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Greater endurance capacity and improved dyspnoea with acute oxygen supplementation in idiopathic pulmonary fibrosis patients without resting hypoxaemia

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Summary at a glance

The effects of supplemental oxygen on oxidative stress, cytokine production, skeletal muscle metabolism and physiological response to exercise in idiopathic pulmonary fibrosis (IPF) were evaluated. Oxygen improved exercise tolerance, prevented exercise-induced hypoxaemia, reduced dyspnoea and decreased xanthine concentrations. Improved muscle metabolism is a possible mechanistic action of oxygen in IPF.

Abstract

Background and objective: Supplemental oxygen is commonly prescribed in people with idiopathic pulmonary fibrosis (IPF) although its benefits have not been proven. The aims of this study were to investigate the effect of oxygen on oxidative stress, cytokine production, skeletal muscle metabolism and physiological response to exercise in IPF.

Methods: Eleven participants with IPF received either oxygen, at a FiO_2 of 0.50, or compressed air for one hour at rest and during a cycle endurance test at 85% of peak work rate. Blood samples collected at rest and during exercise were analysed for markers of oxidative stress, skeletal muscle metabolism and cytokines. The protocol was repeated a week later with the alternate intervention.

Results: Compared to air, oxygen did not adversely affect biomarkers concentrations at rest and significantly improved endurance time (mean difference 99±81s, p=0.002), dyspnoea (-1±1units, p=0.02), systolic BP (-11±11mmHg, p=0.006), nadir SpO_2 (8±6%, p=0.001), SpO_2 at 2 minute (7±6%, p=0.003) and 5 minute isotimes (5±3, p<0.001) and peak exercise xanthine concentrations (-42±73µM, p=0.03). Air significantly increased IL-10 (5±5 pg/ml, p=0.04) at 2 minutes isotime. Thiobarbituric acid reactive substances (TBARs), IL-6, tumor necrosis factor-a (TNF- ±), creatine kinase, lactate, heart rate and fatigue did not differ between the two interventions at any time point.

Conclusion: In patients with IPF, breathing oxygen at FiO_2 of 0.50 at rest seems safe. During exercise, oxygen improves exercise tolerance, alleviates exercise-induced hypoxaemia, and reduces dyspnoea. A potential relationship between oxygen administration and improved skeletal muscle metabolism should be explored in future studies.

Keywords:

Exercise

Idiopathic Pulmonary Fibrosis

Metabolism

Oxidative stress

Oxygen

Short title:

Short term supplemental oxygen in IPF

Introduction

Idiopathic pulmonary fibrosis (IPF) is a devastating lung disease characterized by the destruction of lung parenchyma and the development of fibrosis.[1,2] Oxidative stress, caused by excessive production of damaging oxidants that overwhelm the body's reservoir of antioxidant moieties, is a recognized feature in IPF[2-4] and participates in the alveolar epithelial cell injury believed to drive the fibrotic process.[3,4] In IPF, this increased oxidative stress is further exacerbated by light exercise.[5] Exercise-induced oxidative stress has been shown to increase muscle fatigue[6-9] and compromise muscle function.[6,9] As such, daily physical activity and exercise in IPF may increase oxidative stress and muscle damage. Conversely, exercise training is an important treatment that improves exercise capacity, symptoms and quality of life in IPF.[10,11] It is therefore of interest to identify therapeutic approaches that attenuate exercise-induced oxidative stress and improve training outcomes.

Supplemental oxygen mitigated exercise-induced oxidative stress and ameliorated adenosine triphosphate (ATP) depletion in patients with chronic obstructive pulmonary disease (COPD).[12,13] The prevention of ATP depletion may reduce metabolic stress to the skeletal muscle[8,14] and improve exercise performance. Little is known about the effects of oxygen on oxidative stress in IPF. However randomised trials comparing oxygen to air indicated that oxygen improved endurance time, VO_2 peak, minute ventilation and lactate levels.[15-18] In contrast, one study reported oxygen provided no additional benefit compared to air during the 6-minute walk test (6MWT).[19] Additionally recent evidence suggests that oxygen may even cause hyperoxia-induced oxidative stress;[20-23] thereby potentially contributing to disease progression.

