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Original article

Maximal oxidative capacity during exercise is associated with muscle power output in patients with long coronavirus disease 2019 (COVID-19) syndrome. A moderation analysis

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SUMMARY

Background & aims: Long COVID syndrome (LCS) involves persistent symptoms experienced by many patients after recovering from coronavirus disease 2019 (COVID-19). We aimed to assess skeletal muscle energy metabolism, which is closely related to substrate oxidation rates during exercise, in patients with LCS compared with healthy controls. We also examined whether muscle power output mediates the relationship between COVID-19 and skeletal muscle energy metabolism.

Methods: In this cross-sectional study, we enrolled 71 patients with LCS and 63 healthy controls. We assessed clinical characteristics such as body composition, physical activity, and muscle strength. We used cardiopulmonary exercise testing to evaluate substrate oxidation rates during graded exercise. We performed statistical analyses to compare group characteristics and peak fat oxidation differences based on power output.

Results: The two-way analysis of covariance (ANCOVA) results, adjusted for covariates, showed that the patients with LCS had lower absolute maximal fatty acid oxidation (MFO), relative MFO/fat free mass (FFM), absolute carbohydrates oxidation (CHox), relative CHox/FFM, and oxygen uptake ($\dot{V}O_2$) at maximum fat oxidation (g min^{-1}) than the healthy controls ($P < 0.05$). Moderation analysis indicated that muscle power output significantly influenced the relationship between LCS and reduced peak fat oxidation (interaction $\beta = -0.105$ [95% confidence interval −0.174; −0.036]; $P = 0.026$). Therefore, when muscle power output was below 388 W, the effect of the LCS on MFO was significant (62% in our study sample $P = 0.010$). These findings suggest compromised mitochondrial bioenergetics and muscle function, represented by lower peak fat oxidation rates, in the patients with LCS compared with the healthy controls.

Conclusion: The patients with LCS had lower peak fat oxidation during exercise compared with the healthy controls, potentially indicating impairment in skeletal muscle function. The relationship between peak fat oxidation and LCS appears to be mediated predominantly by muscle power output. Additional research should continue investigating LCS pathogenesis and the functional role of mitochondria.

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1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic has significantly impacted millions of lives globally. Per the World Health Organization (WHO) [1], there have been over 765 million cases and 6 million deaths. Many COVID-19 survivors with long COVID experience persistent cardiopulmonary symptoms and exercise intolerance after infection [2]. Long COVID syndrome (LCS), defined as post-COVID symptoms lasting ≥12 weeks, threatens individuals, populations, and economies, with ~144 million cases estimated globally [3]. Both residual organ damage from acute infection and prolonged inflammation may play a role [4]. Systemic inflammation, physical inactivity, and poor nutrition can lead to muscle dysfunction in patients with LCS [5]. Other factors—such as persistent mitochondrial dysfunction in the heart, kidneys, liver, and lymph nodes—may also have a negative impact [6].

The cardiopulmonary exercise test (CPET) is crucial for assessing the integrated pulmonary, cardiovascular, and skeletal muscle responses during exercise [7]. Peak oxygen uptake (VO_2peak) is significantly lower in patients with LCS compared with healthy controls [8]. In the first months after infection, chronotropic insufficiency and reduced cardiac output can lower VO_2peak through insufficient cardiac output. Peripheral factors like muscle mass, strength, perfusion, mitochondrial function, or arteriovenous oxygen difference may also contribute [9]. These symptoms resemble those in patients with myalgic encephalomyelitis/chronic fatigue syndrome and genetic mitochondrial diseases [10]. Given the close ties between mitochondrial fitness, cardiorespiratory fitness, and physical activity, this assessment is particularly relevant for LCS.

Maximal fatty acid oxidation (MFO) is fundamental to lipid metabolism; it is modulated by exercise, as fatty acids serve as an essential energy source during physical activity [11]. Previous studies have demonstrated that fitter individuals have superior mitochondrial function and a higher rate of MFO during exercise versus less fit people [12]. In particular, muscle power, defined as the force-velocity product of muscle contraction, has been proposed as a determinant of fat oxidation [13,14].

Assessing muscle mass and function can help identify, diagnose, and manage poor muscle health resulting from LCS [15]. The evidence of lower MFO in patients with LCS may explain the inefficient mitochondrial exercise sustainability, while premature lactate accumulation suggests either increased glycolysis or an inability to utilize lactate as an alternative energy source [16]. Although cardiorespiratory fitness is closely associated with the skeletal muscle lipid oxidation capacity [15,16], it remains unknown whether lipid oxidation is reduced in patients with LCS compared with healthy controls. It also remains unclear whether these factors contribute to exercise intolerance, a major manifestation of LCS.

Recent studies suggest that patients with LCS may experience significant mitochondrial dysfunction and impaired fatty acid metabolism [13,17]. Guntur et al. [16] provided preliminary evidence of a metabolomic signature in acute COVID-19, where severity was associated with dyslipidemia and mitochondrial dysfunction markers. These changes suggest impaired pyruvate/lactate metabolism, potentially occurring during mitochondrial catabolism. Guarnieri et al. [6] recently showed that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes COVID-19, can block nuclear- and mitochondrial-encoded mitochondrial genes, inhibiting host mitochondrial function in the heart, liver, kidneys, and lymph nodes. SARS-CoV-2 viral proteins likely inhibit oxidative phosphorylation (OXPHOS) and stimulate glycolysis. Moreover, based on structural and functional analyses of skeletal muscle, researchers have reported bioenergetic changes in muscle biopsies for biomarkers of mitochondrial function, content, and biogenesis in patients with post-acute sequelae of COVID-19 [17]. Other histopathological changes in muscle biopsies, including inflammatory infiltrates and signs of cell damage [18], and impairments in O_2 utilization by skeletal muscle and mitochondrial function [19] have been observed in patients with LCS.

In this context, the objective of our study was two-fold. First, we aimed to assess whether patients with LCS exhibit compromised skeletal muscle oxidation capacity, as evaluated by MFO during graded exercise, compared with healthy controls. Second, we aimed to investigate whether the potential relationship between COVID-19 and skeletal muscle oxidation capacity is mediated by muscle strength. Our hypothesis is that individuals who have experienced COVID-19 demonstrate an impaired capacity for substrate oxidation; moreover, muscle power output in lower body strength plays a significant role in this relationship.

2. Methods

2.1. Participants and study design

This was a secondary, cross-sectional study that used baseline data from "The EXER-COVID Crossover Study" (NCT04797871) [20]. This study comprised patients who had complete data on (i) CPET and (ii) the components included in the MFO during graded exercise. The sample for this exploratory observational study included 71 patients (62% women); the characteristics of the study cohort have been described in a previous publication [21]. The recruitment was carried out by the internal medicine department of the University Hospital of Navarra (HUN), ensuring that each patient met the inclusion criteria and after signing the informed consent. The lead investigator of the project had access to the patients' medical records for confirmation by reverse transcription polymerase chain reaction testing and the control for the vaccination status. The inclusion criteria were diagnosis of LCS according to WHO, mild or moderate symptoms, no hospitalization, no heart disease, and no lung pathology [1]. Patients who presented with atrial fibrillation or acute myocarditis; required home oxygen therapy; were undergoing treatment with the interleukin 6 (IL-6) receptor antagonist tocilizumab; and/or had heart valve disease, chronic obstructive pulmonary disease, muscle myopathy, any form of cancer, or cognitive impairment at the time of evaluation were excluded.

