Title
β-adrenergic receptor blockade blunts post-exercise skeletal muscle mitochondrial protein synthesis rates in humans

Running Head
β-adrenergic signaling and mitochondrial biogenesis

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Abstract

β-adrenergic receptor (β-AR) signaling is a regulator of skeletal muscle protein synthesis and mitochondrial biogenesis in mice. We hypothesized that β-AR blockade blunts post-exercise skeletal muscle mitochondrial protein synthesis rates in adult humans. Six healthy males (age: 26±6 y, maximal oxygen uptake (VO2peak): 39.9±4.9 ml*kg⁻¹*min⁻¹, body mass index 26.7±2.0 kg*m⁻² (mean ± SD)) performed one-hour of stationary cycle ergometer exercise (60% VO2peak) on two separate occasions: 1) during β-AR blockade (intravenous propranolol), and, 2) during administration of saline (control). Skeletal muscle mitochondrial, myofibrillar and sarcoplasmic protein synthesis rates were assessed using ²H₅-phenylalanine incorporation into skeletal muscle proteins post-exercise. The mRNA content of signals for mitochondrial biogenesis was determined using real-time polymerase chain reaction (rtPCR). β-AR blockade decreased mitochondrial protein synthesis rates (0.217±0.076 vs. 0.135±0.031 %*hr⁻¹, p<0.05), but not myofibrillar or sarcoplasmic protein synthesis rates. Peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α) mRNA was increased ~2.5 fold (p<0.05) at five hours compared to one hour post-exercise, but not influenced by β-AR blockade. We conclude that decreased β-AR signaling during cycling can blunt the post-exercise increase in mitochondrial protein synthesis rates without effecting mRNA content.

Keywords: mitochondrial biogenesis, propranolol, stable isotope tracer, aerobic exercise
Introduction

Decreased mitochondrial content and function have been reported with aging and may contribute to chronic disease (30) and muscle wasting (37, 49). Because of their potential role in the pathogenesis of disease, mitochondria are a target of lifestyle and pharmacological therapies. For example, aerobic exercise stimulates mitochondria proliferation (15) and likely contributes to the health benefits of exercise training (22). Conversely, certain drug classes cause mitochondrial dysfunction (9) and may contribute to skeletal muscle myopathies (10). Given the high prevalence of chronic disease and the widespread use of drug therapies and lifestyle recommendations for treatment of chronic diseases, it is necessary to understand how concurrent exercise and drug therapy affect mitochondrial biogenesis.

Recent evidence suggests that mitochondrial biogenesis following aerobic exercise is partly mediated through β-adrenergic receptor (β-AR) signaling (25). In mice, selective β2-AR stimulation under resting conditions increased the mRNA content of a regulator of mitochondrial biogenesis, peroxisome proliferator-activated receptor-gamma coactivator 1α [PGC1-α, (24)]. Additionally, mice that were treated with the non-selective β-AR antagonist propranolol had a blunted increase of PGC1-α mRNA following aerobic exercise (25). Such findings are consistent with human aerobic training studies reporting blunted adaptations of select mitochondrial enzymes and maximal aerobic capacity during non-selective β-AR antagonist treatment (1, 46, 52). β-AR antagonists (β-blockers) are used to treat cardiovascular diseases and are commonly prescribed in conjunction with exercise (39). It is possible that β-blockers may impair mitochondrial adaptations and limit the beneficial effects of aerobic exercise (35). Such a
negative relationship between β-blockers and exercise adaptations implies that drug prescription may be counter-productive to exercise recommendations.

Miura et al. demonstrated mitochondrial biogenesis may be regulated by β-AR signaling, however the assessment was limited to changes in mRNA content for proteins that regulate mitochondrial biogenesis and was performed in mice (25). Changes in mRNA content may not lead to changes in mitochondrial protein content (28); therefore it is necessary to determine mitochondrial biogenesis using additional determinants such as protein synthesis rates. Another recent report using mice found that β2-AR agonist treatment increased mixed skeletal muscle and mitochondrial protein synthesis rates after seven days of treatment (20). However, it is possible that there are inter-species differences in β-AR signaling between mice and humans.

