopsies were alternated between conditions

Table 1 Primer sequences for mRNA expression for 18S ribosomal 1 (18S), phosphofructokinase (PFK), 3-L-hydroxyacyl CoA dehydrogenase (β-HAD), citrate synthase (CS), and succinate dehydrogenase (SDH)

Gene	Gene ID	Forward (5′–3′)	Reverse (5′–3′)
18S	106632259	GGCCCTGTAATTGGAATGAGTC	CCAAGATCCAACTACGAGCTT
PFK	5213	GGTGCCCGTGTCTTCTTTGT	AAGCATCATCGAAACGCTCTC
B-HAD	3033	ACCAGGCAGTTCATGCGTT	ACGTGCTTGACGATTATCTTCTT
CS	1431	ACGTGCTTGACGATTATCTTCTT	CCACCATACATCATGTCCACAG
SDH	6389	ACAGCTCCCCGTATCAAGAAA	GCATGATCTTCGGAAGGTCAA

Muscle protein analysis

Because enzyme activity is measured in the presence of excess reagents, increases in activity could be the result of increased protein content or allosteric regulation. Exercise training-induced increases in muscle enzyme activity reflect increases in enzyme protein content [1, 2]. Western blotting was performed to evaluate if changes in muscle enzyme activity following the acute exercise bout could be attributed to alterations in protein content. Frozen muscle was homogenized on ice in RIPA buffer [1X PBS, 1%Igepal, 0.5% sodium deoxycholate and 0.1% SDS with protease and phosphatase (I and II) inhibitors (Sigma)] as previously described [30]. From muscle homogenates, 30–60 µg of total protein was fractionated on SDS-polyacrylamide gels; transferred to PVDF membrane; and incubated with one of the following primary antibodies: CS from Cell Signaling Technology; PFK, β-HAD (HADHSC), and tubulin from Santa Cruz Biotechnology; total OXPHOS cocktail from Mitosciences (Eugene, OR); and acetyl-coA carboxylate (pan-ACC) and phospho-ACC (Ser79) from Millipore. Phospho-ACC was measured as an indicator of 5′ AMP-activated protein kinase (AMPK) phosphorylation, the main kinase regulator of ACC [31]. AMPK acts as a metabolic fuel gauge to monitor cellular energy charge, is increased with exercise in muscle, and could possibly be responsible for post-translational modification of enzymes. Either florescence-conjugated (LI-COR) or horseradish peroxide-conjugated (Cell Signaling Technology, Danvers, MA) secondary antibodies were incubated with the membrane and detected using florescence or chemiluminescence (Santa Cruz Biotechnology, Dallas, TX) with a FluroChem R system (Proteinsimple, San Jose, CA). Densitometric analysis was performed using Image J software. Results were normalized to GAPDH or tubulin.

Post hoc analysis

After initial data analysis, considerable inter-individual variation was observed in the enzyme activity responses to exercise and linear regression revealed relationships between $\dot{V}O_{2max}$ and several enzyme activities. In response, participants were grouped by $\dot{V}O_{2max}$ to compare enzyme activity responses to exercise between participants with normal (NORM; $N=5$) and low (LOW; $N=7$) $\dot{V}O_{2max}$, where NORM (range 43.4–54.8 $ml \times kg^{-1} \times min^{-1}$) was a $\dot{V}O_{2max} > 50$th percentile and LOW (range 24.6–37.2 $ml \times kg^{-1} \times min^{-1}$) was a $\dot{V}O_{2max} < 30$th percentile [32].

Statistical treatment

One-way repeated measures variance (ANOVA) was used to analyze exercise-induced responses in the larger cohort. After the ANOVA analysis controlled the overall error rate and following a significant F ratio, a Fisher's LSD was used to identify where ANOVA identified changes occurred. Linear regression and Stepwise Forward linear regression ($p < 0.05$ to enter and to stay in the model) were performed to investigate relationships between variables. Student's t test was used to analyze aerobic exercise-induced differences in muscle enzyme activity between LOW and NORM. Significance was established at $p \leq 0.05$ for all statistical sets and data reported are mean ± SE.

Results

Subject characteristics are in Table 2. In several measures ($\dot{V}O_{2max}$, FPG, HOMA-IR, and TC) there was a wide range of values such that some sedentary individuals demonstrated values considered outside healthy ranges. Linear regression revealed significant relationships between subject characteristics ($\dot{V}O_{2max}$, BMI, HOMA-IR) and fasting blood metabolic parameters (FPG, TC) (Table 3). Among these, $\dot{V}O_{2max}$ was significantly related to BMI ($r=-0.76$), FPG ($r=-0.80$), HOMA-IR ($r=-0.57$), and TC ($r=-0.62$). Forward stepwise linear regression between FPG and $\dot{V}O_{2max}$, BMI, and HOMA-IR revealed that $\dot{V}O_{2max}$ explained 64% of the variance in FPG and after controlling for BMI, $\dot{V}O_{2max}$ still explained 56% of the variance in FPG.

