

# Skeletal Muscle Mitophagy In Response To Cold Exposure During Exercise

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## ABSTRACT

**BACKGROUND:** The signaling pathways generated during exercise elicit mitochondrial adaptation, which includes both biogenesis and removal of damaged mitochondria (also known as mitophagy). It has been established that training followed by cold exposure alters the transcription of genes associated with mitochondrial growth and division. However, it is currently unknown whether temperature intervention during exercise influences the mRNA expression of genes associated with mitophagy, which is essential to maintaining the quality of this organelle. **PURPOSE:** To determine the expression of PINK1, PARK2, BNIP3, and BNIP3L mRNA following exercise in a cold environmental temperature with recovery at room temperature compared to exercise and recovery at room temperature. **METHODS:** Eleven recreationally trained males cycled at 65%  $W_{peak}$  for an hour at  $-2\text{ }^{\circ}\text{C}$  (cold) and  $20\text{ }^{\circ}\text{C}$  (room temperature). A muscle biopsy was taken from the *vastus lateralis* before exercise as well as 3h and 6h- post-exercise for gene expression analysis. **RESULTS:** Average exercise heart rate and skin temperature were lower in the cold compared to room temperature ( $154 \pm 4$  bpm,  $160 \pm 4$  bpm,  $P = 0.004$ ;  $28.0 \pm 0.4\text{ }^{\circ}\text{C}$ ,  $33.7 \pm 0.3\text{ }^{\circ}\text{C}$ ,  $P < 0.001$ , respectively), while core temperature was higher in the cold ( $38.9 \pm 0.3\text{ }^{\circ}\text{C}$ ,  $38.4 \pm 0.2\text{ }^{\circ}\text{C}$ ,  $P = 0.016$ ). BNIP3 and BNIP3L mRNA were not influenced by exercise or temperature ( $P > 0.05$ ). PINK1 and PARK2 decreased 3h-post-exercise compared to pre-exercise ( $P = 0.002$ ,  $P = 0.001$ , respectively). This response was diminished 6h-post-exercise, but values were still below baseline ( $P < 0.05$ ). **CONCLUSION:** Gene expression for select genes associated with mitophagy (PINK1, PARK2) is decreased after exercise with no difference between cycling in the cold compared to room temperature.

## INTRODUCTION

- Exercise stimulates regulation of the mitochondrial network, which includes simultaneous biogenesis and mitophagy.
- Previous work from our lab indicates a more favorable response in mRNA expression of mitochondrial biogenesis related genes after exercise and recovery in the cold, but this has not been explored in relation to markers of mitophagy.
- The purpose of this study was to determine the gene expression for mitophagy following an acute bout of cycling at a temperature below freezing compared to that of room temperature.

## METHODS



- 11 recreationally trained males cycled at 65%  $W_{peak}$  for an hour at  $-2\text{ }^{\circ}\text{C}$ , 74% RH and  $20\text{ }^{\circ}\text{C}$ , 66% RH on two separate occasions.
- Heart rate, core temperature, skin temperature, and rating of perceived exertion were measured during exercise trials.
- A muscle biopsy was taken from the *vastus lateralis* pre-exercise, 3h-post-exercise, and 6h-post-exercise for gene expression analysis.
- All mRNA analysis was measured using qRT-PCR normalized using  $2^{-\Delta\Delta CT}$  method.
- Differences were analyzed via repeated measures two-way ANOVA (temperature x time) with a probability of less than 5% for type I error ( $p < 0.05$ ) being deemed significant.

## RESULTS

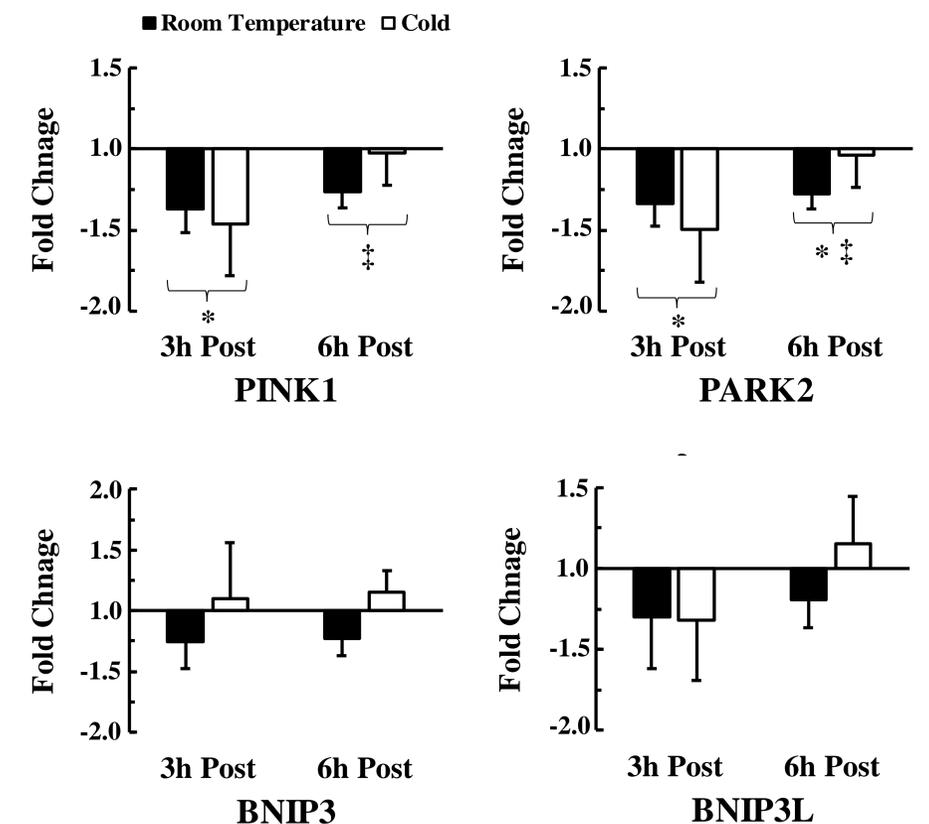
**Table 1.** Subject characteristics. Data are means  $\pm$  SE (n = 11).