Initially, to examine whether the findings of Miura were applicable to humans, we performed a one-hour infusion of the non-selective β-AR agonist isoproterenol (36). Contrary to Miura, we did not increase mitochondrial biogenesis, as measured by mRNA signaling and incorporation of a stable isotope into mitochondrial proteins, or whole body and skeletal muscle protein synthesis (36). Our dose of isoproterenol increased heart rate, increased blood pressure and was previously used to increase metabolic rate above resting values (5). The report of Koopman et al. (20) prompted us to examine further whether β-AR and changes in protein synthesis were unique to mice.

Our previous study was a gain of function design to evaluate if β-AR stimulation could induce mitochondrial biogenesis at rest (36). Here we conduct the loss of function experiment in which β-AR signaling is diminished during exercise. We hypothesized that blocking β1- and β2-ARs prior to and during a one-hour bout of cycling would
decrease mitochondrial protein synthesis and mRNA content of genes related to mitochondrial biogenesis.
Methods

Ethics approval

The Institutional Review Board of Colorado State University approved the protocol. Each volunteer was informed of the potential risks and written consent was obtained prior to enrollment. The study followed the guidelines set forth by the Declaration of Helsinki.

Study overview

Six healthy adult males were studied during two or three days separated by at least three weeks. First, a subset of three subjects was used to confirm the efficacy of our β-AR blockade protocol (see details below). All subjects then performed one-hour of stationary cycle ergometer exercise (60% of unmedicated maximal oxygen uptake (VO₂peak)) on two separate occasions: 1) during β-AR blockade (intravenous propranolol), and, 2) during administration of saline (control). The propranolol trial was always performed before the saline trial to make any adjustments in workload necessary for the subject to complete one-hour of exercise. An identical workload was repeated on the saline control day. Following exercise on both days, the participants rested for five hours during infusion of a stable isotope and subsequent skeletal muscle sampling to determine protein synthesis rates and mRNA content of skeletal muscle.

Subject characteristics

The participants (Table 1) were healthy based on a medical history questionnaire and free of any cardiac abnormalities as determined by an exercising 12 lead electrocardiogram. The three participants (Table 1) who volunteered for verification of β-AR blockade were a subset of the entire group. Exclusion criteria included a resting
heart rate <40 beats*min\(^{-1}\) due to the risk of hypotension during β-AR blockade. \(\text{VO}_2\text{peak}\) was determined during incremental stationary cycle ergometer exercise (Velotron, RacerMate Inc., Seattle WA) via indirect calorimetry (ParvoMedics, Sandy UT). Body composition was determined using dual energy x-ray absorptiometry (DEXA, Lunar Discovery W, GE Medical Systems, Madison WI).

**Verification of β-AR blockade**

Verification of the efficacy of our β-AR blockade procedure was undertaken as previously described (26). Briefly, participants arrived at the laboratory after an overnight fast and 24-hour abstention from vigorous exercise. Subjects were instrumented for the measurement of beat-by-beat heart rate (3-lead electrocardiogram) and blood pressure. An intravenous catheter was inserted into an antecubital vein through which the non-selective β-AR agonist isoproterenol was administered in a continuous and incremental fashion. Dosing rates were increased every five minutes (9, 12, 15, 18, 21 and 24 ng*kg fat free mass\(^{-1}\)*min\(^{-1}\)) until heart rate was increased 25 beats*min\(^{-1}\) above resting values. Next, the non-selective β-AR antagonist propranolol was intravenously infused, first as a priming dose (0.25 mg*kg\(^{-1}\) at 1 mg*min\(^{-1}\)) followed by a maintenance dose (0.006 mg*kg\(^{-1}\)*min\(^{-1}\)) through the one-hour cycling at 60% \(\text{VO}_2\text{peak}\). Following exercise, the infusion was stopped and the participant rested in bed. At one hour post-exercise, the isoproterenol dose that previously raised heart rate to 25 beats*min\(^{-1}\) above resting was repeated to determine if heart rate and blood pressure increased. β-AR blockade was demonstrated by the absence of change in heart rate or blood pressure during β-AR stimulation with isoproterenol.

