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# Physiological and molecular predictors of cycling sprint performance

Victor Galvan-Alvarez[1,2] | Angel Gallego-Selles[1,2] | Miriam Martinez-Canton[1,2] | Ismael Perez-Suarez[1,2] | Eduardo Garcia-Gonzalez[1,2] | Marcos Martin-Rincon[1,2] | Jose A. L. Calbet[1,2,3]

[1]Department of Physical Education, University of Las Palmas de Gran Canaria, Campus Universitario de Tafira s/n, Las Palmas de Gran Canaria, Spain

[2]Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria, Paseo Blas Cabrera Felipe "Físico" s/n, Las Palmas de Gran Canaria, Spain

[3]Department of Physical Performance, The Norwegian School of Sport Sciences, Postboks, Oslo, Norway

**Correspondence**
Jose A. L. Calbet and Marcos Martin-Rincon, Department of Physical Education, University of Las Palmas de Gran Canaria, University Campus of Tafira, Las Palmas de Gran Canaria 35017, Canary Islands, Spain.
Email: lopezcalbet@gmail.com and marcos.martinrincon@gmail.com

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## Abstract

The study aimed to identify novel muscle phenotypic factors that could determine sprint performance using linear regression models including the lean mass of the lower extremities (LLM), myosin heavy chain composition (MHC), and proteins and enzymes implicated in glycolytic and aerobic energy generation (citrate synthase, OXPHOS proteins), oxygen transport and diffusion (myoglobin), ROS sensing (Nrf2/Keap1), antioxidant enzymes, and proteins implicated in calcium handling. For this purpose, body composition (dual-energy X-ray absorptiometry) and sprint performance (isokinetic 30-s Wingate test: peak and mean power output, $W_{peak}$ and $W_{mean}$) were measured in young physically active adults (51 males and 10 females), from which a resting muscle biopsy was obtained from the musculus *vastus lateralis*. Although females had a higher percentage of MHC I, SERCA2, $pSer^{16}/Thr^{17}$-phospholamban, and Calsequestrin 2 protein expressions (all $p < 0.05$), and 18.4% lower phosphofructokinase 1 protein expression than males ($p < 0.05$), both sexes had similar sprint performance when it was normalized to body weight or LLM. Multiple regression analysis showed that $W_{peak}$ could be predicted from LLM, SDHB, Keap1, and MHC II % ($R^2 = 0.62$, $p < 0.001$), each variable contributing to explain 46.4%, 6.3%, 4.4%, and 4.3% of the variance in $W_{peak}$, respectively. LLM and MHC II % explained 67.5% and 2.1% of the variance in $W_{mean}$, respectively ($R^2 = 0.70$, $p < 0.001$). The present investigation shows that SDHB and Keap1, in addition to MHC II %, are relevant determinants of peak power output during sprinting.

**KEYWORDS**

Keap1, muscle mass, muscle phenotype, myoglobin, ROS, SERCA, Wingate test, fatigue

Marcos Martin-Rincon and Jose A. L. Calbet should be considered co-senior authors.

## 1 | INTRODUCTION

Previous studies have reported an association between thigh muscle volume,[1,2] lower extremities lean mass,[3,4] and lower extremities volume[5] and the peak power output reached during maximal sprinting on the cycle ergometer. However, muscle mass leaves a large proportion of the variability in sprint performance unexplained, meaning that other factors should play a role. In this regard, a higher percentage of myosin heavy chain type II (MHC II) has been associated with faster contraction speeds and power generation in vitro and in vivo,[5–9] while the relative area of the muscle occupied by type II fibers has been associated with a higher mean power output during the Wingate test.[10] However, very few attempts have been made to quantify the role played by potential predictive variables beyond the effect of muscle mass and myosin heavy chain composition.[1,5] Even with the inclusion of muscle volume and MHC II %, a large portion of the variance in peak power output remains unexplained,[1,5] implying that more muscle phenotypic features should be implicated.