Oxygen therapy is frequently recommended in the management of IPF however there is limited evidence to support this practice.[24-26] Our study aims were to evaluate the effect of supplemental oxygen on oxidative stress, cytokine and, oxypurine concentrations and physiological responses during exercise in patients with IPF.

Methods

Participants

Patients with a confirmed diagnosis of IPF were recruited from the department of respiratory and sleep medicine at Austin Health. Exclusion criteria were the use of long term oxygen therapy, clinical instability, history of syncope on exertion, another concurrent respiratory disorder, any significant co-morbidities that precluded exercise testing and resting oxyhaemoglobin saturation (SpO_2) d 85% on room air. All participants gave written informed consent. The study was approved by the Austin Health and La Trobe University Human Research Ethics Committees and registered with Australian New Zealand Clinical Trials Registry (ACTRN12611001154998).

Study design and Procedures

This assessor and patient blinded, randomized placebo-controlled crossover trial consisted of five visits conducted over three weeks. On the first visit, respiratory function tests and a maximal, cycle exercise test were performed.[27] A week later, participants received either supplemental oxygen, at a FiO_2 of 0.50, or compressed air, at the equivalent flow rate of 15L.min^{-1}, in random order via a venturi mask for one hour at rest (Figure S1,

Supplementary Information). Participants subsequently performed a cycle endurance test (CET), whilst inhaling the same gas, on an electronically braked cycle ergometer (Corival V2, Lode BV, Netherlands) at 85% of peak work rate achieved on their maximal exercise test. The oxygen dose was prescribed according to an earlier study in COPD which found optimal improvements in exercise tolerance at FiO2 of 0.5.[28] The order of gas allocation was concealed in sealed envelopes until the participants attended the second visit. A researcher independent of the study completed the block randomisation using a web based sequence generator (http://www.randomization.com). The oxygen and air flow meters were screened and an unblinded investigator set up the mask and gas flow and monitored the SpO_2. The participant and the researcher conducting the test were therefore blinded to the gas allocation. The CET was terminated before volitional symptom limitation if SpO_2< 80%. Endurance time, heart rate, blood pressure, dyspnoea and fatigue were recorded. Blood samples were collected via venous cannulation, before and after the hour of rest, at two and five minutes cycling, peak exercise and one hour after the CET. A final blood sample was collected via venepuncture 24hours later. One week later, the entire protocol was repeated with the alternate intervention.

Biochemical Analysis

Plasma was separated, aliquoted and stored at -80^{R}C until use. The biochemical analysis was performed by an investigator blinded to the gas allocation. Blood samples were consequently frozen for > 12months. Each biomarker was analysed at all seven time points except for creatine kinase which was assessed at baseline, peak and 24hours post exercise.

The primary outcome measure was thiobarbituric acid reactive substances (TBARs), a global method for assessing oxidant – mediated lipid damage,[6,29] determined using the

OxiSelect TBARS Assay Kit (Cell Biolabs, San Diego, CA). Secondary outcomes measures were the CET measurements and the following biomarkers. Xanthine, lactate and creatine kinase, indirect markers of muscle metabolism and muscle damage[8, 14, 30-32] determined using Amplex Red Xanthine/Xanthine Oxidase Assay Kit (Molecular Probes, Eugene, OR), EnzyChrom L-lactate and EnzyChrom Creatine Kinase Assay Kit (BioAssay Systems, Hayward, CA). Cytokines IL-6, IL-10 and tumor necrosis factor-a (TNF-a) measured using ELISA kits (Mabtech, Nacka Strand, Sweden). All assays were performed per manufacturer instructions.