**Study protocol (Figure 1)**
Participants refrained from physical activity on the day before each trial and were provided an evening snack to consume the night before each trial. The participants reported to laboratory following an overnight fast and intravenous catheters were inserted into a dorsal hand vein for arterialized blood sampling and antecubital vein for infusions. Beat-by-beat electrocardiograms were continuously recorded throughout the study day and blood pressure was monitored with an automated cuff. On the first trial, propranolol was administered as a priming dose (0.25 mg*kg\(^{-1}\) at 1 mg*min\(^{-1}\)) followed by a maintenance dose (0.006 mg*kg\(^{-1}\)*min\(^{-1}\)) using a precision pump (Harvard Apparatus, Holliston MA) following previously utilized protocols (2-4, 26). Immediately following the priming dose, the participants began cycling for one hour at 60% \(\text{VO}_2\text{peak}\) during infusion of the maintenance dose. Expired air was collected every 15 minutes to confirm steady state oxygen consumption as determined by indirect calorimetry. On the second trial day, the participants received an isovolumetric dose of saline and the same absolute workload was performed. Immediately following exercise on both days, the participants consumed a liquid meal (Ross Laboratories, Abbott Park, IL; comprising 57% carbohydrate, 28% fat, 15% protein) to replace the calories burned during exercise as determined by average oxygen consumption and respiratory exchange ratio. The beverage also contained sufficient protein (>20 grams) to maximally stimulate protein synthesis following exercise (27). A primed continuous infusion of \(^2\text{H}_5\)-phenylalanine (2 \(\mu\text{mol}\)*kg\(^{-1}\) prime, 0.05 \(\mu\text{mol}\)*kg\(^{-1}\)*min\(^{-1}\) continuous infusion) was performed for five hours following exercise. Skeletal muscle samples (~100-150 mg) were collected with a Bergstrom needle under local anesthesia (1% lidocaine) at one hour and five hours following exercise. Intermittent arterialized-venous blood samples were collected from a
heated dorsal hand vein. $^2$H$_5$-phenylalanine (Cambridge Isotope, Cambridge MA) was prepared in sterile isotonic saline by a pharmacy (Medical Center of the Rockies, Loveland CO) and tested for pyrogenicity. As an additional precaution against infection, all solutions were passed through a 0.2 μm filter before being infused intravenously.

Muscle protein synthesis

Skeletal muscle protein synthesis rates were determined for subsarcolemmal mitochondria (MITO), myofibrillar (MYO), and sarcoplasmic (SARC) enriched fractions as previously described (36). We used differential centrifugation to separate protein fractions that are not pure fractions, but predominately contain the proteins of interest. Briefly, ~70 mg of muscle tissue was homogenized and centrifuged at 800g to pellet a crude MYO fraction. The supernatant was centrifuged at 9000g to separate the SARC fraction (supernatant) and a MITO fraction (pellet). The MITO pellet was washed using a series of centrifugations and the final MITO, MYO and SARC pellets were washed with 500 μl of 100% ethanol, centrifuged (1000g, 30 seconds, 4°C), and rinsed with water (repeated twice). Protein pellets were solubilized in 1N NaOH (50°C, 15 minutes) and hydrolyzed into free amino acids (6 M HCl, 120°C, 24 hrs). Free amino acids were added to cation exchange columns then derivatized to their $t$-BDMS derivatives as previously described (12).

Samples were analyzed using gas-chromatography mass spectrometry (GC-MS 7890A GC with 5975C MS, Agilent Technologies Inc, Santa Clara CA) in electron impact mode with selected ion monitoring at m/z at 234 (m+0), 237 (m+3) and 239 (m+5), with m+0 representing the parent ion. Samples were injected in duplicate and the m+0 peak area was kept within standardized limits to minimize differences in abundances
due to concentration differences. Abundances were adjusted to standard curves that were
derivatized from a single set of diluted standards and analyzed before and after each GC-
MS analyses. Internal controls were included within each batch of samples to ensure
consistency between GC-MS analyses. Tracer enrichments of muscle samples were
determined as tracer to tracer ratio from a standard curve using the m+5/m+3 ion ratios
(12). Plasma enrichments were determined from m+5/m+0 ion ratios. The fractional
synthesis rate (FSR) was determined using the standard precursor product relationship
$FSR = \frac{\Delta E_m * E_p}{E_p}$ with $\Delta E_m$ as the change in enrichment of muscle proteins and $E_p$ as the
enrichment of the precursor. We adjusted for non-steady state plasma tracer enrichments
by calculating the precursor enrichment as the integral of the plasma phenylalanine
enrichment over time (54). We used circulating plasma $^2$H$_5$-phenylalanine as the
precursor as reported by others (8, 17, 18, 43), which may underestimate the true
synthesis rates [see (44) for review]. The low natural abundance of the infused tracer and
washout period between separate trials allowed the assumption that background tracer
enrichment is zero (45).