The healthy controls (n = 63, 54% women) were derived from a published cohort of individuals who underwent physical fitness testing in Pamplona, Spain, at the same altitude as our study cohort, prior to the first confirmed case of COVID-19 in Pamplona (on March 3, 2020). The control participants had no flu-like symptoms. The characteristics of both cohorts have been described previously [22].

2.2. Data collection

The participants were instructed to adhere to their normal diet (approximately 55%–60% carbohydrates, 30% fat, and 10–15% protein) and to abstain from physical exercise the day before laboratory tests. The participants were asked to consume a normal meal 3 h prior to the laboratory sessions, and to refrain from alcohol, tobacco, or caffeine consumption 3 h prior to exercise. The health history, including physical activity levels and current medication, was recorded for each participant. Height and weight were measured using a stadiometer and scale (Seca model 799, Electronic Column Scale, Hamburg, Germany) with the participants wearing light clothing and no shoes. Body mass index (BMI) was calculated as the weight (in kilograms) divided by height (in meters) squared. Whole-body fat mass, visceral adipose tissue, and fat

free mass (FFM) were determined with dual-energy X-ray absorptiometry (Discovery Horizon DXA system; Hologic Inc, Marlborough, MA, USA). The sum of the upper and lower limb lean mass, known as appendicular lean mass (ALM), was indexed to height using the formula ALMI = (ALM/height^2).

Physical activity levels were assessed using the self-administered International Physical Activity Questionnaire (IPAQ) [23]. This instrument measures the amount of physical activity performed over the past 7-day period. It includes questions about the duration and frequency of engagement in vigorous physical activities, moderate physical activities, and walking. The responses were converted to metabolic equivalent task minutes per week (MET-min week^{-1}) following the IPAQ scoring protocol. The weighted MET minutes per week were calculated by multiplying the duration (in minutes), frequency (in days), and MET intensity, and then summing across the three domains (vigorous activities, moderate activities, and walking) to obtain a weighted estimate of total physical activity per week (MET-min week^{-1}).

Maximal isometric handgrip strength was measured using a TKK 5101 digital dynamometer (Takei Scientific Instruments Co., Ltd., Tokyo, Japan). The maximal attempt per hand were recorded in an upright position, and the medium reading was used for further analysis [24]. For the evaluation of lower body strength, leg extension measurements were taken using a Smart Strength machine (eGym® GmbH, München, Germany). Dynamic maximal muscle strength was assessed following the recommendations of Haff & Triplett [25]. To warm up, the participant performed joint mobility exercises and 10 repetitions of familiarization with the machine and then rested for 1 min. Next, each participant had up to five attempts to achieve their one-repetition maximum (1RM) load. The 1RM load was considered the maximum weight the participant could lift with proper form before reaching failure. A 3-min rest period was allotted between 1RM attempts. Strong verbal encouragement was given throughout the 1RM test. Muscle power output for lower body strength was determined by using an adapted form of the protocol reported by Sáez de Asteasu et al. [26]. Briefly, the subjects were instructed to perform 10 repetitions with maximum velocity on each set at 50% of 1RM for the leg extension exercise. The mean and peak power output of each set of 10 repetitions was used for further analysis.

Oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$), the respiratory exchange ratio (RER), and ventilation ($\dot{V}_E$) were measured continuously using a breath-by-breath online system (Quark metabolic system, Cosmed S.R.L., Rome, Italy). The protocol was adapted according to the training state of the subjects. The participants started pedaling at 25 W. The pedaling cadence for each subject was set at 50–60 revolutions per minute (rpm). The participants cycled during a 3-min warm-up period at the 25-W workload, after which the workload was increased by 25 W/min every 3 min in an incremented ramp test until exhaustion or the predetermined exclusion criteria were met on the Lode Excalibur Sport electromagnetically braked cycle (Lode BV, Groningen, the Netherlands). The criteria to achieve $\dot{V}O_2$peak and to consider a proper test were as follows: no increase in $\dot{V}O_2$ (plateau) for a given increase in workload, maximum heart rate (HR max) with respect to age (220 beats per minutes (bpm) minus age), and a RER greater than 1.15. $\dot{V}$ O_2peak was determined if the three above-mentioned criteria were met. Calculations were performed for the average oxygen uptake over the last 60 s of the test. The flow sensor and gas analyzers were calibrated using gases of known concentration (16% O_2, 5% CO_2) and volume (3 L syringe) prior to each test. The same gas analyzer instrument was used for all tests. The laboratory room temperature was standardized at 24 °C. Substrate oxidation and energy expenditure (EE) during the exercise test was calculated according to the following stoichiometric correction equations [27], assuming negligible contribution of protein oxidation, for all calculations:

$$\text{Fat oxidation rate}\left(\text{g min}^{-1}\right) = 1.67 \times \dot{V}O_2\left(\text{L min}^{-1}\right) - 1.67 \times \dot{V}CO_2\left(\text{L min}^{-1}\right)$$

$$\text{Carbohydrate oxidation rate}\left(\text{g min}^{-1}\right) = 4.55 \times \dot{V}CO_2\left(\text{L min}^{-1}\right) - 3.21\,\dot{V}O_2\left(\text{L min}^{-1}\right)$$

$$\text{Energy expenditure}\left(\text{kcal min}^{-1}\right) = 0.550 \times \dot{V}CO_2\left(\text{L min}^{-1}\right) + 4.47 \times \dot{V}O_2\left(\text{L min}^{-1}\right)$$

If the RER values were above 1, then fat oxidation was accepted as zero and the data were calculated as such [28,29]. For each individual, the mathematical model (sine model, SIN) [30], which includes three independent variables that represent the main quantitative characteristics of the curve (dilatation, symmetry, and translation), was constructed for the MFO rate (expressed as a percentage) and normalized for fat free mass (FFM) (expressed as mg kg FFM^{-1} min^{-1}) versus exercise intensity (expressed as a percentage of the predictive VO_2peak). The MFO rate was determined at intervals of 5% between 20% and 85%. The MFO zone was determined by calculating the range of exercise intensities with fat oxidation rates within 10% MFO as described previously [31]. % HR_{max} and RER (RER_{Fatmax}) at MFO and carbohydrates (CHO) rates (g min^{-1}) and normalized for FFM (mg kg FFM^{-1} min^{-1}) versus exercise intensity (expressed as a percentage of the predictive VO_2peak) were calculated using a third polynomial curve. Other endpoints were the ventilatory threshold (VT_1 and VT_2) determined by the V-slope method, and the minute ventilation to carbon dioxide production ($\dot{V}E/\dot{V}CO_2$) slope was calculated using the entire exercise data. In addition, $\dot{V}O_{2peak}$, $\dot{V}O_{2peak}$ relative to body mass, HR max, W, $\dot{V}$, oxygen pulse, and chronotropic index, was computed from the CPET, using previously described methods [21,32]. HR was monitored continuously throughout the duration of the tests (Polar RS800cx monitor, Polar, Finland).