Statistical Analysis

A sample size of 12 was planned for this study. Nine were required to detect a mean (SD) difference in TBARs of 1.1 (0.6) μM, with 90% power, between oxygen and air[12] and we aimed to recruit an additional three subjects to allow for premature test termination due to oxyhaemoglobin desaturation. This sample size was also powered to detect a mean (SD) difference in endurance time of 3.3 (1.9) minutes (n=6).[28, 33] Paired t-tests were used to compare the differences between air and oxygen at each time point and the differences between pre and post exercise concentrations for each gas. Two way repeated measures ANOVA was used to evaluate exercise-induced biochemical changes and the interaction between exercise time and gas. Logarithmic transformed value of one was used for levels below the assay detection limit. Data are expressed as mean (SD) or mean (95% CI). All analyses were performed using SPSS V.20 (SPSS, Chicago, Illinois, USA). A $p < 0.05$ was considered statistically significant.

Results

Between January and December 2013, 14 participants were recruited and 11 participants completed the entire protocol. Three participants were withdrawn due to: (i) an unrelated inpatient admission, (ii) difficulty in obtaining blood samples and (iii) premature termination of the maximal exercise test due to face mask discomfort. The blood samples from three CETs were unsuitable, preventing the paired statistical analysis for three participants and reducing the sample size for the biochemical analysis to eight. There were no adverse events like pulmonary artery hypertension or cardiac dysfunction. Baseline participant characteristics are presented in Table 1.

Physiological response

Oxygen significantly improved endurance time, with a mean (SD) increase compared to air of 99 (81) seconds, dyspnoea, peak systolic BP and baseline, nadir, 2 minute and 5 minute SpO_2 (Table 2). There were no significant differences in heart rate or fatigue (Table 2).

Biochemical and metabolic response

At Rest

There were no significant baseline differences between air and oxygen or significant changes after one hour of rest in TBARs, IL-6, IL10, TNF-±, xanthine, creatine kinase or lactate (Table S1, Supplementary Information).

Exercise-related response

Oxidative Stress. Concentrations of TBARs did not significantly differ between air and oxygen at any time point (Figure 1a) or significantly change during exercise with either intervention (Figure 1b). Concentrations of TBARs were greater following exercise with both interventions but this was not significant (Figure 1a).

Cytokine production.

There were no significant differences in IL-6 or TNF-± between air and oxygen at any time point (Figure 2a and 2c). A significant increase in IL-10 at 2 minutes occurred with air (Figure 2b). Regardless of intervention received, IL-6 and IL-10 declined towards peak exercise (Figure 3a and 3b); this decline reached significance only for IL-6 (p= 0.02, exercise effect). Concentrations of TNF-± decreased with air and increased with oxygen, however this difference between interventions during exercise was not significant (Figure 3c, p=0.06, interaction effect). Post exercise concentrations of IL-6, IL10 and TNF-± were not significantly different to pre exercise concentrations for either intervention (Figure 2a, 2b and 2c). Thirty-eight percent of TNF-± and 19% of IL-6 concentrations were below the immunoassay's detection limit. A greater proportion of TNF-± concentrations below the detection limit (13 pg/ml) occurred with air compared to oxygen (77% vs. 46%) particularly at peak (63% vs 25%) and 1-hour post (63% vs 25%) exercise.

Skeletal muscle metabolism. Xanthine was elevated at 5 minutes and peak exercise with air, although only the latter reached significance (Figure 4a). There was a significant decrease in this exercise-induced xanthine response with oxygen (Figure 4b, p=0.004, interaction effect). There were no significant differences in creatine kinase and lactate between air and oxygen at any time point (Figure S2a and S2b, Supplementary Information). Creatine kinase and lactate decreased during exercise with oxygen but these changes were not significant (Figure S3a and S3b, Supplementary Information). Xanthine, creatine kinase, and lactate concentrations following exercise were not significantly different to pre-exercise concentrations except for lactate at 24hours post exercise with air (Figure S2a, Supplementary Information).

Discussion

This study is the first to assess the effect of supplemental oxygen on oxidative stress, cytokine production and skeletal muscle metabolism in patients with IPF. We found that breathing oxygen at rest did not increase oxidative stress or cytokine concentrations. During exercise, oxygen improved exercise tolerance, prevented exercise-induced hypoxaemia and reduced exertional dyspnoea. These improvements were not associated with changes in oxidative stress or cytokine response but were associated with a decrease in xanthine levels.