**Real-time PCR**

Real-time PCR was used to determine changes in mRNA content for PGC-1α and
downstream targets (Table 1). Total RNA was extracted from ~10 mg of skeletal muscle
using standard Trizol® methods (Invitrogen, Carlsbad CA) and reverse transcribed to
cDNA as previously described (36). Approximately 10 ng of cDNA were amplified
using 20 μl reactions with a manufactured master mix (Thermo Fisher, Rockford IL) in
triple in clear 96 well plates using Taqman® probes and 7300 Real-time PCR system
(Applied Biosystems, Carlsbad CA). PCR conditions were a hot start (2 minutes at 50°C,
15 minutes at 95°C) followed by 40 cycles of denaturing and annealing (15 seconds 95°C, 1 minute 60°C). The relative quantity of each gene target was normalized to a reference gene (β2-microglobulin) and fold changes determined using the $2^{-\Delta\Delta C_T}$ method (31).

**Statistics**

Heart rate, blood pressure, and VO2, were compared between trials using two-way (Trial x Time) analysis of variance (ANOVA) with repeated measures. Skeletal muscle FSR was compared using a one-way ANOVA. Changes in mRNA content were determined using two-way (Trial x Time) ANOVA with repeated measures on the ΔCt values (53) and are expressed as fold increase compared to the one hour biopsy on the saline control day. Multiple comparisons were performed using the Bonferroni correction. Statistical significance was set at p=0.05. Power analysis revealed that a sample size of six with standard deviation of 0.06, α at 0.05, and β approximately 0.83 is able to detect a difference of 75% between treatments.
Results

Verification of $\beta$-AR blockade

Stimulation of $\beta$-AR with isoproterenol increased heart rate and blood pressure above resting values. Repeating the $\beta$-AR stimulation following $\beta$-AR blockade (propranolol infusion) and one-hour cycling had no effect on heart rate or blood pressure, indicating that the efficacy of our $\beta$-AR blockade procedure (Figure 2).

Exercise response during exercise

Compared with the control condition (saline administration), exercising heart and blood pressure were decreased during $\beta$-AR blockade (Figure 3A and 3B). Absolute workload was identical between conditions resulting in a VO$_2$ that was not different between trials (p>0.05; Figure 3C). Respiratory exchange ratio was not different between trials (data not shown).

Fractional synthesis rates (FSR)

Plasma enrichment of $^2$H$_5$-phenylalanine was not different between saline or propranolol trials (Figure 4A). Skeletal muscle enrichments are reported in Table 3. Skeletal muscle fractional synthesis rate (FSR, Figure 4B) of subsarcolemmal mitochondrial was decreased with $\beta$-AR blockade [MITO Saline: 0.217±0.076 vs. MITO PROP: 0.135±0.031 (p<0.05) %*hr$^{-1}$]. The FSR was not different between trials for myofibrillar proteins [MYO Saline: 0.154±0.061 vs. MYO PROP: 0.102±0.038 (p>0.05) %*hr$^{-1}$] or sarcoplasmic proteins [SARC Saline: 0.122±0.034 vs. SARC PROP: 0.0898±0.025 (p>0.05) %*hr$^{-1}$]. The 95% confidence intervals for the difference with propranolol were MITO: -0.15 to -0.01, MYO: -0.13 to 0.02 and SARC: -0.10 to 0.04 %*hr$^{-1}$.
Real-time PCR

Real-time PCR revealed PGC-1α mRNA was increased at five hours compared to one hour after cycling, with no differences between β-AR blockade or control condition (p<0.01 for time, Figure 5A). Downstream mRNA signals for mitochondrial biogenesis were not different between time points or between conditions (Figure 5B-F).
Discussion

Propranolol was infused during a one-hour bout of cycling to determine if non-selective β-AR blockade could alter skeletal muscle protein synthesis and mitochondrial biogenesis. Post-exercise mitochondrial protein synthesis was greater during the saline than propranolol trial. PGC-1α mRNA was increased at five hours post-exercise compared to one hour, but was not different between trials. Other downstream mRNA signals for mitochondrial biogenesis were not different between one and five hours after exercise or between trials. These data imply that β-AR signaling is an important physiological determinant of post-exercise mitochondrial biogenesis in adult humans.