In theory, the peak power output may depend on muscle features allowing the achievement of higher peak force or muscle shortening speed during the sprint. Since peak power output depends on the optimal combination of force and muscle contraction velocity, the proteins implicated in $Ca^{2+}$ handling and $Ca^{2+}$ sensitivity could be determinants of peak and mean power output.[11] In addition, all processes implicated in energy metabolism could affect peak and mean power output. During the Wingate test, 20%–30% of the energy is provided by oxidative phosphorylation while the rest depends on substrate-level phosphorylation,[12–14] with a greater contribution by glycolysis than phosphagens (ATP and phosphocreatine).[13–15]

During sprint exercise, substantial amounts of reactive oxygen and nitrogen species (RONS) are produced, which may be deleterious for sprint performance[16,17] unless timely counteracted by redox-regulating proteins and enzymes like nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and kelch-like ECH-associated protein 1 (Keap1). Keap1 is a cysteine-rich protein that acts as an oxidative and electrophilic stress sensor. Under resting unstressed conditions, most Keap1 is bound to Nrf2, preventing Nrf2 translocation to the nucleus. Keap1 is an adaptor protein for a Cul3/Rbx1 E3 ubiquitin ligase complex, which ubiquitinates Nrf2 for proteasomal degradation. However, in the presence of oxidative or electrophilic stress, several Keap1 cysteine residues undergo covalent modifications leading to Keap1 detachment from Nrf2.[18–20] Free Nrf2 can then translocate to the nucleus, where it binds to antioxidant response elements (AREs) to activate the expression of genes involved in the antioxidant response and mitochondrial biogenesis.[21,22]

Therefore, this research aimed to determine in male and female adults which muscle phenotypic factors could determine sprint performance using a linear regression model assessing proteins and enzymes implicated in glycolytic and aerobic energy generation (citrate synthase, OXPHOS proteins), oxygen transport and diffusion (myoglobin), RONS sensing (Nrf2/Keap1), antioxidant enzymes, and proteins implicated in calcium handling.

The central hypothesis is that, given the role attributed to RONS in fatigue during high-intensity exercise,[16,17] muscle molecular components enhancing redox regulation like Nrf2/Keap1 and antioxidant enzymes regulated by Nrf2 could also be determinants of sprint performance. We also hypothesized that higher expression of enzymes implicated in $Ca^{2+}$ handling (Serca1, Serca2, and Phospholamban, PLB), glycolytic energy production (Phosphofructokinase 1, PFKM), $O_2$ diffusion (Myoglobin), and $O_2$ utilization (Citrate synthase, oxidative phosphorylation proteins) will be associated with increased sprint performance.

## 2 | METHODS

### 2.1 | General overview of the study

This study is based on the analysis of resting muscle biopsies obtained in research projects carried out in our laboratory to determine mechanisms of fatigue during high-intensity exercise.[23–25] The studies included a pretesting and familiarization phase, which were followed by the assessment of $VO_{2max}$ and the performance of an isokinetic 30s Wingate test, all on separate days (Figure 1).

### 2.2 | Subjects

Fifty-one males and ten females were included in the current research (Table 1). To be eligible for participation, volunteers had to be physically active and healthy, body mass index above 18 and below 30, without contraindications for maximal exercise, be non-smokers and not taking any drug or medication. All subjects were sport sciences students, some were participating in team sports, primarily soccer, while others were involved in triathlon, cross-fit, or running. None would qualify as an elite athlete. All participants volunteered to participate in the corresponding studies and signed written informed consent after receiving complete information regarding the aims of the studies and potential side effects of the procedures.