Several studies have found oxygen increases oxidative stress in both healthy subjects and patients with COPD[20-23] suggesting oxygen may independently cause oxidant induced lung injury.[34] By contrast, this and a previous study in COPD,[12] found oxygen did not affect resting oxidative stress or cytokine levels. This discordance may be attributed to the variable concentrations and duration of oxygen exposure. The threshold at which point oxygen becomes toxic is still not clear[20] and may differ across different disease groups. Breathing oxygen at FiO_2 of 0.50 for an hour at rest appears to avoid oxygen toxicity in patients with IPF.

We found oxygen improved exercise performance, strengthening previous findings[15-18] and we provided the first evidence that oxygen effectively reduces exertional dyspnoea in IPF. Furthermore, the improvement in endurance time exceeded the estimated minimum important difference (MID) of 70 seconds for a CET at 85% of peak work rate[35], which

was used in this study, and was slightly below the lower limit of the MID range for CETs at lower workloads (100- 200 seconds).[33,36] The improvement in dyspnoea was equivalent to MID of 1-unit for the modified Borg scale.[37] This suggests the acute benefits of oxygen supplementation are clinically meaningful. These results conflict with a recent study evaluating oxygen during the 6MWT in IPF.[19] The CET is more sensitive than the 6MWT in evaluating the response of interventions[36,38] and the self-paced nature of the 6MWT and the additional weight of the oxygen cylinder may have contributed to the reduced benefit. Whilst the 6MWT may better reflect the impact of ambulatory oxygen, our results provide support for the benefits of oxygen during exercise training, permitting increased endurance training time.

As ATP, the immediate energy source for muscle contraction, is consumed its metabolic by-products such as purine nucleosides and oxypurines (hypoxanthine, xanthine) increase.[32] Therefore, the elevation of xanthine at peak exercise with air and the concomitant reduction with oxygen raises the possibility oxygen could facilitate improved skeletal muscle metabolism in IPF. A similar improved metabolic response with oxygen has also been found in patients with COPD.[12,39] It is important to note however that the accurate evaluation of ATP metabolism cannot be determined with plasma levels of xanthine alone and would require a more objective measure of ATP depletion such as Adenosine diphosphate. Nonetheless, larger, clinical trials of oxygen therapy need to occur to fully elucidate this potentially mechanistic action of oxygen in IPF.

Contrary to previous reports in IPF[5] and COPD,[8,12,13,39] we did not observe an exercise-induced rise in oxidative stress and oxygen did not alter oxidative stress levels.[12,13] This inconsistency may be related to the timing of blood samples or the lesser disease severity

and hypoxaemia. Peak oxidative stress levels can occur at any time up to 72 hours after exercise.[40] Additionally, two studies in COPD found a significant increase in oxidative stress only at 6 hours following exercise.[6,29] Oxidative stress increases in parallel with disease severity[3] and exercise-induced increases are greater in hypoxaemic compared to non-hypoxaemic patients, with levels increasing proportional to the magnitude of hypoxaemia.[40-42] Our cohort had less impaired lung function compared to the previous study in IPF,[5] no resting hypoxaemia and only three participants with clinically significant exercise-induced hypoxaemia[43] despite exercise-induced oxyhaemoglobin desaturation in 90% of participants. Additionally, our study may not have been optimally powered to clearly evaluate the exercise-related oxidative stress response in IPF.