2.3. Statistical analysis

Two-way analysis of covariance (ANCOVA) followed by contrasts was performed to compare substrate oxidation and the RER at each exercise intensity [expressed as a percentage of the predictive VO_2peak: exercise intensity [20%–85% × group [patients with LCS vs. healthy controls]). Age, sex, body mass, FFM and physical activity levels were entered as covariates in the analysis. The significance was determined with a t-test, with Bonferroni adjustment where appropriate, when ANCOVA revealed significant interaction effects. The SIN model included variables (dilatation, symmetry, and translation) of the whole-MFO kinetics obtained during CPET. To determine differences in participants characteristics between the LCS and control groups, Student's t-test, or the Mann–Whitney test when assumptions required for the t-test were not met, was performed. Homoscedasticity was analyzed using the Fisher–Snedecor test. Statistical significance was set at $P \leq 0.05$.

As illustrated in Fig. 1, the moderating effect of muscle power output in lower body strength (moderator, M) on the relationship between groups (healthy controls vs. patients with LCS, independent variable, X) and MFO (dependent variable, Y) was examined

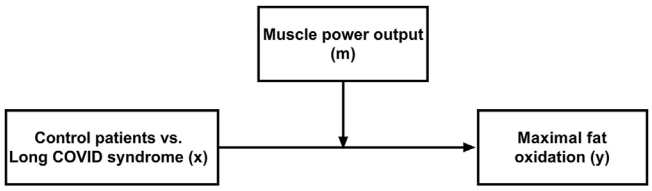

Fig. 1. A conceptual diagram for moderation models for the relationship between groups (control patients vs. long COVID syndrome, path **x**), and maximal fat oxidation (path **y**) moderated by muscle power output in lower body strength (path **m**). Beta (β) expressed as unstandardized regression coefficients and 95% confidence interval.

with Andrew Hayes' PROCESS macro [33]. This relationship uses ordinary least squares regression analysis when predicting continuous variables (muscle power output and MFO in the study). A simple slope plot was used to visualize the effect of the moderator. Johnson–Neyman plots visualize interactions between a continuous exposure and modifier without using categorical modifier values by graphing the modifier values (x-axis) against the beta for the exposure and outcome association (y-axis). The analysis was adjusted for age, sex, body mass, FFM, and physical activity levels, which have previously been identified as determinants of MFO [34]. The statistical significance of the model was tested with bias-corrected bootstrapping ($n = 5000$) and 95% confidence intervals (CIs). For all other tests, statistical significance was set at $P < 0.05$. SPSS Statistics version 27.0 for Windows (IBM Corp., Armonk, NY, USA) was used for statistical analysis.

3. Results

3.1. Patient characteristics

The clinical characteristics of the study sample are shown in Table 1. The cohort consisted of 134 individuals: 71 patients with LCS (44 females, 62%) and 63 healthy controls (34 females, 54%). We assessed the participants at an average of 123 (standard deviation [SD] 38) days after they had experienced their first COVID-19-related symptom. The vaccination rate for two doses was 57% at the time of enrollment. The patients with LCS exhibited a significantly higher body mass, body fat mass, and visceral adipose tissue compared with the healthy controls ($P < 0.05$). Conversely, age, FFM (%), muscle strength parameters (power output and 1RM), $\dot{V}O_2$ and HR at the first VT_1, as well $\dot{V}O_2$peak and the chronotropic index at maximum load were significantly lower in the patients with LCS ($P < 0.05$). Furthermore, the prevalence of muscle mass loss was

Table 1
Clinical characteristics of the study patients.

	LCS patients ($n = 71$)	Control patients ($n = 63$)	*P*-value
Sex (n, women/men)	44/27	34/29	0.348
Age (y)	47.47 (9.05)	52.35 (12.1)	0.008
Height (m)	1.67 (0.08)	1.66 (0.10)	0.230
Body mass (kg)	76.84 (17.05)	70.45 (16.06)	0.031
Body mass index (kg/m^2)	27.61 (26.22–29.00)	26.14 (25.21–27.08)	0.091
Nutritional status by BMI, (%)			
Normal weight	32	40	0.051
Overweight	38	48	
Obesity	30	12	
Body fat mass (%)	38.79 (7.82)	34.79 (8.37)	0.006
Visceral adiposity tissue (cm^3)	1243 (998.27)	415.66 (616.58)	<0.001
Fat free mass (%)	59.01 (7.25)	62.87 (7.86)	0.005
Appendicular lean mass index (kg/m^2)	7.28 (1.38)	7.46 (1.34)	0.466
Low muscle mass, (%)[a]			
<6.0 kg/m^2 for women	25	15	0.148
<7.0 kg/m^2 for men			
Overall physical activity (MET-min/wk)[b]	1026.19 (1239.10)	1403.63 (1182.83)	0.075
Physical activity levels, (%)[b]			
Light-intensity activity	53	43	0.072
Moderate-intensity activity	43	41	
Vigorous-intensity activity	4	16	
Grip strength (kg)	28.20 (9.64)	31.67 (9.08)	0.084
Low muscle strength, (%)[c]			
<18 kg for women	17	2	0.003
<28 kg for men			
Leg extension 1RM (kg)	74.96 (31.463)	91.79 (29.362)	0.002
Mean muscle power output (w)	348.04 (179.76)	416.84 (192.73)	0.034
VT_1 responses			
$\dot{V}O_2$ (mL/kg^{-1} min^{-1})	9.50 (2.18)	10.49 (2.53)	0.017
HR (bpm)	106.93 (15.47)	97.10 (16.18)	0.001
Watts (w)	42.14 (14.46)	44.05 (19.94)	0.526
VT_2 responses			
$\dot{V}O_2$ (mL/kg^{-1} min^{-1})	20.10 (4.55)	22.26 (5.60)	0.016
HR (bpm)	143.90 (21.20)	133.67 (18.74)	0.032
Watts (w)	100.00 (34.16)	118.63 (34.48)	0.150
Peak exercise responses			
$\dot{V}O_2$ (mL/kg^{-1} min^{-1})	21.58 (5.07)	25.01 (6.42)	0.021
$\dot{V}O_2$ peak relative to leg mass (mL/kg^{-1} min^{-1})	108.30 (24.82)	116.98 (25.18)	0.053
HR (bpm)	149.59 (22.47)	154.53 (24.31)	0.240
Watts (w)	126.91 (32.89)	134.52 (38.74)	0.223
Oxygen pulse (mL/beat)	11.32 (3.20)	12.00 (4.35)	0.316
Chronotropic index[d]	86.77 (12.91)	92.14 (13.53)	0.025

Table 1 (*continued*)

	LCS patients ($n = 71$)	Control patients ($n = 63$)	*P*-value
Laboratory measures[e]			
Glucose (mg/dL)	89.76 (14.20)	96.31 (13.99)	0.008
Creatinine (mg/dL)	2.81 (12.44)	0.98 (1.14)	0.247
Aspartate aminotransferase (U/l)	20.88 (8.92)	25.04 (6.30)	0.002
Alanine aminotransferase (U/l)	25.76 (25.69)	26.81 (9.11)	0.758
C-reactive protein (mg/dL)	4.62 (7.61)	1.71 (1.74)	0.003
Fibrinogen (mg/dL)	428.82 (88.97)	429.02 (69.81)	0.988
Vaccination rate at enrolment (%)[e]			
Dose 1	77	–	–
Dose 2	57	–	–
Booster	13	–	–
Do not know, no response	20	–	–
Most common persistent symptoms (%)			
Fatigue	96	–	–
Headaches	83	–	–
Attention problems	81	–	–
Concentration problems	79	–	–
Sleep disorders	76	–	–
Mean post-COVID time, days	123 (38)	–	–

Data are mean (SD) or percentage (%). Two-tailed Student's t tests for two samples of equal variance were performed between control patients and long-COVID-19 syndrome group. The chi-square test was performed for sex-variable groups.