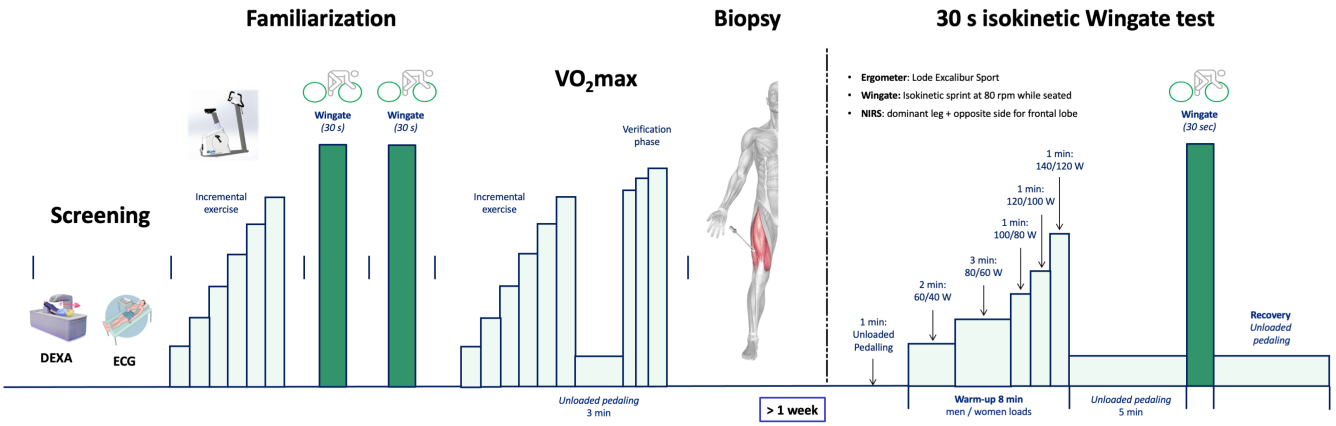


**FIGURE 1** Experimental protocol (vertical lines indicate separate days).

**TABLE 1** Physical characteristics, ergospirometric variables, and myosin heavy chain composition (mean ± SD).

| | Males ($n = 51$) | Females ($n = 10$) | $p$ |
|---|---|---|---|
| Age (years) | 22.8 ± 2.9 | 22.1 ± 1.6 | 0.449 |
| Height (cm) | 175.9 ± 7.3 | 162.0 ± 4.5 | 0.000 |
| Weight (kg) | 74.0 ± 8.6 | 57.8 ± 7.0 | 0.000 |
| % body fat | 18.2 ± 5.3 | 27.1 ± 3.5 | 0.000 |
| Lean body mass (kg) | 57.2 ± 5.7 | 39.6 ± 3.3 | 0.000 |
| LLM (kg) | 19.8 ± 2.1 | 14.3 ± 1.5 | 0.000 |
| $HR_{max}$ (Beats·min$^{-1}$) | 192.1 ± 7.7 | 196.0 ± 6.8 | 0.133 |
| $VO_{2max}$ (mL·min$^{-1}$) | 3591 ± 454 | 2363 ± 226 | 0.000 |
| $VO_{2max}$ (mL·kg$^{-1}$·min$^{-1}$) | 48.9 ± 6.7 | 41.1 ± 2.9 | 0.000 |
| $VO_{2max}$ (mL·kg LLM$^{-1}$·min$^{-1}$) | 182.2 ± 25.2 | 166.6 ± 15.6 | 0.018 |
| $W_{max}$ (W) | 289.9 ± 54.4 | 193.2 ± 26.8 | 0.000 |
| MHC I (%) | 39.0 ± 13.6 | 54.4 ± 12.2 | 0.002 |
| MHC IIa (%) | 48.5 ± 10.5 | 35.7 ± 6.9 | 0.001 |
| MHC IIx (%) | 12.5 ± 9.1 | 9.9 ± 7.0 | 0.403 |

*Note*: $p$-values from t-test for independent groups.

Abbreviations: $HR_{max}$, maximal heart rate; LLM, lean mass of the lower extremities; MHC, myosin heavy chain; $VO_{2max}$, maximal oxygen uptake; $W_{max}$, maximal intensity during the incremental exercise test to exhaustion.

Gender and sex identification were self-disclosed during the recruitment phase. All respondents identified themselves as cis-gender. All female participants were eumenorrheic, not on oral contraceptives, and were assessed randomly during their menstrual cycle phases.[26] This methodology aligns with studies indicating consistent sprint and high-intensity performance outcomes across menstrual phases.[27–29] All experiments were performed per the Declaration of Helsinki after ethical approval (CEIH-2017-13). Participants were instructed to refrain from engaging in intense physical activity 48 h prior to all laboratory evaluations, including the muscle biopsy. Additionally, they were advised to avoid sparkling beverages, caffeine, and alcohol for 24 h before all tests. Throughout the study, no dietary supplements or vitamins were allowed.

## 2.3 | Pre-testing and familiarization

During pre-testing and familiarization phases subjects reported to the laboratory early in the morning, following a 12-h overnight fast for assessment of their body composition using dual-energy X-ray absorptiometry (Lunar iDXA, GE Healthcare, Milwaukee, WI, USA). The anatomical regions of interest were delineated manually from the whole-body scans, as previously reported.[30] On subsequent visits to the laboratory, the volunteers performed an incremental exercise to exhaustion and two isokinetic Wingate tests at 80 rpm for familiarization purposes. Adjustments to the seat and handlebar were carefully customized to the subject's anthropometric measurements during the first visit and were kept consistent throughout the following visits. Special care was taken into familiarize the participants with the performance of sprinting in isokinetic mode at 80 rpm while remaining seated in the saddle. Exercise tests took place in an air-conditioned laboratory with an ambient temperature of ~21°C, a relative humidity of 60%–80%, and ~735 mmHg atmospheric pressure.