To identify if the mode, duration, and intensity of acute exercise employed were able to activate metabolically relevant pathways, phosphorylation of ACC was measured (Fig. 2). When expressed relative to total tubulin, phosphorylation of ACC tended ($p=0.06$) to be increased following AEx and was significantly increased by A + REx compared to PRE. When expressed relative to total ACC, there was a trend toward an increase with exercise ($p=0.07$).

Table 2 Participant characteristics

	Mean ± SE	Range
Age, years	22.3 ± 1.7	18–30
Height, m	1.80 ± 0.02	1.73–1.85
Mass, kg	75.5 ± 4.4	57.7–90.9
BMI, kg/m^2	23.3 ± 1.4	17.3–29.7
$\dot{V}O_{2max}$, l × min^{-1}	2.93 ± 0.20	1.88–3.68
$\dot{V}O_{2max}$, ml × kg^{-1} × min^{-1}	39.0 ± 4.0	24.36–54.8
KE, kg	35.6 ± 3.1	27.3–50.0
KE, kg × kg^{-1}	0.47 ± 0.04	0.36–0.68
Fasting glucose, mg/dl	88.9 ± 3.6	75–99
Fasting insulin, μIU/ml	10.6 ± 2.0	6–18
HOMA-IR	2.4 ± 0.5	1.2–4.4
HOMA-β	154.2 ± 26.4	72.2–271.7
Total cholesterol, mg/dl	169.3 ± 9.3	140–211
HDL, mg/dl	46.1 ± 4.4	33–63
LDL, mg/dl	105.5 ± 9.5	67–149
Triglycerides, mg/dl	88.1 ± 16.9	50–168

$N = 12$

BMI body mass index, $\dot{V}O_{2max}$ maximal oxygen consumption, *KE* maximal knee extensor strength, *HOMA-IR* homeostasis model assessment-insulin resistance, *HOMA-β* homeostasis model assessment-β cell function, *HDL* high density lipoprotein, *LDL* low density lipoprotein

Table 3 Correlation matrix for subject characteristics

	$\dot{V}O_{2max}$	BMI	FPG	HOMA-IR
BMI	−0.76*			
FPG	−0.80*	0.50		
HOMA-IR	−0.57*	−0.53	0.59*	
TC	−0.64*	0.42	0.70*	0.26

$\dot{V}O_{2max}$ maximal oxygen consumption (ml × kg^{-1} × min^{-1}), *BMI* body mass index, *FPG* fasting plasma glucose, *HOMA-IR* homeostatic model assessment-insulin resistance, *TC* total cholesterol

*$p \leq 0.05$

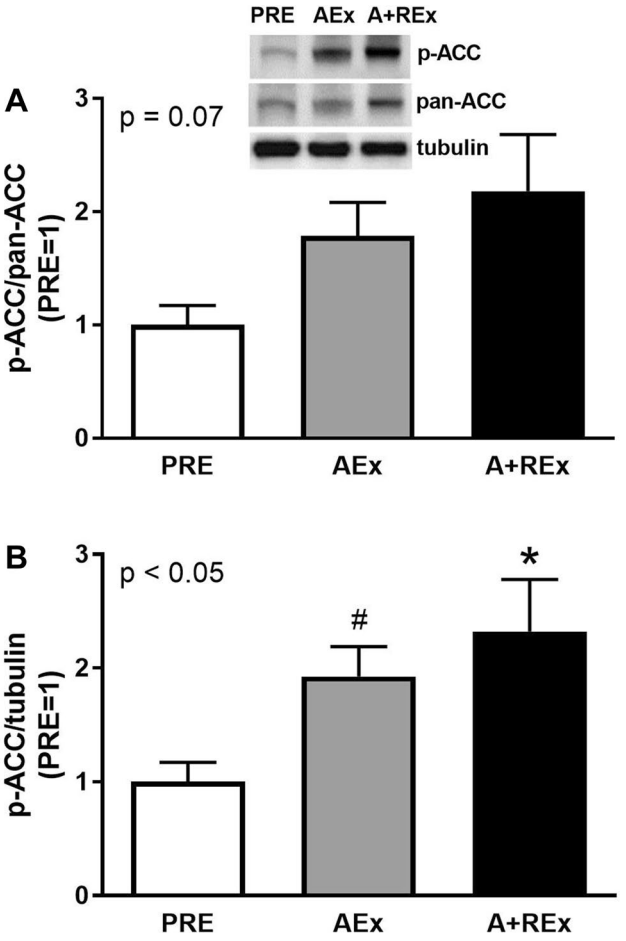

Fig. 2 Skeletal muscle responses to acute aerobic (AEx) and aerobic + resistance (A + REx) exercise for acetyl coA carboxylase (ACC) phosphorylation to pan-ACC (**a**) and tubulin (**b**). Mean ± SE. $N = 12$. *Significantly different than PRE ($p \leq 0.05$). #Trend versus PRE ($p = 0.06$)

In contrast to our hypothesis, no muscle enzyme activity was uniformly altered by acute exercise (AEx or A + REx) (Fig. 3). To gain a broader understanding of the response to acute exercise, mRNA and protein were also measured for PFK, CS, β-HAD, or SDH/mitochondrial complex I–V. There were no differences in mRNA or protein for any of the measured enzymes with acute exercise.