Exercise elicits a strong anti-inflammatory cytokine response initiated by IL-6, a commonly classified pro-inflammatory cytokine.[44] Exercise-induced IL-6 upregulates anti-inflammatory cytokines IL-1ra and IL-10 and suppresses pro-inflammatory cytokine TNF-±.[45] Furthermore, exercise is likely to suppress TNF-± independently of IL-6-, for instance by the induction of high levels of epinephrine.[44] Therefore, a normal anti-inflammatory exercise response was initially observed for air. The subsequent IL-6 and IL-10 decline however contrasts with the reported exponential increase towards peak exercise.[44,45]It is possible an impaired IL-6 and IL-10 exercise response may exist in IPF. Oxygen did not alter the IL-6 and IL-10 response however it was associated with an increase in TNF-±. This suggests oxygen may interfere with the normal anti-inflammatory response to exercise. There were though a substantial number of IL-6 and TNF-± concentrations below the immunoassay detection limit. In addition, cytokines can peak rapidly after induction and then drop to undetectable levels as they are rapidly cleared from the blood making assessment in plasma challenging. [46,47] This may be more apparent in short

exhaustive bicycle exercise. Therefore, these results may not reflect a true cytokine exercise response or a true effect of oxygen. Nevertheless, considering the cardinal role TNF-± plays in the pathogenesis of IPF;[48] and the positive effects of muscle derived IL-6[44,45], further research utilising more sensitive methods such as urine samples or muscle biopsies[47] is essential to elucidate this cytokine exercise response in IPF.

This study had several limitations. Reduced statistical power due to the omission of blood samples, reduced immunoassay sensitivity and the absence of antioxidant capacity or ADP evaluation consequently preventing firm conclusions on the biochemical exercise response and the corresponding effect of oxygen. Additionally, our study did not include a disease-free control group however a recent study[5] found oxidative stress at rest was increased in patients with IPF compared to age matched healthy controls and was further exacerbated by light exercise. Despite these limitations, this small pilot study demonstrated clinically meaningful benefits of oxygen, suggesting that further investigation of the mechanistic action of oxygen is warranted in larger trials. Further research should investigate if the provision of supplemental oxygen during exercise training facilitates long term improvements in muscle metabolism, exercise tolerance and physical activity in daily life, and corresponding increases in health-related quality of life.

In conclusion, this study shows that oxygen improves exercise tolerance, alleviates exercise-induced hypoxaemia and reduces exertional dyspnoea in patients with IPF. Supplemental oxygen may facilitate improved skeletal muscle metabolism through increased oxygen delivery. Supplemental oxygen may therefore be a beneficial adjunct to exercise training in IPF, optimising physiological improvements through increased endurance training time.

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Table 1 Baseline characteristics of participants

Sample, n	11
Age, years	71(11)
Gender – male	9 (81%)
Treatment	
Pirfenidone	1 (9%)
Nintedanib	0 (0%)
N-Acetylcysteine	0 (0%)
Bronchodilaors	3 (27%)
Exertional Oxygen therapy	1 (9%)
FVC, % pred	76 (13)
TLCO, % pred	49 (10)
Nadir SpO_2	90 (5)
HR Peak, bpm	128 (18)
HR Peak, % pred	87 (11)
Ve Peak, $L.min^{-1}$	63 (15)
Ve Peak, % pred	80 (21)
Peak Workate, Watts	80 (37)
Peak Workrate, % pred	61 (22)
Vo_2 peak, ml.kg.min	15 (3)
Vo_2 peak, % pred	56 (20)

*Values are mean (SD) or **n (%)**,*

%pred, per cent predicted; FVC, forced vital capacity; HR, heartrate; SpO_2, oxyhaemoglobin saturation; TLCO, carbon monoxide transfer factor; VO_2peak, peak oxygen uptake.

Table 2 Comparison of physiological responses to supplemental oxygen and compressed air

	Compressed Air	Supplemental Oxygen	P value
n=11			
Endurance time (s)	425 (228)	524 (240)	0.002
HR (bpm)			
Isotime 2mins	110 (17)	108 (12)	0.4
Isostime 5 mins	121 (22)	121 (18)	0.5
Peak	126 (20)	127 (19)	0.5
SpO_2 (%)			
Isotime 2mins	91 (6)	98 (1)	0.003
Isostime 5 mins	92 (4)	97 (2)	<0.001
Nadir	88 (7)	96 (3)	0.001
Peak Systolic BP (mmHg)	155 (17)	144 (14)	0.006
Peak Diastolic BP (mmHg)	71 (12)	72 (11)	0.9
Borg Dyspnea	4(1)	3 (1)	0.02
Borg Fatigue	4(2)	4 (1)	0.8

Values are mean (SD).