## 2.4 | Assessment of $VO_{2max}$

The $VO_{2max}$, maximal power output ($W_{max}$), and maximal hear rate ($HR_{max}$) were determined in normoxia ($F_IO_2$: 0.21, $P_IO_2$: 144 mmHg) using an incremental exercise test until exhaustion, including a verification phase.[31] The incremental exercise was performed in a post-absorptive state, at least 4 h after a light meal using a cycle ergometer

(Lode BV, Groningen, The Netherlands). In 40 subjects (30 M/10 W), the test began with a 3-min stage at 20 W, which was subsequently increased every 3 min by 15 W for females and 20 W for males until the respiratory exchange ratio (RER) reached or exceeded 1.00. Following this, the intensity was raised every minute by 10 W in females and 15 W in males until exhaustion. In the remaining 21 male subjects, the initial load was set at 80 W, which was increased by 30 W every 2 min until exhaustion. The peak intensity achieved was denoted as the $W_{max}$. Upon exhaustion, the ergometer was unloaded, and the subjects continued pedaling at a low cadence (30–40 rpm) for 3 minutes to facilitate recovery. Right after, the verification phase began at $W_{max}$ +5 W for a minute, increased every 20 s by 4 W in females and 5 W in males, continuing until participants could no longer sustain the effort. Exhaustion was defined either by the inability to sustain a pedaling rate of more than 50 rpm for 5 s or by an abrupt cessation of pedaling. Verbal encouragement was provided throughout the last phases of every test.

During the incremental exercise test, the $VO_2$ was measured breath-by-breath by indirect calorimetry (Vyntus, Jaeger-CareFusion, Höchberg, Germany), which has been validated by a butane combustion test.[32] The metabolic cart was calibrated immediately before each with room air (20.93% $O_2$ and 0.05% $CO_2$) and calibration gases purchased from the manufacturer (16% $O_2$ and 5% $CO_2$). The flowmeter was calibrated for low (0.2 L/s) and high (2 L/s) ventilation flows immediately before each test. Breath-by-breath data were averaged every 20 s, and the highest 20-s averaged $VO_2$ achieved during either the incremental test or the verification phase was taken as the $VO_{2max}$.[33] Heart rate (HR) was registered every 1 s (RS400 and RS800, Polar Electro, Woodbury, NY, USA).

## 2.5 | Wingate test

The Wingate test consisted of a 30-s all-out sprint performed on a separate testing day, at least 1 h after a light meal (Lode Excalibur Sport 925 900, Groningen, The Netherlands). The Wingate test was preceded by a standardized warm-up consisting of 1 min of unloaded pedaling, 2 min at 40 W or 60 W, 3 min at 60 or 80 W, 1 min at 80 or 100 W, 1 min at 100 or 120 W, and 1 min at 120 or 140 W for females and males, respectively. At the end of the warm-up, the participants recovered by pedaling at a low cadence (20–40 rpm) for 5 min with the ergometer unloaded. The Wingate test was performed in isokinetic mode at a fixed cadence of 80 (41 males and 10 females) or 100 rpm (10 males) and performed under strong verbal encouragement to ensure that a maximal effort was provided. Participants were requested to remain seat on the saddle during the whole Wingate test. About 30 s before the start of the sprint, participants accelerated the flywheel with the ergometer unloaded near to the prescribed cadence and waited ready to sprint as hard and fast as possible after a 5-s countdown. Utilizing the ergometer configured in isokinetic mode, the resistance presented is continuously adjusted through an automated servo-control mechanism. This apparatus exclusively permits a consistent pedaling cadence of 80 (±1) rpm, ensuring that any diminution in the force applied to the pedals leads to a corresponding reduction in the ergometer's resistance, and, conversely, an augmentation in force results in an increase in resistance. All participants, regardless of the sport cycling footwear chosen, were tightly attached to the pedals using additional fastening straps. Instantaneous power values were obtained using Lode Ergometry Manager Software (LEM; Lode BV, Groningen, The Netherlands) from which instantaneous peak power output ($W_{peak}$) was obtained, while the mean power output ($W_{mean}$) was calculated.