[a] The recent European working group on sarcopenia in older people (EWGSOP) guidelines suggest an appendicular lean mass index <6.0 kg/m^2 in women and appendicular lean mass index < 7.0 kg/m^2 in men as diagnostic cut-off values to define low muscle mass.

[b] The short, self-administered International Physical Activity Questionnaire (IPAQ) was used to assess overall physical activity. The short form records four types of physical activity: vigorous activity such as aerobics; moderate-intensity activity such as leisure cycling; walking, and sitting, in the last seven days. Responses were converted to Metabolic Equivalent Task minutes per week (MET-min/wk) according to the IPAQ scoring protocol: total minutes over last seven days spent on vigorous activity, moderate-intensity activity, and walking (light-intensity activity) were multiplied by 8.0, 4.0, and 3.3, respectively, to create MET scores for each activity level. MET scores across the three sub-components were summed to indicate overall physical activity.

[c] Low muscle strength was defined as grip strength <18 kg in women and <28 kg in men, according to EWGSOP guidelines.

[d] Chronotropic index was calculate as (peak HR−resting HR)/(220−age−resting HR)].

[e] Vaccination rate at enrolment and medical history examination findings were determined by study investigators based on medical record review, and patient interview at first study visit. One-repetition maximum (1RM); w (watts); METs, metabolic equivalents; $\dot{V}O_2$peak, oxygen uptake peak; HR, heart rate; VCO_2, carbon dioxide produced; oxygen pulse (VO_2/HR); VT_1, first ventilatory threshold; VT_2, second ventilatory threshold. The variables VO_2peak (mL/kg^{-1} min^{-1}), oxygen pulse (VO_2/HR), $\dot{V}O_2$ peak relative to leg mass and chronotropic index were determined at maximum load using flow analysis and concentrations of inhaled and exhaled respiratory gases in the mixing chamber (QUARK CPET, Cosmed, Italy).

significantly higher in the patients with LCS compared with the healthy controls ($P = 0.003$).

3.2. Whole body fat and carbohydrate oxidation and exercise data

The two-way ANCOVA results, adjusted for covariates (Table 2), showed that the patients with LCS had lower absolute MFO, relative MFO/FFM, absolute CHox, relative CHox/FFM, and VO_2 at MFO (mL min^{-1}) compared with the healthy controls ($P < 0.05$).

MFO, expressed as g min^{-1}, showed a significant interaction effect ($P < 0.0001$) and was significantly lower in patients with LCS compared with the healthy controls ($P = 0.009$). MFO was ~13 to 20% lower at 30%–40% predicted VO_2peak in patients with LCS (Fig. 2A). Similarly, MFO, expressed as mg kg FFM^{-1} min^{-1}, was lower in the patients with LCS (Fig. 2B, $P < 0.01$); it was ~13 to 24% at 30%–40% predicted VO_2peak versus the healthy controls (Fig. 2B, $P < 0.05$). Whole-body fat oxidation kinetics in the patients with LCS exhibited similar translation, lower dilatation, and left-shift symmetry relative to the healthy controls (Fig. 2C). CHox (g min^{-1}) and CHox/FFM (mg kg FFM^{-1} min^{-1}) showed a significant interaction effect ($P < 0.0001$), except at ~30% predicted VO_2peak ($P < 0.05$), which was similar between the groups (Figure s2D-2E).

3.3. Moderation analysis

Fig. 3 depicts our ordinary least squares regression moderation analysis, which revealed a relationship between the patients with LCS and the healthy controls (path x) on MFO (path y). This path (m), known as a direct effect ($\beta = -0.105$ [95% CI −0.174; −0.036]; $P = 0.026$), was moderated by relative muscle power output (leg extension). Therefore, the effect of patients with LCS on MFO was moderated by muscle power output (interaction $\beta = 0.002$ [95% CI 0.001; 0.004]; $P = 0.014$).

Table 2
Substrate oxidation at maximum fat oxidation and CPET values.

	LCS patients ($n = 71$)	Control patients ($n = 63$)	*P*-value
MFO (g min^{-1})	0.212 (0.011)	0.252 (0.010)	0.009
MFO/FFM (mg kg FFM^{-1} min^{-1})	4.735 (0.25)	5.890 (0.25)	0.002
MFO (% of $\dot{V}O_2$)	28.87 (1.56)	38.16 (1.61)	<0.001
CHox (g min^{-1})	1.178 (0.04)	1.609 (0.04)	<0.001
CHox/FFM (mg kg FFM^{-1} min^{-1})	26.04 (1.00)	36.02 (1.02)	<0.001
EE (kcal min^{-1})	3.70 (0.13)	4.07 (0.13)	0.059
HR (bpm)	106.07 (2.00)	101.60 (2.16)	0.148
VO_2 (mL min^{-1})	756.05 (27.89)	842.09 (28.88)	0.041
Power (W)	35.41 (1.876)	40.56 (1.943)	0.069

Data are mean (SD). Two-tailed Student's t tests for two samples of equal variance were performed between control and long-COVID-19 syndrome groups. MFO, fatty acid oxidation; CHox, carbohydrate oxidation; FFM, fat free mass; EE, energy expenditure; HR, hearth rate; $\dot{V}O_2$peak, oxygen uptake peak.