Verification of β-AR blockade

Non-selective β-AR blockade was confirmed in three participants. The ability for isoproterenol to stimulate heart rate and blood pressure was determined before and after cycling with propranolol. The lack of cardiovascular stimulation at one hour following cycling with propranolol indicates non-selective β-AR blockade during the acute period following cycling, which is when skeletal muscle protein synthesis rates and signals for mitochondrial biogenesis are elevated (14, 33, 51). Our biopsies were collected at one and five hours following propranolol infusion, which is during the three to six hour half-life of propranolol (34). We did not determine if there was complete blockade of all β-ARs on skeletal muscle. However, the demonstration of non-selective β-AR blockade combined with the half-life of propranolol indicates that β-AR signaling was decreased during our measures of skeletal muscle protein synthesis and mRNA. As expected, the magnitude of increase in heart rate and blood pressure was decreased during cycling with β-AR blockade suggesting that β-AR signaling was decreased in all participants.
Previous work has shown increased catecholamine signaling during exercise at 60% VO2 peak (19). Thus, our cycling workload was sufficient to increase β-AR signaling and propranolol was able to blunt β-AR signaling.

Our study was designed as an integrative approach to assess mitochondrial biogenesis during recovery from aerobic exercise. PGC-1α and downstream mRNA content are increased by two hours and have remained elevated for eight hours following exercise (32, 33). Additionally, skeletal muscle protein synthesis is increased in the hours following exercise and can remain elevated for 72 hours (23, 51). We are confident that the timing of muscle sampling was adequate to capture any changes in mRNA content for mitochondrial biogenesis or changes in protein synthesis rates. We assessed mitochondrial biogenesis as changes in mRNA transcript content of select mitochondrial signaling proteins and mitochondrial protein synthesis. Previous studies of β-AR signaling and mitochondrial biogenesis have reported changes in mRNA content of mitochondrial signaling proteins (24, 25), however such measurements do not take into account post-transcription or translational regulation. Our measurement of mitochondrial protein synthesis represents the cumulative regulation of signals for mitochondrial biogenesis.

Fractional synthesis rate (FSR)

The synthesis rate of skeletal muscle subsarcolemmal mitochondrial proteins was blunted in the several hours following cycling with β-AR blockade. Our results provide short-term evidence that non-selective β-AR blockade during cycling can impair mitochondrial adaptations, and supports long-term studies that showed decreased mitochondrial enzyme activity during training with β-blocker drugs (1). A blunted
response of mitochondrial protein synthesis to individual training sessions may lead to decreased adaptations over long-term.

The regulation of β-AR signaling on mitochondrial protein synthesis appears to be mediated through β_2_-ARs. Previous reports showed that selective β_2_-AR agonists, but not β_1_ or α agonists, increased PGC-1α mRNA at rest and selective β_2_-AR blockers abolished the post-exercise increase of PGC-1α mRNA (25). Additionally, the blunted increase of mitochondrial enzymes during aerobic training was reported during treatment with non-selective β-AR blockers (e.g. atenolol or propranolol), but not selective β_1_-AR blockers (e.g. metoprolol or atenolol) (1, 16, 46). Others have reported no difference in VO_{2peak} adaptations between selective or non-selective β-blocker treatments (42, 52). It is possible that different degrees of β-AR blockade due to various drug doses or binding preferences within selective or non-selective classes can explain variable training adaptations. It is important to note that studies with a greater focus on specific mitochondrial responses (e.g. mitochondrial enzyme activity) appear able to detect impairments with non-selective β-blockers that are not detected with whole body measures (e.g. VO_{2peak}). Our approach was to measure acute changes in protein synthesis that may explain long-term differences in mitochondrial adaptations with non-selective β-AR blockade.