## 2.6 | Muscle biopsies

*Vastus lateralis* muscle biopsies were performed in the morning after a 12-h overnight fast from the middle third of one of the two thighs, using Bergstrom's needles and applying suction, as previously reported.[23] The leg to be biopsied was assigned randomly, and the skin and subcutaneous tissue were infiltrated with 2% lidocaine (1–2 mL), taking special care to avoid anesthetic injection below the fascia. Ten minutes later, a 5 mm incision was performed, and the needle was introduced with a 45° inclination toward the foot to position the biopsy window of the needle about 2 cm below the fascia. All biopsies were immediately frozen in liquid nitrogen and store at −80°C until analyzed.

### 2.6.1 | Protein extraction and western blotting

Muscle lysates preparation was carried out as previously reported.[34] Briefly, lysates protein concentration was determined by the bicinchoninic acid assay.[35] Then, approximately 10 mg of muscle was homogenized in urea lysis buffer (6 M urea, 1% SDS), 50X Complete protease inhibitor and 10X PhosStop phosphatase inhibitor cocktails (Roche). This mixture was centrifuged for 12 min at 25 200 g at 16°C, and the supernatant was diluted with electrophoresis loading buffer (160 mM Tris–HCl,

pH 6.8, 5.9% SDS, 25.5% glycerol, 15% β-mercaptoethanol-bromophenol blue). All proteins were assayed at optimal amounts, that is, in the linear portion of the antigen–antibody reaction, as revealed by optical density response for protein amounts ranging from 1 to 35 μg. Equal amounts of protein of each sample (1.5–15 μg) were loaded for SDS-PAGE using the system of Laemmli,[36] followed by transference onto the polyvinylidene fluoride (PVDF) membranes for protein blotting (Bio-Rad Laboratories, Hercules, CA, USA). To reduce the variability between gels, test samples were run with an equal protein amount from an internal human control sample (non-experimental and same as during linearity optimization) loaded in triplicate or quadruplicate. The densitometric value of the protein of interest was normalized to the mean value of the internal control sample included onto the gel.

Membrane blocking was achieved by incubation for 1 h in 4% bovine serum albumin or 5% non-fat dried milk powder (blotting-grade blocker) diluted in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) (BSA or Blotto blocking buffer) and incubated overnight at 4°C with primary antibodies. Afterward, the membranes were washed and incubated for 1 h at room temperature with an HRP-conjugated anti-rabbit or anti-mouse antibody (diluted 1:5000 to 1:20000 in 5% Blotto blocking buffer in all instances) followed by chemiluminescent visualization with Clarity™ Western ECL Substrate (Bio-Rad Laboratories) with the ChemiDoc™ Touch Imaging System (Bio-Rad Laboratories). Band density was determined using the Image Lab© software 5.2.1 (Bio-Rad Laboratories). Reactive Brown 10 (Sigma-Aldrich, St. Louis, MO, USA) staining was used to control for equal loading and transfer efficiency. The corresponding catalogue numbers of primary antibodies were as follows: anti-SOD1 (no. ab16831), anti-pSer$^{40}$ Nrf2 (no. ab76026), anti-Nrf2 (no. ab62352), and the anti-OXPHOS premixed cocktail antibody (total OXPHOS human antibody cocktail, no. ab110411) which includes antibodies against Complex I subunit NADH dehydrogenase [ubiquinone] 1 beta subcomplex (NDUFB8, ab110242), Complex II subunit succinate dehydrogenase [ubiquinone] iron–sulfur (SDHB, ab14714), Complex III subunit cytochrome b-c1 complex subunit 2 (UQCRC2, ab14745), Complex IV subunit cytochrome c oxidase subunit 2 (COXII, ab110258), and ATP synthase subunit alpha (ATP5A, ab14748) were purchased from Abcam (Cambridge, UK). Anti-Myoglobin (no.25919), anti-SOD2 (no. 13141), anti-pSer$^{16}$/Thr$^{17}$ Phospholamban (no. 8496), and anti-Catalase (no. 14097) were obtained from Cell Signalling Technology (Danvers, MA, USA). Anti-Calsequestrin1 (CSQ1) (no. C0618), anti-Calsequestrin2 (CSQ2), (no. 3868), anti-SERCA1 (no. WH0000487M1), and anti-SERCA2 (no. S1439) were ordered from Sigma-Aldrich. Anti-Citrate synthase (16131-1-AP), anti-Keap1 (no. 10503-2-AP), anti-PFKM 1 (55028-1-AP), and Glutathione reductase (GR) (18257-1-AP) were acquired from Proteintech (Rosemont, IL, USA). Secondary HRP-conjugated goat anti-mouse (no.115–035-003) and goat anti-rabbit (no. 111–035-144) antibodies were purchased from Jackson ImmunoResearch Inc. (West Grove, PA, USA). See Table S1 for complementary information. No additional muscle proteins were measured.