We next investigated if muscle enzyme activity was related to $\dot{V}O_{2max}$, BMI, and HOMA-IR (Table 4). Resting skeletal muscle enzyme activity was not related to $\dot{V}O_{2max}$, BMI, or HOMA-IR. However, $\dot{V}O_{2max}$ was related to skeletal muscle PFK, β-HAD, CS, and SDH after AEx and CS after A + REx; BMI was related to skeletal muscle PFK, CS, and SDH after AEx and CS after A + REx; and HOMA-IR was related to SDH after AEx and CS after A + REx. There was a relationship following AEx between p-ACC and SDH activity ($r = 0.65$; $p = 0.02$).

After separation into groups by $\dot{V}O_{2max}$ (16 ml × kg^{-1} × min^{-1} difference between NORM and LOW), LOW demonstrated higher fasting glucose and trends toward greater total cholesterol and triglycerides than NORM (Table 5). NORM had greater AEx-induced changes in skeletal muscle PFK, β-HAD, CS, and SDH compared to LOW (Fig. 4). There was no difference in AEx-induced changes in p-ACC between groups. Characteristics for participants grouped by $\dot{V}O_{2max}$ are in Table 5.

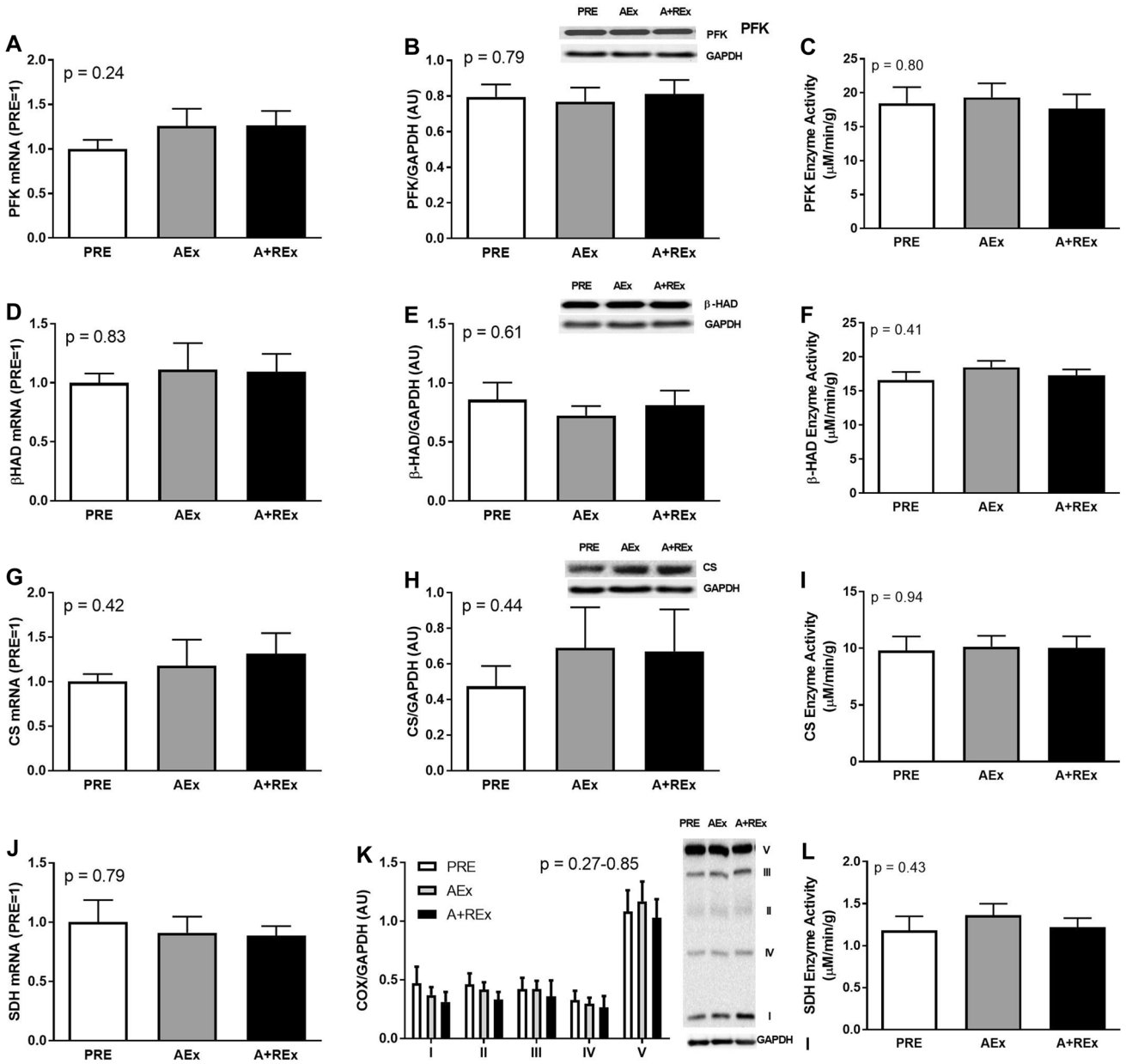

Fig. 3 Skeletal muscle mRNA, protein, and enzyme activity responses to acute aerobic (AEx) and aerobic + resistance (A + REx) exercise for phosphofructokinase (PFK) (**a**–**c**), 3-L-hydroxyacyl CoA dehydrogenase (β-HAD) (**d**–**f**), citrate synthase (CS) (**g**–**i**), and succinate dehydrogenase (SDH) (**j**–**l**). There was no effect of AEx or A + REx on any mRNA, protein, or enzyme activity. Mean ± SE. $N = 12$