Myofibrillar protein synthesis was not different between the propranolol and saline trials. While β-AR antagonists are used to treat cardiovascular disease, β-AR agonists have been used for the treatment of muscle loss, such as with aging or disease [see (21) for review]. In particular, β_2_-selective agonists stimulate skeletal muscle protein gains over several weeks in aging rats (41). We have shown that acute infusion of
isoproterenol (non-selective $\beta$-AR agonist) did not acutely stimulate skeletal muscle protein synthesis in humans (36). Recent data in mice showed that chronic treatment with a selective $\beta_2$-AR agonist increased skeletal muscle protein synthesis of myofibrillar, mitochondrial, and sarcoplasmic fractions at seven days, but not acutely or at 28 days of treatment (20). It is possible that our acute treatment with propranolol would result in significant decreases in myofibrillar and sarcoplasmic proteins if carried out for an extended period of time.

The primary outcome of our current study was mitochondrial protein synthesis for which we demonstrate a difference in fractional synthesis rate. However, we were potentially underpowered to detect differences in the secondary outcomes of myofibrillar (-33%) and sarcoplasmic (-26%) fractional synthesis rates. The 95% confidence intervals for the difference between treatments are weighted towards a decrease in protein synthesis with propranolol treatment. It is possible that including additional participants would reveal decreased protein synthesis in myofibrillar and sarcoplasmic fractions. Such a global regulation of protein synthesis rates is consistent with the increase in protein synthesis following $\beta_2$-selective agonist treatment shown by Koopman et al. (20) and could be mediated through $\beta$-adrenergic regulation of the mammalian target of rapamycin pathway (11).

Our values of skeletal muscle fractional synthesis rates were determined following exercise in the fed state. The fractional synthesis of skeletal muscle mitochondria is higher than myofibrillar proteins at rest (36, 38), following exercise (13) and is increased with intravenous amino acid infusion (6). Our measured mitochondrial protein synthesis rates are slightly higher than those reported by others due to the
experiment conditions (i.e. post-exercise and feeding) and calculating rates using a zero background labeling (45). The high rate of mitochondrial protein synthesis reported here and by others indicates a high rate of mitochondrial protein turnover that can be stimulated by exercise (51) and nutrition (6).

We included post-exercise nutritional supplementation to avoid a negative energy balance following exercise and to increase external validity. After reporting to the lab overnight fasted, and given the length of the protocol, the subjects would have been fasted approximately 18 hours. It is known that negative energy balance decreases nitrogen balance (48). Further, a prolonged fast and exercise would likely result in AMPK activation and therefore PGC-1α activation. Even though our study did not include an exercise plus fasting group, our results indicate that β-blockade can impair mitochondrial protein synthesis in the fed state following exercise.

Exercise VO$_2$

The one-hour cycling workload was adjusted to maintain 60% VO$_{2\text{peak}}$ during the first trial (propranolol) and the same absolute workload was repeated for the second trial (saline). The respiratory exchange ratios were not different between trials indicating that substrate utilization was similar during the trials. Others have reported similar indirect calorimetry results during cycling exercise following propranolol treatment (50). Post-exercise signals for mitochondrial biogenesis can be influenced by dietary habits that alter glycogen content (32) but are not related to circulating glucose or free fatty acid availability (40). Thus, the potential influence of β-AR signaling on mitochondrial biogenesis is likely to be within skeletal muscle and not due to changes in circulating substrate metabolism during exercise.
Contrary to our hypothesis and previous studies with mice (25), mRNA content of markers of mitochondrial biogenesis were not different between exercise with propranolol compared to saline. PGC-1α mRNA was increased in both trials at five hours post exercise compared to one hour, which is similar to previous reports in human exercise trials (33). To minimize subject burden, we did not collect a muscle sample before exercise and cannot determine the change in mRNA content from resting values. However, our biopsy time points at one hour and five hours after exercise provide two times points to compare between propranolol and saline trials and are within the timeframe that PGC-1α and downstream mRNA are elevated (32).

Exercise-induced changes of PGC-1α mRNA content are dependent on exercise intensity (47) and appear to be regulated by the relative intensity rather than the absolute intensity (29). Despite an acute blunting of heart rate response and increased perceived effort with propranolol, our exercise bouts were performed at the same relative intensities. Support for the same relative exercise intensity was that subjects were tested within four weeks with no changes in exercise patterns in the intervening period, and equal respiratory exchange ratio, which scales to relative exercise intensity (7), between trials.