### 2.6.2 | Myosin heavy chain analysis

Myosin heavy chain isoform composition analysis was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), as previously reported.[25] Briefly, 7.5–10 μg of the same western blot-ready protein extracts used for muscle signaling was loaded in triplicate onto the same gel, together with two internal control samples of known MHC composition (used for quality control). SDS-PAGE gels containing a 3% acrylamide (v/v) phase (stacking gel) were run at 4°C for ~12 h at 70 V and afterward on a 6% acrylamide (v/v) and 30% glycerol (v/v) phase (resolving gel) for ~20 h at 350 V. Bands were identified by staining with Coomassie Blue for ~1 h, subsequently distained with a 40% methanol (v/v) and 10% glacial acetic acid (v/v) solution for ~1 h and finally submerged in distilled water ~1 h to optimize background subtraction. The MHC composition was determined by scanning the gel with a densitometry scanner (GS-800 Imaging Densitometer, Bio-Rad Laboratories, Hercules, CA, USA) followed by quantification (Image Lab© software 5.2.1, Bio-Rad Laboratories).

## 2.7 | Statistics

The normal distribution of variables was verified using the Shapiro-Wilks test, and variables that were not normally distributed were logarithmically transformed before further analysis. Descriptive data are reported as the mean ± standard deviation (SD). Sex differences were analyzed using a two-tailed unpaired t-test, with appropriate correction in case of failure to pass Levene's test for equality of variances. Linear relationships between variables were determined using simple and multiple linear regression analyses, including sex as a categorical variable in the prediction models. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using IBM SPSS Statistics v.21 for Mac (SPSS Inc., Chicago, IL, USA) and Jamovi v1.8.1. (Jamovi project, 2021).

# 3 | RESULTS

## 3.1 | Physical characteristics and performance

The physical characteristics of the participants are reported in Table 1. Females had lower height, body mass, and whole-body lean mass (LM) than males and a higher body fat percentage. When $VO_{2max}$ was expressed in absolute values, normalized to whole-body mass and normalized to lean mass of the lower extremities (LLM), values were 34.2, 16.1, and 8.6% lower in females than males, respectively. Males had superior sprint performance than females in absolute values, although those sex differences disappeared after normalization to body weight, LM, and LLM (Table 2).

## 3.2 | Sex differences in skeletal muscle phenotype

Females had a higher percentage of MHC I than males, while males had a higher percentage of MHC IIa than females (Table 1). No between-sex differences in MHC IIx % were observed (Table 1). Keap1, Nrf2, and the antioxidant proteins assessed had similar levels of expression in males and females (Figure 2). The OXPHOS mitochondrial proteins were expressed similarly in males and females (Figure 2). Citrate synthase and myoglobin were also similarly expressed in both sexes. Phosphofructokinase protein expression was 18.4% lower in females than in males ($p=0.049$). Marked sex differences were observed in $Ca^{2+}$ handling proteins (Figure 2), where SERCA2, $pSer^{16}$/$Thr^{17}$ PLB (26 kDa + 12 kDa isoforms), and CSQ2 protein expressions were 2.8 and 1.7, 1.7-fold higher in females than males, respectively ($p<0.05$). Representative immunoblots are depicted in Figure 3.

**TABLE 2** Wingate test performance (mean ± SD).

| | Males ($n=51$) | Females ($n=10$) | $p$ |
|---|---|---|---|
| $W_{peak}$ (W) | 984 ± 173 | 751 ± 117 | 0.000 |
| $W_{mean}$ (W) | 562 ± 84 | 402 ± 61 | 0.000 |
| $W_{peak}$ (W·kg $BW^{-1}$) | 13.3 ± 2.1 | 13.1 ± 2.2 | 0.744 |
| $W_{mean}$ (W·kg $BW^{-1}$) | 7.6 ± 0.9 | 7.0 ± 1.2 | 0.069 |
| $W_{peak}$ (W·kg $LM^{-1}$) | 17.2 ± 2.8 | 19.0 ± 2.6 | 0.071 |
| $W_{mean}$ (W·kg $LM^{-1}$) | 9.8 ± 1.2 | 10.2 ± 1.4 | 0.430 |
| $W_{peak}$ (W·kg $LLM^{-1}$) | 49.6 ± 7.4 | 52.8 ± 7.1 | 0.215 |
| $W_{mean}$ (W·kg $LLM^{-1}$) | 28.3 ± 2.9 | 28.3 ± 4.0 | 0.980 |

*Note*: $p$-values from t-test for independent groups.