Discussion

In the current report, we investigated if acute, moderate intensity, cycle exercise increases skeletal muscle PFK, β-HAD, CS, and SDH activity and if the addition of fatiguing acute knee extension resistance exercise alters the response in sedentary men. While as a group we did not observe any changes in muscle enzyme activity from rest to acute exercise in sedentary men, individuals with normal $\dot{V}O_{2max}$ had greater aerobic exercise-induced changes (50–100% increase) in PFK, β-HAD, CS, and SDH activity versus those with low $\dot{V}O_{2max}$. These findings provide potential links between skeletal muscle and the metabolic health benefits associated with a higher $\dot{V}O_{2max}$ [33].

Skeletal muscle enzyme activity and exercise

Increases in enzyme activity are routinely used to quantify skeletal muscle responses to exercise training [1]. Previous work in men demonstrated increases in CS activity

Table 4 Correlation matrix between subject characteristics and skeletal muscle metabolic enzyme activity

	$\dot{V}O_{2max}$	BMI	HOMA-IR
PFK PRE	−0.10	0.19	0.07
PFK AEx	0.74*	−0.68*	−0.47
PFK A + REx	0.45	−0.36	−0.06
β-HAD PRE	−0.25	0.05	−0.29
β-HAD AEx	0.62*	−0.52	−0.56
β-HAD A + REx	−0.08	−0.10	−0.06
CS PRE	0.22	−0.12	−0.46
CS AEx	0.71*	−0.62*	−0.48
CS A + REx	0.66*	−0.61*	−0.72*
SDH PRE	0.31	−0.12	−0.43
SDH AEx	0.62*	−0.66*	−0.60*
SDH A + REx	0.52	−0.40	−0.48

$\dot{V}O_{2max}$ maximal oxygen consumption ($ml \times kg^{-1} \times min^{-1}$), *BMI* body mass index, *HOMA-IR* homeostatic model assessment-insulin resistance, *PFK* phosphofructokinase, *β-HAD* 3-L-hydroxyacyl CoA dehydrogenase, *CS* citrate synthase, *SDH* succinate dehydrogenase, *PRE* prior to exercise, *AEx* aerobic exercise, *A + REx* aerobic + resistance exercise

*$p \leq 0.05$

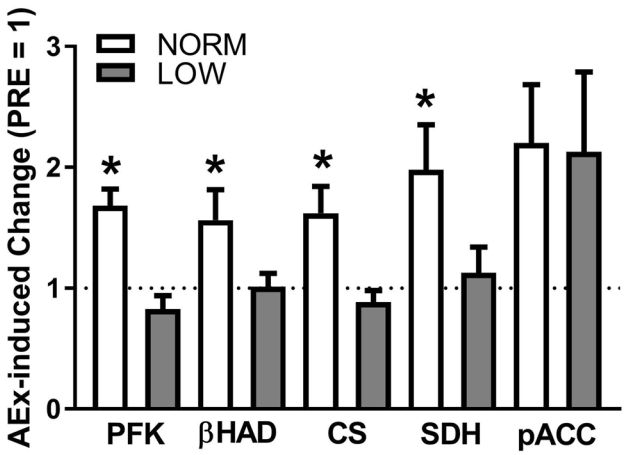

Fig. 4 Skeletal muscle responses to acute aerobic (AEx) exercise-induced for phosphofructokinase (PFK), 3-L-hydroxyacyl CoA dehydrogenase (βHAD), citrate synthase (CS), succinate dehydrogenase (SDH), and phosphorylation of acetyl coA carboxylase to tubulin ratio (pACC) in normal (NORM) and low (LOW) $\dot{V}O_{2max}$. The change with acute aerobic exercise was greater in NORM than LOW for PFK, BHAD, CS, and SDH enzyme activities. There was no difference in pACC. Mean ± SE. $N = 5–7$/group. *Significantly different than LOW

Table 5 Participant characteristics for normal (NORM) and low (LOW) $\dot{V}O_{2max}$