Perspectives and Significance

We previously reported that acute non-selective β-AR stimulation during resting did not increase mitochondrial protein synthesis or mRNA markers of mitochondrial biogenesis. Our current investigation indicates that β-AR signaling can modulate exercise-induced mitochondrial protein synthesis. The post-exercise recovery period is a
critical time for skeletal muscle remodeling and decreasing sympathetic signals to skeletal muscle may attenuate adaptations. Further work should consider if chronic intake of β-AR blockers would decrease mitochondrial protein synthesis and contribute to impaired mitochondrial adaptations during exercise training.

We conclude that non-selective β-AR blockade can blunt the post-exercise increase in mitochondrial protein synthesis rates. Non-selective β-AR blockade did not alter the mRNA content for signals of mitochondrial biogenesis, suggesting that the impaired protein synthesis response is independent of changes to mRNA transcripts. Our results indicate that non-selective β-AR antagonists can impair mitochondrial adaptations to acute aerobic exercise and may lead to decreased training adaptations.

Acknowledgements

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References


Table 1: Subject characteristics of subjects who volunteered for the verification of β-blockade using isoproterenol and propranolol (ISO+PROP) and the propranolol study (PROP). The three subjects for ISO+PROP were from final PROP group.

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Table 2: PCR Sequences.

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Table 3: Muscle enrichments (tracer to tracee ratio) for mitochondrial (MITO), myofibrillar (MYO) and sarcoplasmic (SARC) fractions between saline (Saline) and propranolol (PROP) trials. Data are mean±SD.

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**Figure legends**

**Figure 1:** Study schematic. β-adrenergic receptor blockade was verified in three participants using a graded isoproterenol (ISO) infusion to raise HR and BP, then infused propranolol (PROP) during cycling and repeated the ISO infusion at one hour post exercise (A). Six participants complete two trials with either propranolol (PROP) or saline infused during cycling followed by stable isotope infusion and muscle biopsies (B).

**Figure 2:** Efficacy of β-adrenergic receptor blockade protocol was demonstrated in three subjects by a lack of a heart rate (HR, A), systolic (B) or diastolic blood pressure (C) response to β-adrenergic stimulation with isoproterenol. Data are expressed as change from resting following isoproterenol (ISO) infusion or propranolol followed by isoproterenol (PROP+ISO).

**Figure 3:** Heart rate (HR, A), systolic (SYS), and diastolic (DIA) blood pressure (BP, B) were all lower during exercise with β-adrenergic receptor blockade (propranolol: PROP). Absolute workloads were identical resulting in similar steady state oxygen consumption (C). *p<0.01

**Figure 4:** Plasma enrichment of $^2$H$_5$-phenylalanine (panel A) increased over time (p<0.001) but was not different between saline (Saline) or propranolol (PROP). β-adrenergic receptor blockade (propranolol: PROP) during cycling decreased skeletal muscle fractional synthesis (FSR, B) for subsarcolemmal mitochondrial (MITO) but not myofibrillar (MYO) or sarcoplasmic (SARC) proteins. *p<0.05

**Figure 5:** Real-time PCR revealed PGC-1α mRNA was increased at five hours following cycling (*p<0.01 for time, A) but not different between saline (open) or β-adrenergic receptor blockade (propranolol: PROP, shaded) trials. Downstream mRNA signals for mitochondrial biogenesis were not different at one hour and five hours following cycling between saline or β-adrenergic receptor blockade trials (B-F). Data are expressed as fold change compared to one-hour saline.
A.

- Continuous HR and BP
- Cycling
  - ISO Infusion
  - PROP Infusion
- Feeding

B.

- Primed, continuous infusion of \textit{ring-[^2}H_{5}]Phe
- Cycling
  - PROP or SALINE Infusion
- Feeding
  - Skeletal muscle biopsies
- Heart rate, blood pressure and intermittent blood samples
A. PGC-1α

* p<0.01 for time

B. TFAM

C. NRF1

D. NRF2

E. COX7a

F. mtND4

Fold from 1-hr Saline

1-hr  5-hr

Fold from 1-hr Saline

1-hr  5-hr

Fold from 1-hr Saline

1-hr  5-hr

Fold from 1-hr Saline

1-hr  5-hr