Age (yr)	Height (cm)	Weight (kg)	Body fat (%)	$VO_{2peak}$ (l/min)	$VO_{2peak}$ ( $ml \cdot kg^{-1} \cdot min^{-1}$ )	Power at $VO_{2peak}$ (W)
25 $\pm$ 2	179 $\pm$ 2	82.7 $\pm$ 3.8	17.9 $\pm$ 1.8	3.84 $\pm$ 0.16	47.0 $\pm$ 2.2	238 $\pm$ 12

**Table 2.** Study parameters measured during exercise. Data are means  $\pm$  SE (n = 11). Values represent 15 minute averages during exercise and 1h average of entire exercise bout. HR, heart rate; RPE, rating of perceived exertion; RT, room temperature. †  $P < 0.05$  from RT.

	Rest	15 m	30 m	45 m	60 m	Avg Exs
<b>RT, <math>^{\circ}\text{C}</math> (20.1 <math>\pm</math> 0.1)</b>						
HR	77 $\pm$ 4	150 $\pm$ 5	162 $\pm$ 5	164 $\pm$ 5	166 $\pm$ 5	160 $\pm$ 4
Core temperature, $^{\circ}\text{C}$	36.7 $\pm$ 0.2	37.8 $\pm$ 0.2	38.4 $\pm$ 0.1	38.5 $\pm$ 0.2	38.8 $\pm$ 0.2	38.4 $\pm$ 0.2
Skin temperature, $^{\circ}\text{C}$	32.8 $\pm$ 0.2	32.9 $\pm$ 0.3	33.7 $\pm$ 0.4	34.0 $\pm$ 0.4	34.0 $\pm$ 0.4	33.7 $\pm$ 0.3
RPE		12 $\pm$ 0.6	13 $\pm$ 1.3	16 $\pm$ 0.4	17 $\pm$ 0.6	14 $\pm$ 0.5
<b>Cold, <math>^{\circ}\text{C}</math> (-2.0 <math>\pm</math> 0.5)</b>						
HR	84 $\pm$ 6	144 $\pm$ 5	156 $\pm$ 4	158 $\pm$ 4	159 $\pm$ 4	154 $\pm$ 4†
Core temperature, $^{\circ}\text{C}$	36.5 $\pm$ 0.1	38.7 $\pm$ 0.4	39.1 $\pm$ 0.3	39.1 $\pm$ 0.2†	39.0 $\pm$ 0.3	38.9 $\pm$ 0.3†
Skin temperature, $^{\circ}\text{C}$	32.8 $\pm$ 0.2	30.5 $\pm$ 0.5†	28.3 $\pm$ 0.6†	27.0 $\pm$ 0.5†	26.3 $\pm$ 0.4†	28.0 $\pm$ 0.4†
RPE		11 $\pm$ 0.6	14 $\pm$ 0.6	15 $\pm$ 0.5	15 $\pm$ 0.7	14 $\pm$ 0.5

## RESULTS



**Figure 3.** mRNA response normalized to pre-exercise conditions. Data are expressed as means  $\pm$  SE (n = 11). \*  $P < 0.05$  from pre-exercise; ‡  $P < 0.05$  from 3h-post-exercise.

## CONCLUSIONS

- Acute aerobic exercise downregulates signaling to synthesize mitophagy-related proteins, which may serve as an immediate protective mechanism to preserve mitochondrial quantity.
- Though not significant, it appears that the decrease in transcription of PINK1 and PARK2 is greater in the cold at 3h-post-exercise but then returns more quickly to baseline. Future research should further examine this tendency.
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