	NORM ($N = 5$)	LOW ($N = 7$)	p Value
Age, years	21.6 ± 1.8	22.7 ± 1.5	0.64
Height, m	1.79 ± 0.01	1.81 ± 0.02	0.51
Mass, kg	66.7 ± 3.2	81.8 ± 2.1	< 0.01
BMI, kg/m^2	20.8 ± 1.0	25.1 ± 0.9	< 0.01
$\dot{V}O_{2max}$, $l \times min^{-1}$	3.18 ± 0.06	2.75 ± 0.20	0.11
$\dot{V}O_{2max}$, $ml \times kg^{-1} \times min^{-1}$	48.0 ± 1.9	32.5 ± 1.5	< 0.01
1-RM, kg	35.5 ± 3.9	35.7 ± 2.3	0.95
1-RM, $kg \times kg^{-1}$	0.53 ± 0.04	0.44 ± 0.02	0.07
Fasting glucose, mg/dl	82.2 ± 2.9	93.7 ± 1.9	< 0.01
Fasting insulin, μIU/ml	9.2 ± 2.0	11.6 ± 1.8	0.40
HOMA-IR	1.9 ± 0.5	2.7 ± 0.4	0.28
Total cholesterol, mg/dl	156.0 ± 4.8	178.9 ± 8.2	0.06
HDL, mg/dl	46.6 ± 5.1	45.7 ± 3.7	0.88
LDL, mg/dl	96.0 ± 9.7	112.3 ± 7.4	0.20
Triglycerides, mg/dl	66.8 ± 7.1	103.3 ± 16.0	0.10

Mean ± SE

BMI body mass index, $\dot{V}O_{2max}$ maximal oxygen consumption, *KE* maximal knee extensor strength, *HOMA-IR* homeostasis model assessment-insulin resistance

*Significantly different than LOW ($p \leq 0.05$)

following acute aerobic knee extensor [5] and cycle exercise to fatigue [6], but not following lower intensity (60% of $\dot{V}O_{2peak}$) cycle exercise for 90 min [34]. We chose acute aerobic exercise for 45 min at 55% of $\dot{V}O_{2max}$ as the uniqueness of previous designs (aerobic knee extensor, exercise to fatigue, or exercise of 90 min) make them difficult to translate to current exercise recommendations [6]. Finally, we chose to sample 1 h after the completion of exercise as this time post-exercise demonstrated the largest increase in CS activity following exercise in humans [5]. Considerable variation in the enzyme response to acute exercise does occur where women, but not men (trained or untrained), increased CS activity 8% immediately following a 90-min cycle bout at 60% of $\dot{V}O_{2peak}$ [34]. In the current report, variability in the muscle enzyme response to acute AEx was explained in part by the variability in $\dot{V}O_{2max}$. The current results suggest there are individuals with exercise-induced increases in muscle enzyme activity at 1 h post-exercise. Additional time points of muscle sampling or different intensities of exercise might provide results that a response to acute exercise does occur for those lacking such a response in the current report.

During exercise, skeletal muscle ATP turnover increases more than 100-fold [35] and there is direct (Ca^{2+}, AMP/ATP ratio, inorganic phosphate, etc.) regulation of metabolic flux related to increases in contractile and metabolic activity [36]. Phosphorylation is an important post-translational mechanism to regulate protein activity. Global phosphoproteomic analysis revealed phosphorylation of CS (Ser190), SDH (Tyr119), and PFK (15 sites of Ser, Thr, Tyr) after a single bout of high-intensity (85–92%

of $\dot{V}O_{2max}$) exercise in human skeletal muscle [37]. To address this, we attempted to measure phosphorylated Ser/Thr on PFK, β-HAD, CS, and SDH in our samples, hoping to link changes in muscle enzyme activities with exercise-induced allosteric modifications of the enzymes. However, we were unable to produce reliable results perhaps due to the detection limit of immunoprecipitation/Western blotting compared to phosphoproteomic analysis.

Exercise activates several kinase signaling pathways that in turn regulate mitochondrial activity including p38 MAPK, CaMK, and AMPK [38]. To investigate AMPK activation in response to exercise and the possible role of AMPK in enzyme activity regulation, ACC phosphorylation was measured as an indicator of AMPK as AMPK is the primary kinase phosphorylating ACC [39]. As a group, the increase in ACC phosphorylation by acute exercise suggests that the exercise bouts employed here did produce discernable responses. Combined with the lack of increases in muscle enzyme activity, the results suggest AMPK likely is not responsible for exercise-induced PFK, β-HAD, CS, and SDH activity changes. Consistent with this, there was no difference in ACC phosphorylation between NORM and LOW suggesting that exercise-induced changes in muscle enzyme activity are not due to activation by AMPK.

$\dot{V}O_{2max}$ and health

In the experimental design, we had questioned the physiological relevance of post-exercise enzyme activity. It is possible that greater skeletal muscle metabolic enzyme activities following exercise could contribute in part to higher $\dot{V}O_{2max}$ in otherwise untrained individuals. Limits in $\dot{V}O_{2max}$ are multifactorial and can occur at many steps along the oxygen delivery and use cascade [40, 41]. In the current study, $\dot{V}O_{2max}$ was related to skeletal muscle PFK, β-HAD, CS, and SDH activities following AEx, but not at rest suggesting that exercise activation may be an important determinant of aerobic capacity. This is in contrast to previous work where acute-exercise-induced increases in muscle CS activity were not related to $\dot{V}O_{2max}$ [6]. A critical difference between the current work and Tonkongi et al. (mean $\dot{V}O_{2max}$: 52.1 ml $O_2 \times kg^{-1} \times min^{-1}$; range 35.8–68.9) is the unresponsive low $\dot{V}O_{2max}$ that suggests there may be an aerobic capacity threshold below which metabolic enzymes are unresponsive to acute exercise. Conversely, it is possible that exercise-related metabolic enzyme activities are aerobic capacity dependent.