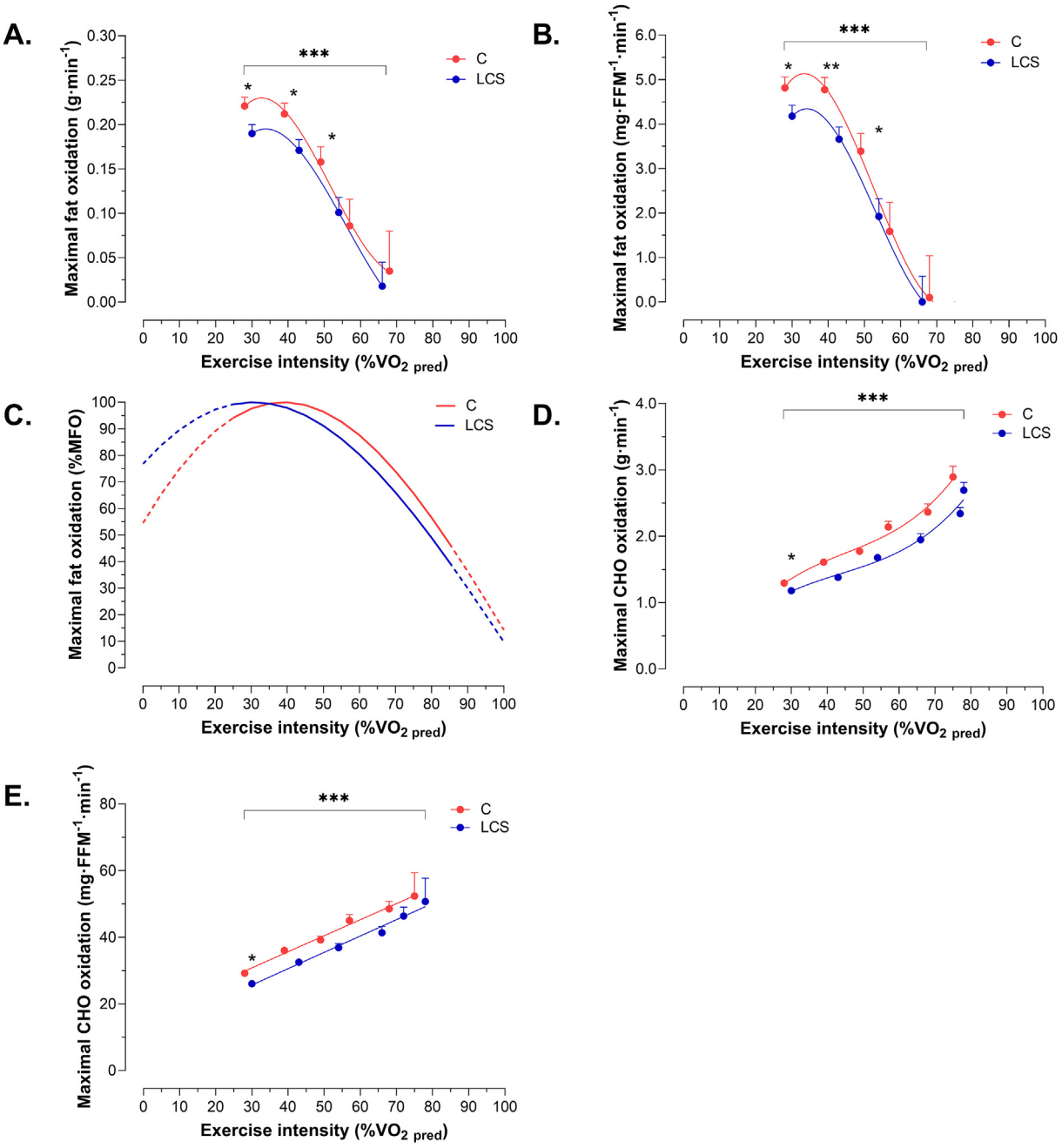

Fig. 2. Mean whole-body fat and carbohydrate oxidation kinetics in absolute [g·min^{-1} (Panel A and D) and relative mg·FFM^{-1} min^{-1} (Panel B and E)]. The percentage of maximal fat oxidation (MFO) are shown in Panel C)], and values determined with the sinusoidal (SIN) model and during the submaximal incremental test in long COVID syndrome (LCS: blue, n = 71) and control (C: red, n = 63) patients. Whole-body fat oxidation kinetics were characterized by similar right-shift symmetry (both = 0.9), significantly lower dilatation (0.4 *vs.* 0.6) and translation (0.4 *vs.* 0.2) in LCS compared with C (Panel C). Values are the means ± SE. All analysis were adjusted for age, sex, body mass, FFM and physical activity levels which have been previously identified as determinants of MFO [34]. Peak oxygen uptake. *P < 0.05 for differences with control; **P < 0.01 for differences with control; ***P < 0.0001 for significant group interaction effect.

To elucidate a possible estimate point from which the moderator value has a moderating effect, we used the Johnson–Neyman statistical approach (Fig. 4). The slope shows the continuum of the moderator (mean muscle power output expressed as m) and the region of significance (the gray line). Therefore, when muscle power output was below 388 W, the effect of LCS on MFO was significant (62% in our study sample $P = 0.010$).

4. Discussion

In this study, we selected patients with LCS to investigate the physiopathology of exercise intolerance. This approach allowed us to simultaneously assess muscular fitness and MFO during exercise as indirect measures of the oxidative capacity. This study provides evidence of metabolic and functional impairments of muscle

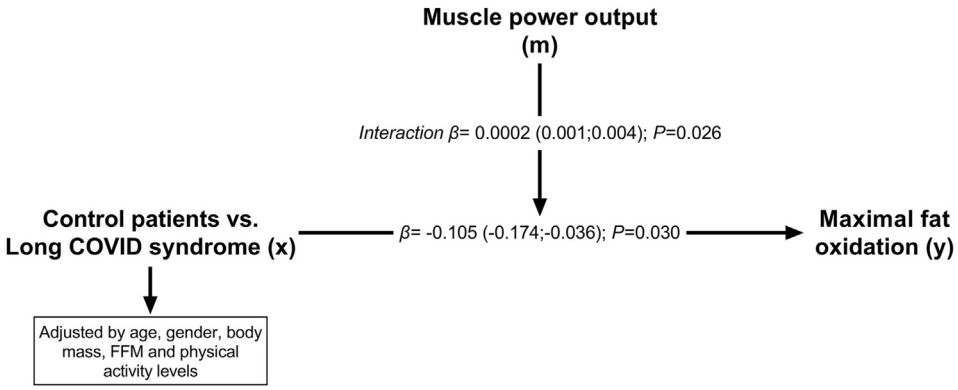

Fig. 3. Moderation analyses for MFO rate (path **y**) moderate the association between groups (control patients vs. long COVID syndrome, path **x**), and muscle power output in lower body strength (path **m**). Beta (β) expressed as unstandardized regression coefficients and 95% confidence interval. Adjusted for age, sex, body mass, FFM and physical activity levels.

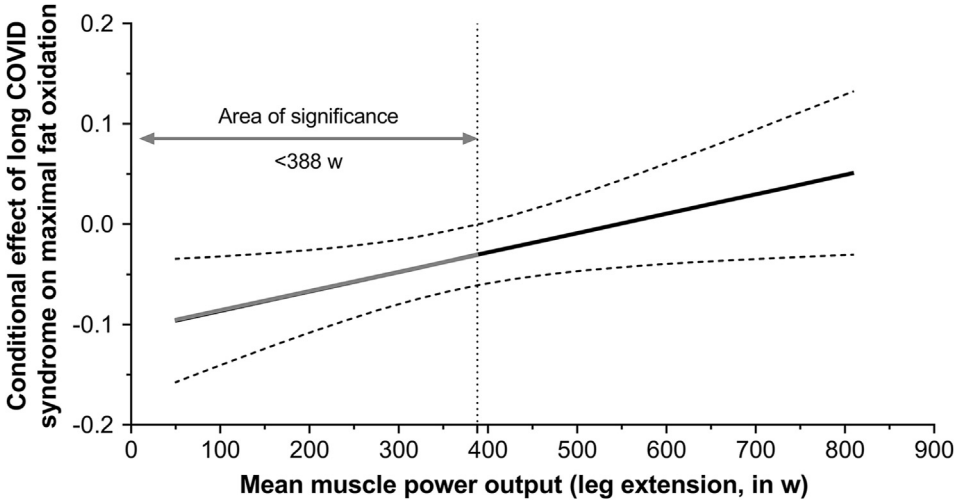

Fig. 4. Regression slope estimate and 95% confidence intervals for the relationship between moderator variable (mean muscle power output) and conditional effect of LCS on MFO rate based on the Johnson–Neyman procedure. Gray line indicates negative region of significance at moderator value (<388 W by muscle power output in leg extension exercise). Regression slope analysis was adjusted for age, sex, body mass, FFM and physical activity levels which have been previously identified as determinants of MFO [34].