BP, blood pressure; HR, heartrate; SpO_2, oxyhaemoglobin saturation.

Figure Legends

Figure 1 Comparison of TBAR concentrations between compressed air and supplemental oxygen for a) each time point and b) exercise-related response

*Data are (95%CI), n=8**,** no significant differences between compressed air or supplemental oxygen or exercise responses for each gas. Pre exercise refers to the blood sample collected at the end of the hour rest and before the cycle test (BS2). Figure (a), n=6 for 5 minute isotime, 2 participants total exercise time < 5 minutes. Figure (b), Isotime refers to 2 minutes cycling, 5 minutes was excluded since not all participants reached this isotime.*

TBARS, thiobarbituric acid–reactive substances.

Figure 2 Comparison of cytokine concentrations between compressed air and supplemental oxygen at each time point for a) *IL-6 b) IL-10 and c) TNF-*±

*Data are mean (95%CI), n=8, *p<0.05 compressed air vs supplemental oxygen. Pre exercise refers to the blood sample collected at the end of the hour rest and before the cycle test (BS2). n=6 for 5 minute isotime, 2 participants total exercise time < 5 minutes.*

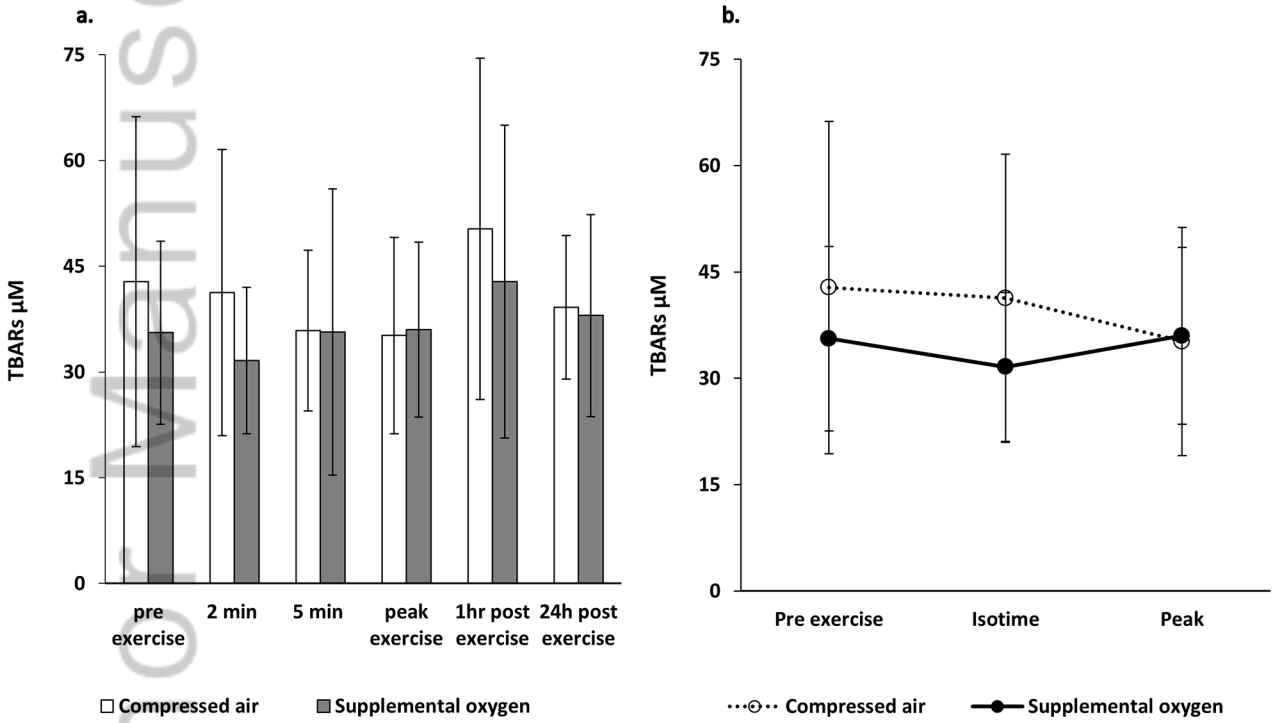
IL, interleukin; TNF- ±, tumor necrosis factor alpha.