Abbreviations: BW, body weight; LLM, lean mass of the lower extremities; LM, whole-body lean mass; $W_{mean}$, mean power output; $W_{peak}$, instantaneous peak power output.

## 3.3 | Relationships between sprint performance and skeletal muscle phenotype

### 3.3.1 | Myosin heavy chain composition

In the whole group of subjects, a higher percentage of MHC II was associated with greater $W_{peak}$ and $W_{mean}$ per body mass (both $r=0.32$, $p<0.05$). This association was attenuated when expressed per kg of LM ($r=0.26$, $p=0.041$; $r=0.27$, $p=0.033$, respectively). However, when expressed per kg of LLM, the correlation did not reach statistical significance ($r=0.24$, $p=0.06$). No significant associations were observed between Wingate test performance and the percentage of MHC IIx or MHC I + IIa.

### 3.3.2 | The lean mass of the lower extremities is the main determinant of sprint performance

There was a linear relationship between $W_{peak}$ or $W_{mean}$ and the LM ($r=0.60$ and $r=0.76$, respectively, both $p<0.001$) and the LLM ($r=0.68$, $r=0.82$, respectively, both $p<0.001$) (Figure 4), indicating a higher predictive value for LLM. This relationship was similar in males and females, with no sex interaction. Since there is collinearity between LM and LLM ($r=0.91$, $p<0.001$), LLM was retained for multiple regression models to predict sprint performance.

Multiple regression analysis showed that $W_{peak}$ could be predicted from LLM, SDHB, Keap1, and MHC II % ($R^2=0.62$, $p<0.001$), each variable contributing to explain 46.4, 6.3, 4.4, and 4.3% of the variance in $W_{peak}$, respectively (Table 3). Multiple regression analysis showed that the main variables contributing to explain the variance of $W_{mean}$ were LLM and MHC II % ($R^2=0.70$, $p<0.001$), which explained 67.5% and 2.1% of the variance in $W_{mean}$, respectively (Table 4). None of the other phenotypic variables examined made any significant contribution to enhance the predictive value of LLM and MHC II %. Sex did not significantly enhance the predictive value of these two models (Tables 3 and 4).

In females, there was an association between $W_{peak}$ normalized to LLM and PFKM protein expression ($r=0.81$, $p=0.005$, $n=10$) and Log MHC II % ($r=0.69$, $p=0.027$, $n=10$). Similar associations were observed between $W_{mean}$ and PFKM protein expression ($r=0.67$, $p=0.033$, $n=10$), Log MHC II % ($r=0.82$, $p=0.004$, $n=10$), and SERCA1