oxidative metabolism in patients with long COVID compared with healthy controls. We also report preliminary data suggesting the MFO–LCS association may be influenced by lower body muscle power output. Prior research reported the peripheral limitation to exercise is indicated by impairment in skeletal muscle function underlined by lower in vivo fractional O_2 extraction and impaired muscle oxidative capacity, substantial reductions in biomarkers of mitochondrial function and content, and overall reduced mitochondrial sensitivity to adenosine diphosphate in post-acute sequelae of COVID-19 [17]. Thus, we can speculate that compared with the healthy controls, the patients with LCS presented more damaged mitochondria [35]. Our findings align with the observations made by Boer et al. [15], who reported decreased MFO during exercise in patients with LCS. This supports the notion that low fat oxidation and altered lactate production may contribute to the functional limitations experienced by patients with LCS. Boer et al. [15] proposed that dysfunctional mitochondria, exhibiting a reduced lipid oxidation capacity and a premature shift from fat to carbohydrate oxidation, may underlie these findings. At the molecular level, a reduction in mitochondrial function occurs as a result of the following changes: (i) lower fractional O_2 extraction and impaired muscle oxidative capacity, (ii) substantial reductions in biomarkers of mitochondrial function and content, and (iii) inflammation may also alter mitochondrial dynamics [17]. In turn, these changes result in reduced adenosine-5′-triphosphate (ATP) production while still consuming excess oxygen, leading to increased production of reactive oxygen species, which are produced as a by-product of OXPHOS.

In a related study, Guntur et al. [16] analyzed metabolite levels in venous plasma of patients with LCS, comparing them to individuals who had recovered from COVID-19 but had not developed LCS as well as healthy individuals. They discovered that patients with LCS exhibited significantly higher concentrations of free- and carnitine-conjugated mono-, poly-, and highly unsaturated fatty acids in their plasma, along with markedly lower levels of mono-, di-, and tricarboxylates, polyamines and taurine [16]. These plasma metabolites suggest altered fatty acid metabolism and impaired mitochondria-dependent lipid catabolism in patients with LCS. In addition, Ajaz et al. [36] demonstrated that patients with COVID-19 experienced compromised mitochondrial function and an energy deficit, which was compensated by a metabolic shift to glycolysis. To gain a comprehensive understanding of metabolic disturbances in LCS, future studies should encompass a broader assessment of plasma metabolites. Overall, our study and the findings from Boer et al. [16], Guntur et al. [16], and Ajaz et al. [36] underscore the importance of investigating mitochondrial dysfunction and

metabolic derangement in patients with LCS, offering potential insights into therapeutic strategies for this condition [37]. Similarly, Longobardi et al. [38] found that patients with LCS experience greater oxidative metabolic inertia and higher metabolic demand during submaximal exercise compared with controls matched for sex, age, comorbidities, and physical activity without a prior history of SARS-CoV-2 infection. Furthermore, Trinity et al. [19] described a pre-symptomatic COVID-19 case in which SARS-CoV-2 infection significantly impaired both vascular and mitochondrial function (ADP-stimulated respiration assessed in vitro).

The presence of lower MFO in patients with LCS may explain the mitochondrial inefficiency observed during exercise. This notion is supported by Pleguezuelos et al. [39], who identified similar mechanical inefficiencies in patients with COVID-19, comparable to those observed in individuals with chronic obstructive pulmonary disease and ischemic heart disease. The authors proposed that these inefficiencies, linked to reduced mitochondrial efficiency, could play a crucial role in identifying decreased delta efficiency (defined as the relationship between the change in external work and the change in total energy expenditure) in patients with COVID-19. Another alternative explanation for the occurrence of muscle bioenergetic dysfunction and lower values in muscular fitness in patients with LCS could be persistent, low-grade inflammation in skeletal muscle [4,40]. In a recent study, the authors reported sustained inflammation and activation of the immune system several months (8 months) after the initial mild-to-moderate infection [40]. Additionally, we cannot entirely rule out that the connection between LCS and reduced muscle oxidative capacity may be influenced by factors such as inactivity and/or reduced muscle mass, both of which are known determinants of MFO.

We conducted a moderation analysis to explore the potential involvement of muscle power output in lower body strength as a mediator in the relationship between mitochondrial dysfunction (assessed through MFO) and LCS. Our findings suggest that muscle power output in lower body strength, particularly when below 388 W during leg extension exercises, may predominantly mediate this connection. It is worth noting that catabolic molecules are often released in individuals with basal sarcopenia and obesity, conditions commonly seen in patients with LCS, which can be attributed to factors such as inflammation and malnutrition. These factors may contribute to lower body strength in patients with COVID-19. Furthermore, it is important to consider that cytokine storms induced by SARS-CoV-2 have also been associated with muscular and/or peripheral abnormalities in oxygen extraction [17,41]. Using invasive CPET, Singh et al. [10] found reduced peripheral oxygen extraction, and other researchers [15,42] reported alterations in metabolism and lactate production. Consistently, in patients with LCS, abnormally high blood lactate levels have been found after even mild exertion, suggesting metabolic dysfunction and muscle acidosis [16,37]. Based on these findings, Van der Togt and Rossman [43] hypothesize that an inflammatory acid-base disruption underpins post-acute sequelae of COVID-19 and that viral proteins, both acutely and persistently expressed, cause disease symptomology by impairing microvascular circulation, resulting in hypoxia. This condition, coupled with virally induced metabolic reprogramming, increases cellular anaerobic respiration. Because patients with LCS have higher fasting levels of lactate blood, we hypothesize that LCS represents a polarization of energy toward glycolytic metabolism. However, we did not measure blood lactate levels during and after exercise, so we could not test this hypothesis. In addition, it is possible that LCS is not the only pathogen-induced inflammatory acid-base disorder, as several other pathogens cause persistent disease. Impairment in O_2 muscular peripheral extraction cannot be excluded [44]. Overall, the hypothetical muscle function impairment in patients with LCS may be related to documented endurance impairment [45,46]. Taken together, our findings suggest that altered mitochondrial function might be a phenomenon that occurs early after infection and lasts long after recovery.

The authors of previous studies have concluded that high mitochondrial—and cardiorespiratory—fitness may protect against viral infections, including by SARS-CoV-2 [47]. Exercise training can enhance cardiorespiratory fitness and mitochondrial function, as shown in other populations [48]. This therapeutic potential underlies exercise interventions for LCS. Strategies targeting mitochondrial function, muscle health, and adiposity should be integrate into LCS treatment to improve mitochondrial fitness, metabolic flexibility, and immune function. However, controversy remains regarding exercise interventions for patients with post-exertional malaise, which must be considered.