Figure 3 Exercise-related cytokine response between compressed air and supplemental oxygen for a) *IL-6 b) IL-10 and c) TNF-*±

*Data are mean (95%CI), n=8, *p<0.05 exercise effect. There were no significant intervention or intervention*exercise effects. Pre exercise refers to the blood sample collected at the end of the hour rest and before the cycle test (BS2). Isotime refers to 2 minute cycling, 5 minutes was excluded since not all participants reached this isotime. IL, interleukin; TNF- ±, tumor necrosis factor alpha*

Figure 4 Comparison of xanthine concentrations between compressed air and supplemental oxygen a) each time point and b) exercise-related response

*Data are (95%CI), n=8, *p<0.05 compressed air vs supplemental oxygen, †p<0.05 exercise* intervention effect. There were no significant intervention or exercise effects. Pre exercise refers to the blood sample collected at the end of the hour rest and before the cycle test (BS2). Figure (a), n=6 for 5 minute isotime, 2 participants total exercise time < 5minutes. Figure (b), Isotime refers to 2 minutes cycling, 5 minutes was excluded since not all participants reached this isotime.*



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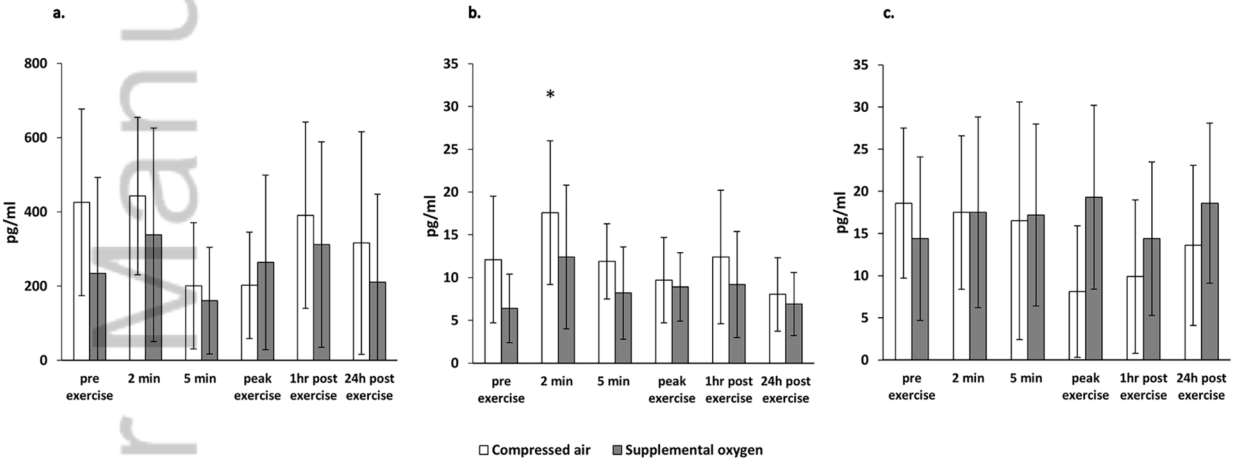