Current recommendations on physical activity include performing both aerobic and resistance exercise [11]. Initial work on concurrent exercise questioned if the performance of both aerobic and resistance exercise training limited the benefits observed with either exercise modality performed alone [42, 43]. These reports identified reduced strength gains, but no interference in the increases in $\dot{V}O_{2max}$. Interestingly, the relationships between exercise muscle activity and $\dot{V}O_{2max}$ are no longer significant with the addition of resistance exercise suggesting some level of resistance exercise interference in the current study.

Acute exercise is a metabolic stress requiring flexibility in substrate utilization and metabolic flexibility during sub-maximal exercise is impaired in obesity [21, 22]. In the current report, lower $\dot{V}O_{2max}$ was related to higher blood glucose, higher HOMA-IR, and lower exercise-induced changes in muscle enzyme activity. In addition, lower SDH activity after AEx was related to higher HOMA-IR possibly providing a mechanism connecting muscle electron transport chain flexibility, aerobic capacity, and insulin sensitivity. Lower $\dot{V}O_{2max}$ and higher HOMA-IR are linked to increased risk of chronic disease [44–46], while increases in $\dot{V}O_{2max}$ in response to exercise training are linked with beneficial changes in insulin sensitivity both in normoglycemic and T2D individuals [47]. A lack of changes in skeletal muscle enzyme activity in response to exercise as occurred in the low $\dot{V}O_{2max}$ group may be a predictor of increased risk of chronic disease.

Conclusion

The current report found changes in enzyme activity in response to acute exercise are dependent upon aerobic capacity; though as a group moderate intensity and duration acute, systemic, aerobic exercise did not increase skeletal muscle enzyme activity. There were positive linear relationships between aerobic exercise PFK, β-HAD, CS, and SDH activity and $\dot{V}O_{2max}$ suggesting higher aerobic capacity is linked to exercise-induced muscle enzyme responsiveness. However, whether this is a causal relationship or the direction (greater enzyme activity supports greater $\dot{V}O_{2max}$ or vice versa) of a causal relationship requires further investigation.

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Author contributions YN and JSS assisted with data collection, data analysis, contributed to the discussion, and wrote/edited the manuscript. JAW, RTG, SK, and JS assisted with data collection and analysis. TPG designed the experiment, assisted with data collection and data analysis, contributed to the discussion, and wrote/edited the manuscript. TPG is the guarantor of this work, and as such, takes responsibility for the integrity of the data and accuracy of the data analysis.