The present study has certain limitations that warrant consideration. The sample size, while larger than a previous study [15], was still limited. Larger cohorts are needed to further assess mitochondrial dysfunction in patients with COVID-19. In addition, the results may not be equally generalizable to other patients with LCS who have characteristics that are different from the current sample. Moreover, the cross-sectional design confined us to one time point; hence, we could not estimate changes in MFO over time. Physical activity levels were self-reported; objective daily activity measures like actimetry would be useful. Moreover, the method for assessing MFO provides an indirect measure of oxidative capacity. Likewise, the lack of blood lactate measurements limits interpretation of the fat oxidation rate data [12]. Finally, although the control group was well-characterized, the use of a control group analyzed in a different setting is a potential limitation of our study [49].

Notwithstanding these limitations, our study provides a plausible hypothesis that impaired skeletal muscle function and alterations in mitochondrial function and mitochondrial dynamics can be a major determinant of limited muscle and cardiorespiratory capacity in patients with LCS. These novel findings contribute to our understanding of the pathogenesis of LCS and offer valuable guidance to sports medicine practitioners and clinical exercise physiologists regarding exercise prescription for managing this condition.

Further investigation is warranted to comprehensively explore dysregulated lipid oxidation and to deepen our knowledge of the pathophysiology of LCS. Additionally, it would be intriguing to design a rehabilitation program to assess the progression of mitochondrial dysfunction in patients with LCS.

5. Conclusions

In summary, we have provided a preliminary report of lower MFO during exercise in patients with LCS compared with healthy controls, potentially indicating mitochondrial dysfunction. Furthermore, the relationship between MFO and LCS appears to be predominantly mediated by muscle power output in lower body strength. To advance the current understanding and facilitate therapeutic interventions aimed at improving the functional status of patients with LCS, further research investigating the mechanisms of mitochondrial dynamics and muscle function in LCS is warranted.

Author contributions

JO, MI and RRV conceived and designed study; RRV and MCR performed statistical analysis; all authors interpreted results of analysis; SOO, JO and GLG prepared figures; MCR, AEL, NGA, YGA,

MI, and R.R.V. drafted manuscript; all authors edited and revised manuscript; all authors approved final version of manuscript.

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Ethics approval and consent to participate

The study was conducted in accordance with the principles of the Declaration of Helsinki and received approval from the Ethics Committee on Human Research (CEIH, Procotol No. PI_2020/140) of the Hospital Universitario de Navarra (HUN) (Pamplona, Spain). Written consent was obtained from all participants, and they were provided with information regarding Spain's data protection laws.

Availability of data and materials

The datasets used in this study are available from the corresponding author on reasonable request.

Declaration of competing interest

The authors declare that they have no competing interests.