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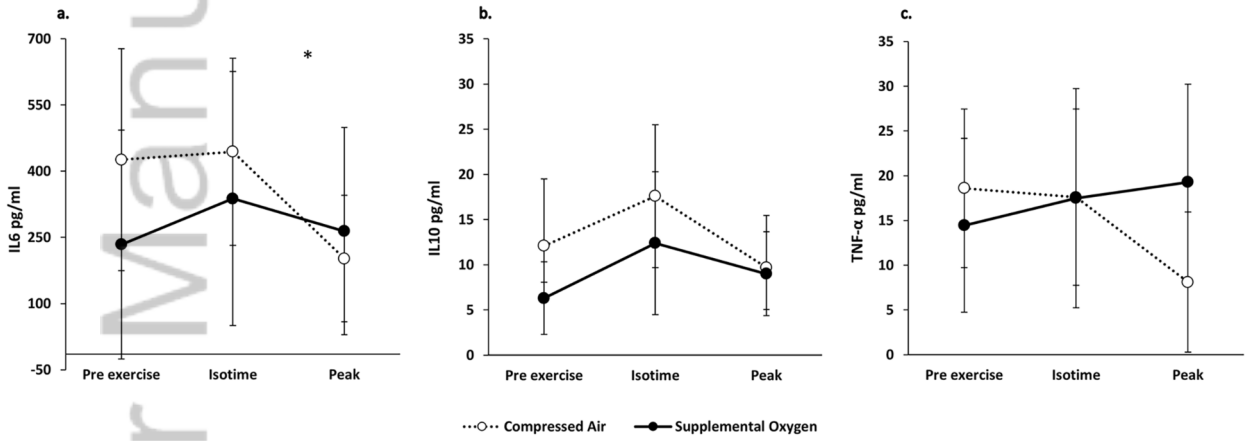

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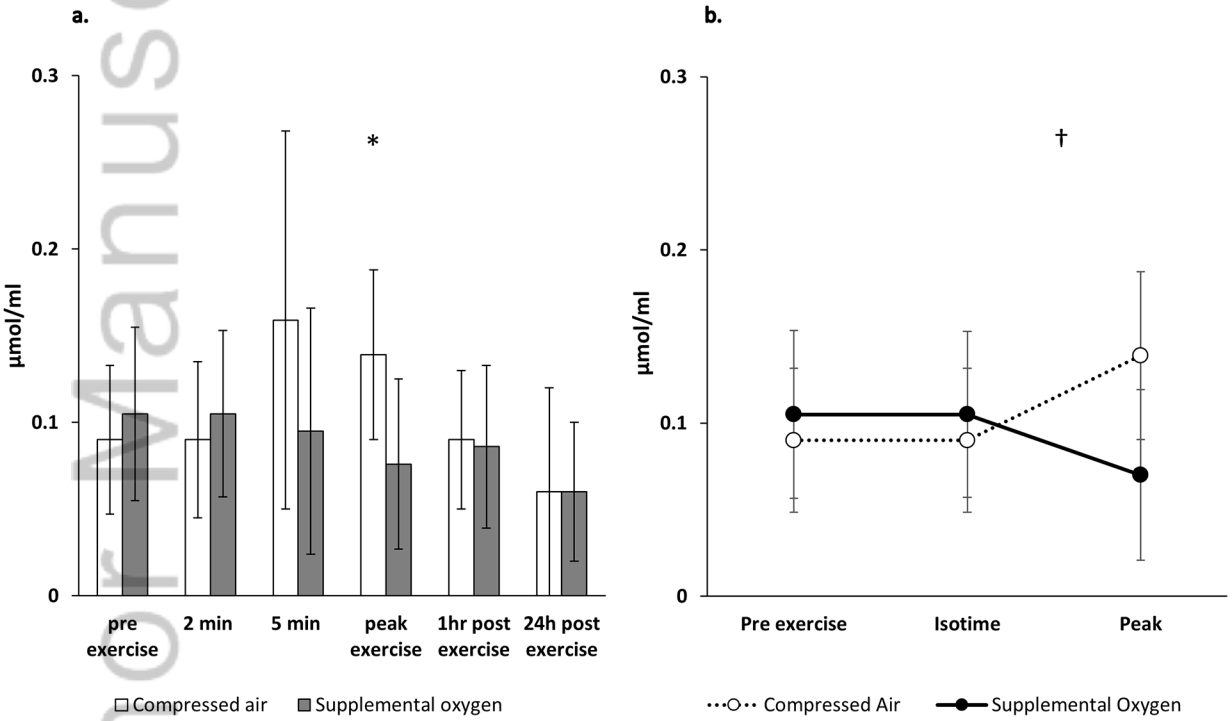

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