Compliance with ethical standards

Conflict of interest TPG is on the Advisory Board for *Sport Sciences for Health*. YN, JSS, JAW, RTG, SK, JS, and TPG report no potential conflicts of interest relevant to this article.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the University Institutional Review Board and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Henriksson J, Reitman JS (1976) Quantitative measures of enzyme activities in type I and type II muscle fibres of man after training. Acta Physiol Scand 97(3):392–397. https://doi.org/10.1111/j.1748-1716.1976.tb10279.x
2. Simoneau JA, Lortie G, Boulay MR, Marcotte M, Thibault MC, Bouchard C (1987) Effects of two high-intensity intermittent training programs interspaced by detraining on human skeletal muscle and performance. Eur J Appl Physiol Occup Physiol 56(5):516–521
3. Tang JE, Hartman JW, Phillips SM (2006) Increased muscle oxidative potential following resistance training induced fibre hypertrophy in young men. Appl Physiol Nutr Metab 31(5):495–501. https://doi.org/10.1139/h06-026
4. Komi PV, Viitasalo JT, Rauramaa R, Vihko V (1978) Effect of isometric strength training of mechanical, electrical, and metabolic aspects of muscle function. Eur J Appl Physiol Occup Physiol 40(1):45–55
5. Leek BT, Mudaliar SR, Henry R, Mathieu-Costello O, Richardson RS (2001) Effect of acute exercise on citrate synthase activity in untrained and trained human skeletal muscle. Am J Physiol Regul Integr Comp Physiol 280(2):R441–R447
6. Tonkonogi M, Harris B, Sahlin K (1997) Increased activity of citrate synthase in human skeletal muscle after a single bout of prolonged exercise. Acta Physiol Scand 161(3):435–436. https://doi.org/10.1046/j.1365-201X.1997.00233.x
7. Dohm GL, Huston RL, Askew EW, Fleshood HL (1973) Effects of exercise, training, and diet on muscle citric acid cycle enzyme activity. Can J Biochem 51(6):849–854
8. Ji LL, Stratman FW, Lardy HA (1988) Enzymatic down regulation with exercise in rat skeletal muscle. Arch Biochem Biophys 263(1):137–149
9. Lawler JM, Powers SK, Visser T, Van Dijk H, Kordus MJ, Ji LL (1993) Acute exercise and skeletal muscle antioxidant and metabolic enzymes: effects of fiber type and age. Am J Physiol 265(6 Pt 2):R1344–R1350. https://doi.org/10.1152/ajpregu.1993.265.6.R1344
10. Gollnick PD, Bertocci LA, Kelso TB, Witt EH, Hodgson DR (1990) The effect of high-intensity exercise on the respiratory capacity of skeletal muscle. Pflugers Arch 415(4):407–413
11. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, Nieman DC, Swain DP, American College of Sports M (2011) American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. Med Sci Sports Exerc 43(7):1334–1359. https://doi.org/10.1249/MSS.0b013e318213fefb
12. Sale DG, MacDougall JD, Jacobs I, Garner S (1990) Interaction between concurrent strength and endurance training. J Appl Physiol (1985) 68(1):260–270
13. Sigal RJ, Kenny GP, Boule NG, Wells GA, Prud'homme D, Fortier M, Reid RD, Tulloch H, Coyle D, Phillips P, Jennings A, Jaffey J (2007) Effects of aerobic training, resistance training, or both on glycemic control in type 2 diabetes: a randomized trial. Ann Intern Med 147(6):357–369
14. Church TS, Blair SN, Cocreham S, Johannsen N, Johnson W, Kramer K, Mikus CR, Myers V, Nauta M, Rodarte RQ, Sparks L, Thompson A, Earnest CP (2010) Effects of aerobic and resistance training on hemoglobin A1c levels in patients with type 2 diabetes: a randomized controlled trial. JAMA 304(20):2253–2262. https://doi.org/10.1001/jama.2010.1710
15. Sparks LM, Johannsen NM, Church TS, Earnest CP, Moonen-Kornips E, Moro C, Hesselink MK, Smith SR, Schrauwen P (2013) Nine months of combined training improves ex vivo skeletal muscle metabolism in individuals with type 2 diabetes. J Clin Endocrinol Metab 98(4):1694–1702. https://doi.org/10.1210/jc.2012-3874
16. Myers J, Kaykha A, George S, Abella J, Zaheer N, Lear S, Yamazaki T, Froelicher V (2004) Fitness versus physical activity patterns in predicting mortality in men. Am J Med 117(12):912–918. https://doi.org/10.1016/j.amjmed.2004.06.047
17. Gavin TP, Van Meter JB, Brophy PM, Dubis GS, Potts KN, Hickner RC (2012) Comparison of a field-based test to estimate functional threshold power and power output at lactate threshold. J Strength Cond Res 26(2):416–421. https://doi.org/10.1519/JSC.0b013e318220b4eb
18. Harms CA, Babcock MA, McClaran SR, Pegelow DF, Nickele GA, Nelson WB, Dempsey JA (1997) Respiratory muscle work compromises leg blood flow during maximal exercise. J Appl Physiol 82(5):1573–1583
19. Hoppeler H (1990) The different relationship of $\dot{V}O_2$max to muscle mitochondria in humans and quadrupedal animals. Respir Physiol 80(2–3):137–145
20. Goodpaster BH, Sparks LM (2017) Metabolic flexibility in health and disease. Cell Metab 25(5):1027–1036. https://doi.org/10.1016/j.cmet.2017.04.015
21. Goodpaster BH, Wolfe RR, Kelley DE (2002) Effects of obesity on substrate utilization during exercise. Obes Res 10(7):575–584. https://doi.org/10.1038/oby.2002.78
22. Prior SJ, Ryan AS, Stevenson TG, Goldberg AP (2014) Metabolic inflexibility during submaximal aerobic exercise is associated with glucose intolerance in obese older adults. Obesity (Silver Spring) 22(2):451–457. https://doi.org/10.1002/oby.20609
23. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP (1981) The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes 30(12):1000–1007
24. Gavin TP, Stallings HW III, Zwetsloot KA, Westerkamp LM, Ryan NA, Moore RA, Pofahl WE, Hickner RC (2005) Lower capillary density but no difference in VEGF expression in obese vs. lean young skeletal muscle in humans. J Appl Physiol 98(1):315–321
25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
26. Spinazzi M, Casarin A, Pertegato V, Salviati L, Angelini C (2012) Assessment of mitochondrial respiratory chain enzymatic activities on tissues and cultured cells. Nat Protoc 7(6):1235–1246. https://doi.org/10.1038/nprot.2012.058
27. Shan C, Elf S, Ji Q, Kang HB, Zhou L, Hitosugi T, Jin L, Lin R, Zhang L, Seo JH, Xie J, Tucker M, Gu TL, Sudderth J, Jiang L, DeBerardinis RJ, Wu S, Li Y, Mao H, Chen PR, Wang D, Chen GZ, Lonial S, Arellano ML, Khoury HJ, Khuri FR, Lee BH,