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References

[1] The World Health Organization (WHO). Coronavirus disease (COVID-19) pandemic. 2022. Available from: https://www.who.int/europe/emergencies/situations/covid-19 [Accessed 2022 Sep 23].
[2] Singh I, Joseph P, Heerdt PM, Cullinan M, Lutchmansingh DD, Gulati M, et al. Persistent exertional intolerance after COVID-19: Insights from invasive cardiopulmonary exercise testing. Chest 2022;161(1):54–63.
[3] Astin R, Banerjee A, Baker MR, Dani M, Ford E, Hull JH, et al. Long COVID: mechanisms, risk factors and recovery. Exp Physiol 2023;108(1):12–27.
[4] Castanares-Zapatero D, Chalon P, Kohn L, Dauvrin M, Detollenaere J, Maertens de Noordhout C, et al. Pathophysiology and mechanism of long COVID: a comprehensive review. Ann Med 2022;54:1473.
[5] Kuchler T, Günthner R, Ribeiro A, Hausinger R, Streese L, Wöhnl A, et al. Persistent endothelial dysfunction in post-COVID-19 syndrome and its associations with symptom severity and chronic inflammation. Angiogenesis 2023;26(4):547–63.
[6] Guarnieri JW, Dybas JM, Fazelinia H, Kim MS, Frere J, Zhang Y, et al. Core mitochondrial genes are down-regulated during SARS-CoV-2 infection of rodent and human hosts. Sci Transl Med 2023;15(708):eabq1533.
[7] Guazzi M, Bandera F, Ozemek C, Systrom D, Arena R. Cardiopulmonary exercise testing: what is its value? J Am Coll Cardiol 2017;70:1618–36.
[8] Schwendinger F, Knaier R, Radtke T, Schmidt-Trucksäss A. Low cardiorespiratory fitness post-COVID-19: a narrative review. Sports Med Auckl NZ 2023;53:51–74.
[9] Chen T-H, Chang C-J, Hung P-H. Possible pathogenesis and prevention of long COVID: SARS-CoV-2-Induced mitochondrial disorder. Int J Mol Sci 2023;24:8034.
[10] Singh I, Joseph P, Heerdt PM, Cullinan M, Lutchmansingh DD, Gulati M, et al. Persistent exertional intolerance after COVID-19: insights from invasive cardiopulmonary exercise testing. Chest 2022;161:54–63.
[11] Gemmink A, Schrauwen P, Hesselink MKC. Exercising your fat (metabolism) into shape: a muscle-centred view. Diabetologia 2020;63:1453–63.
[12] San-Millán I, Brooks GA. Assessment of metabolic flexibility by means of measuring blood lactate, fat, and carbohydrate oxidation responses to exercise in professional endurance athletes and less-fit individuals. Sports Med 2018;48:467–79.
[13] Maunder E, Plews DJ, Kilding AE. Contextualising maximal fat oxidation during exercise: determinants and normative values. Front Physiol 2018;9.
[14] Purdom T, Kravitz L, Dokladny K, Mermier C. Understanding the factors that effect maximal fat oxidation. J Int Soc Sports Nutr 2018;15:3.
[15] de Boer E, Petrache I, Goldstein NM, Olin JT, Keith RC, Modena B, et al. Decreased fatty acid oxidation and altered lactate production during exercise in patients with post-acute COVID-19 syndrome. Am J Respir Crit Care Med 2022;205:126–9.
[16] Guntur VP, Nemkov T, de Boer E, Mohning MP, Baraghoshi D, Cendali FI, et al. Signatures of mitochondrial dysfunction and impaired fatty acid metabolism in plasma of patients with post-acute sequelae of COVID-19 (PASC). Metabolites 2022:12.
[17] Colosio M, Brocca L, Gatti M, Neri M, Crea E, Cadile F, et al. Structural and functional impairments of skeletal muscle in patients with post-acute sequelae of SARS-CoV-2 infection. J Appl Physiol 1985:2023. https://doi.org/10.1152/japplphysiol.00158.2023.
[18] Hejbøl EK, Harbo T, Agergaard J, Madsen LB, Pedersen TH, Østergaard LJ, et al. Myopathy as a cause of fatigue in long-term post-COVID-19 symptoms: evidence of skeletal muscle histopathology. Eur J Neurol 2022;29(9):2832–41.
[19] Trinity JD, Craig JC, Fermoyle CC, McKenzie AI, Lewis MT, Park SH, et al. Impact of presymptomatic COVID-19 on vascular and skeletal muscle function: a case study. J Appl Physiol (1985) 2021;130(6):1961–70.
[20] Ramírez-Vélez R, Oteiza J, de Tejerina JMCF, García-Alonso N, Legarra-Gorgoñon G, Oscoz-Ochandorena S, et al. Resistance training and clinical status in patients with postdischarge symptoms after COVID-19: protocol for a randomized controlled crossover trial "The EXER-COVID Crossover Study. Trials 2022;23.
[21] Ramírez-Vélez R, García-Alonso N, Legarra-Gorgoñón G, Oscoz-Ochandorena S, Oteiza J, Izquierdo M. Ventilatory efficiency in response to maximal exercise in persistent COVID-19 syndrome patients: a cross-sectional study. Rev Española Cardiol 2023;76(3):206–9.
[22] Ramírez-Vélez R, García-Alonso N, Oteiza J, Izquierdo M. Cardiopulmonary exercise test in patients with post SARS-CoV-2 sequelae: need to create a multicenter working group. Response. Rev Esp Cardiol. 2023;76(6):489–90.
[23] Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 2003;35:1381–95.
[24] Jaric S. Muscle strength testing: use of normalisation for body size. Sports Med 2002;32:615–31.
[25] Haff GG, Triplett NT. Essentials of strength training and conditioning. 4th ed. Champaign, IL, USA: Human Kinetics; 2016.
[26] Sáez de Asteasu ML, Martínez-Velilla N, Zambom-Ferraresi F, Ramírez-Vélez R, García-Hermoso A, Cadore EL, et al. Changes in muscle power after usual care or early structured exercise intervention in acutely hospitalized older adults. J Cachexia Sarcopenia Muscle 2020;11:997–1006.
[27] Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol 1983;55:628–34.
[28] Jeukendrup AE, Wallis GA. Measurement of substrate oxidation during exercise by means of gas exchange measurements. Int J Sports Med 2005;26(Suppl 1):S28–37.
[29] Macfarlane DJ. Open-circuit respirometry: a historical review of portable gas analysis systems. Eur J Appl Physiol 2017;117:2369–86.
[30] Chenevière X, Malatesta D, Peters EM, Borrani F. A mathematical model to describe fat oxidation kinetics during graded exercise. Med Sci Sports Exerc 2009;41:1615–25.
[31] Lanzi S, Codecasa F, Cornacchia M, Maestrini S, Salvadori A, Brunani A, et al. Fat oxidation, hormonal and plasma metabolite kinetics during a submaximal incremental test in lean and obese adults. PLoS One 2014;9:e88707.
[32] Hetlelid KJ, Plews DJ, Herold E, Laursen PB, Seiler S. Rethinking the role of fat oxidation: substrate utilisation during high-intensity interval training in well-trained and recreationally trained runners. BMJ Open Sport Exerc Med 2015;1:e000047.
[33] Hayes AF. Partial, conditional, and moderated mediation: quantification, inference, and interpretation 2017;85:4–40.
[34] Venables MC, Achten J, Jeukendrup AE. Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study. J Appl Physiol Bethesda Md 1985 2005;98:160. –107.
[35] Raman B, Bluemke DA, Lüscher TF, Neubauer S. Long COVID: post-acute sequelae of COVID-19 with a cardiovascular focus. Eur Heart J 2022;43:1157–72.
[36] Ajaz S, McPhail MJ, Singh KK, Mujib S, Trovato FM, Napoli S, et al. Mitochondrial metabolic manipulation by SARS-CoV-2 in peripheral blood mononuclear cells of patients with COVID-19. Am J Physiol Cell Physiol 2021;320:C57–65.
[37] Skjørten I, Ankerstjerne OAW, Trebinjac D, Brønstad E, Rasch-Halvorsen Ø, Einvik G, et al. Cardiopulmonary exercise capacity and limitations 3 months after COVID-19 hospitalisation. Eur Respir J 2021;58. https://doi.org/10.1183/13993003.00996-2021.
[38] Longobardi I, do Prado DML, Goessler KF, Meletti MM, de Oliveira Júnior GN, de Andrade DCO, et al. Oxygen uptake kinetics and chronotropic responses to exercise are impaired in survivors of severe COVID-19. Am J Physiol Heart Circ Physiol 2022;323:H569–76.

[39] Pleguezuelos E, Del Carmen A, Llorensi G, Carcole J, Casarramona P, Moreno E, et al. Severe loss of mechanical efficiency in COVID-19 patients. J Cachexia Sarcopenia Muscle 2021;12:1056–63.
[40] Phetsouphanh C, Darley DR, Wilson DB, Howe A, Munier CML, Patel SK, et al. Immunological dysfunction persists for 8 months following initial mild-to-moderate SARS-CoV-2 infection. Nat Immunol 2022;23:210–6.
[41] Durstenfeld MS, Sun K, Tahir P, Peluso MJ, Deeks SG, Aras MA, et al. Use of cardiopulmonary exercise testing to evaluate long COVID-19 symptoms in adults: a systematic review and meta-analysis. JAMA Netw Open 2022;5:E2236057.
[42] Mohr A, Dannerbeck L, Lange TJ, Pfeifer M, Blaas S, Salzberger B, et al. Cardiopulmonary exercise pattern in patients with persistent dyspnoea after recovery from COVID-19. Multidiscip Respir Med 2021:16.
[43] van der Togt V, Rossman JS. Hypothesis: inflammatory acid-base disruption underpins Long Covid. Front Immunol 2023;14:1150105.
[44] Long Q, Li J, Hu X, Bai Y, Zheng Y, Gao Z. Follow-ups on persistent symptoms and pulmonary function among post-acute COVID-19 patients: a systematic review and meta-analysis. Front Med 2021;8.
[45] Ezzatvar Y, Ramírez-Vélez R, Izquierdo M, Garcia-Hermoso A. Physical activity and risk of infection, severity and mortality of COVID-19: a systematic review and non-linear dose-response meta-analysis of data from 1 853 610 adults. Br J Sports Med 2022 Aug 22. bjsports-2022-105733. Online ahead of print.
[46] Ramírez-Vélez R, Legarra-Gorgoñon G, Oscoz-Ochandorena S, García-Alonso Y, García-Alonso N, Oteiza J, et al. Reduced muscle strength in patients with long-COVID-19 syndrome is mediated by limb muscle mass. J Appl Physiol Bethesda Md 1985 2023;134:50–8.
[47] Burtscher J, Burtscher M, Millet GP. The central role of mitochondrial fitness on antiviral defenses: an advocacy for physical activity during the COVID-19 pandemic. Redox Biol 2021;43:101976.
[48] Pedersen BK, Saltin B. Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. Scand J Med Sci Sports 2015;25(Suppl 3):1–72.
[49] Chrzanowski-Smith OJ, Edinburgh RM, Thomas MP, Haralabidis N, Williams S, Betts JA, et al. The day-to-day reliability of peak fat oxidation and FATMAX. Eur J Appl Physiol 2020;120:1745–59.