Brat DJ, Ye K, Boggon TJ, He C, Kang S, Fan J, Chen J (2014) Lysine acetylation activates 6-phosphogluconate dehydrogenase to promote tumor growth. Mol Cell 55(4):552–565. https://doi.org/10.1016/j.molcel.2014.06.020
28. Jaworowski A, Porter MM, Holmback AM, Downham D, Lexell J (2002) Enzyme activities in the tibialis anterior muscle of young moderately active men and women: relationship with body composition, muscle cross-sectional area and fibre type composition. Acta Physiol Scand 176(3):215–225. https://doi.org/10.1046/j.1365-201X.2002.t01-2-01004.x
29. Nie Y, Sato Y, Wang C, Yue F, Kuang S, Gavin TP (2016) Impaired exercise tolerance, mitochondrial biogenesis, and muscle fiber maintenance in miR-133a-deficient mice. FASEB J 30(11):3745–3758. https://doi.org/10.1096/fj.201600529R
30. Zwetsloot KA, Westerkamp LM, Holmes BF, Gavin TP (2008) AMPK regulates basal skeletal muscle capillarization and VEGF expression, but is not necessary for the angiogenic response to exercise. J Physiol 586:6021–6035
31. Park SH, Gammon SR, Knippers JD, Paulsen SR, Rubink DS, Winder WW (2002) Phosphorylation-activity relationships of AMPK and acetyl-CoA carboxylase in muscle. J Appl Physiol (1985) 92(6):2475–2482. https://doi.org/10.1152/japplphysiol.00071.2002
32. ACSM (2000) ACSM's guidlelines for exercise testing and prescription, 6th edn. American College of Sports Medicine, Baltimore
33. Blaha MJ, Hung RK, Dardari Z, Feldman DI, Whelton SP, Nasir K, Blumenthal RS, Brawner CA, Ehrman JK, Keteyian SJ, Al-Mallah MH (2016) Age-dependent prognostic value of exercise capacity and derivation of fitness-associated biologic age. Heart. https://doi.org/10.1136/heartjnl-2015-308537
34. Roepstorff C, Schjerling P, Vistisen B, Madsen M, Steffensen CH, Rider MH, Kiens B (2005) Regulation of oxidative enzyme activity and eukaryotic elongation factor 2 in human skeletal muscle: influence of gender and exercise. Acta Physiol Scand 184(3):215–224. https://doi.org/10.1111/j.1365-201X.2005.01442.x
35. Gaitanos GC, Williams C, Boobis LH, Brooks S (1993) Human muscle metabolism during intermittent maximal exercise. J Appl Physiol (1985) 75(2):712–719
36. Hargreaves M (2015) Exercise, muscle, and CHO metabolism. Scand J Med Sci Sports 25(Suppl 4):29–33. https://doi.org/10.1111/sms.12607
37. Hoffman NJ, Parker BL, Chaudhuri R, Fisher-Wellman KH, Kleinert M, Humphrey SJ, Yang P, Holliday M, Trefely S, Fazakerley DJ, Stockli J, Burchfield JG, Jensen TE, Jothi R, Kiens B, Wojtaszewski JF, Richter EA, James DE (2015) Global phosphoproteomic analysis of human skeletal muscle reveals a network of exercise-regulated kinases and AMPK substrates. Cell Metab 22(5):922–935. https://doi.org/10.1016/j.cmet.2015.09.001
38. Jornayvaz FR, Shulman GI (2010) Regulation of mitochondrial biogenesis. Essays Biochem 47:69–84. https://doi.org/10.1042/bse0470069
39. Wojtaszewski JF, Jorgensen SB, Hellsten Y, Hardie DG, Richter EA (2002) Glycogen-dependent effects of 5-aminoimidazole-4-carboxamide (AICA)-riboside on AMP-activated protein kinase and glycogen synthase activities in rat skeletal muscle. Diabetes 51(2):284–292
40. Ferretti G (2014) Maximal oxygen consumption in healthy humans: theories and facts. Eur J Appl Physiol 114(10):2007–2036. https://doi.org/10.1007/s00421-014-2911-0
41. Wagner PD (1996) Determinants of maximal oxygen transport and utilization. Annu Rev Physiol 58:21–50
42. Hickson RC (1980) Interference of strength development by simultaneously training for strength and endurance. Eur J Appl Physiol Occup Physiol 45(2–3):255–263
43. Dudley GA, Djamil R (1985) Incompatibility of endurance- and strength-training modes of exercise. J Appl Physiol (1985) 59(5):1446–1451
44. Abbasi F, Brown BW Jr, Lamendola C, McLaughlin T, Reaven GM (2002) Relationship between obesity, insulin resistance, and coronary heart disease risk. J Am Coll Cardiol 40(5):937–943
45. Wei M, Kampert JB, Barlow CE, Nichaman MZ, Gibbons LW, Paffenbarger RS Jr, Blair SN (1999) Relationship between low cardiorespiratory fitness and mortality in normal-weight, overweight, and obese men. JAMA 282(16):1547–1553
46. Laaksonen DE, Lakka HM, Salonen JT, Niskanen LK, Rauramaa R, Lakka TA (2002) Low levels of leisure-time physical activity and cardiorespiratory fitness predict development of the metabolic syndrome. Diabetes Care 25(9):1612–1618
47. Koivisto VA, Yki-Jarvinen H, DeFronzo RA (1986) Physical training and insulin sensitivity. Diabetes Metab Rev 1(4):445–481