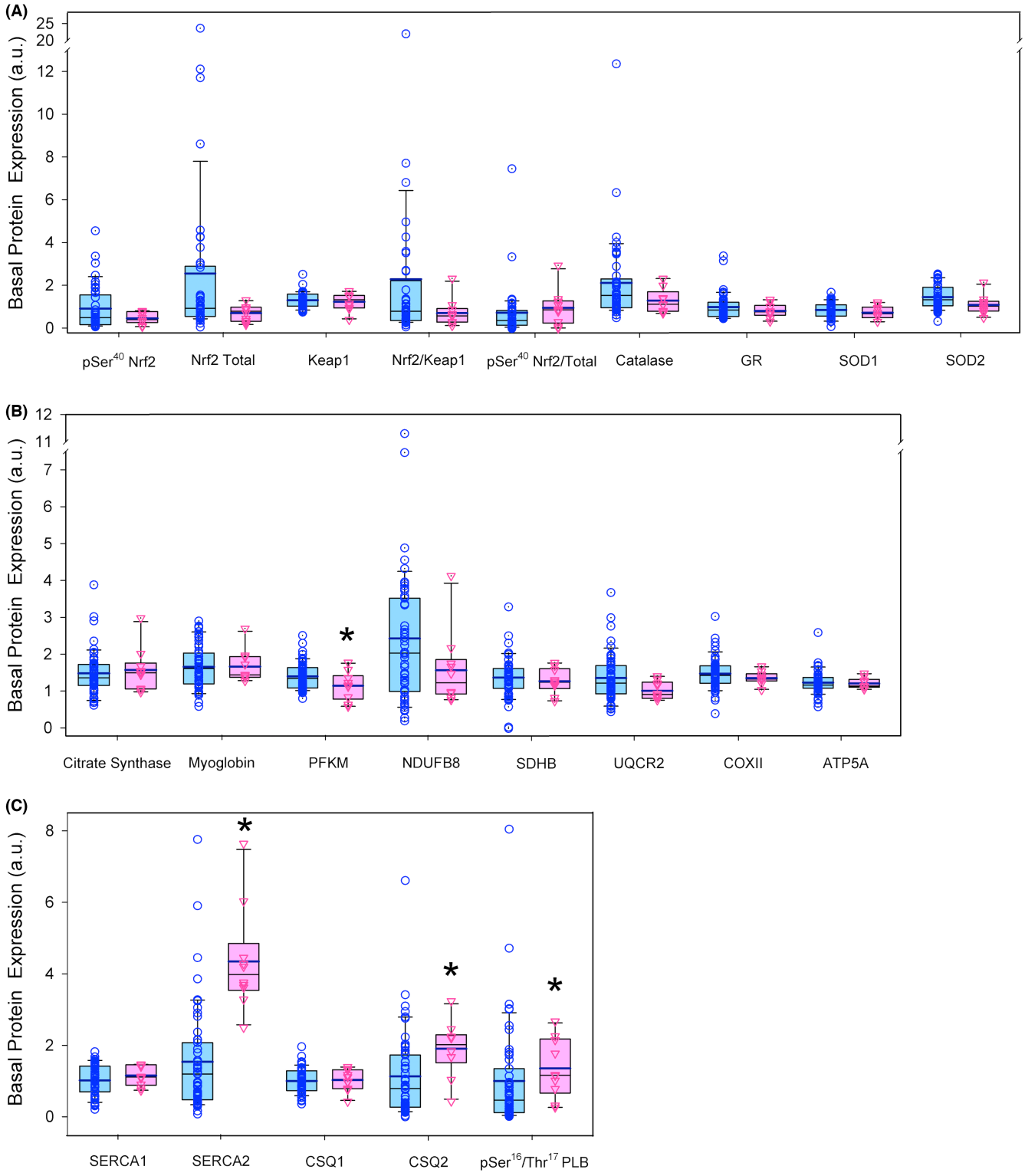


**FIGURE 2** Sex differences in basal skeletal muscle protein expression. Antioxidant enzymes (41 M, 10 F) and RONS-sensing proteins (51 M, 10 F) (A), proteins involved in muscle energy metabolism and oxygen transport (51 M, 10 F) (B), and proteins involved in regulation of sarcoplasmic calcium concentration (51 M, 10 F) (C). The extremes of the whiskers represent the limits of the 5th and 95th percentiles, respectively; the thick and thin horizontal lines inside the boxes correspond to the mean and median values, respectively; and the lower and upper limits of the box delimit the first and third quartiles, respectively. The statistical analysis was performed with logarithmically transformed data, when appropriate. Males are represented by blue circles and females by pink triangles. *$p < 0.05$ compared to males.

protein expression ($r=0.70$, $p=0.024$, $n=10$). In males, $W_{peak}$ normalized to LLM was associated with NDUFB8 protein expression ($r=-0.29$, $p=0.046$ $n=51$), while $W_{peak}$ normalized to LLM was associated with the ratio $pSer^{40}$ Nrf2/Nrf2 total ($r=-0.29$, $p=0.046$ $n=51$) and Log MHC II % ($r=0.31$, $p=0.029$, $n=51$). Log MHC II % did not correlate with SDHB ($r=-0.17$, $p=0.23$, $n=51$) nor Keap1 ($r=0.09$, $p=0.55$, $n=51$).

## 4 | DISCUSSION

The present investigation shows that the main two variables determining sprint performance during exercise on the cycle ergometer are the active muscle mass and the percentage of type II fibers. The main novelty of the present investigation is that, despite the large number of phenotypic variables examined, which included proteins

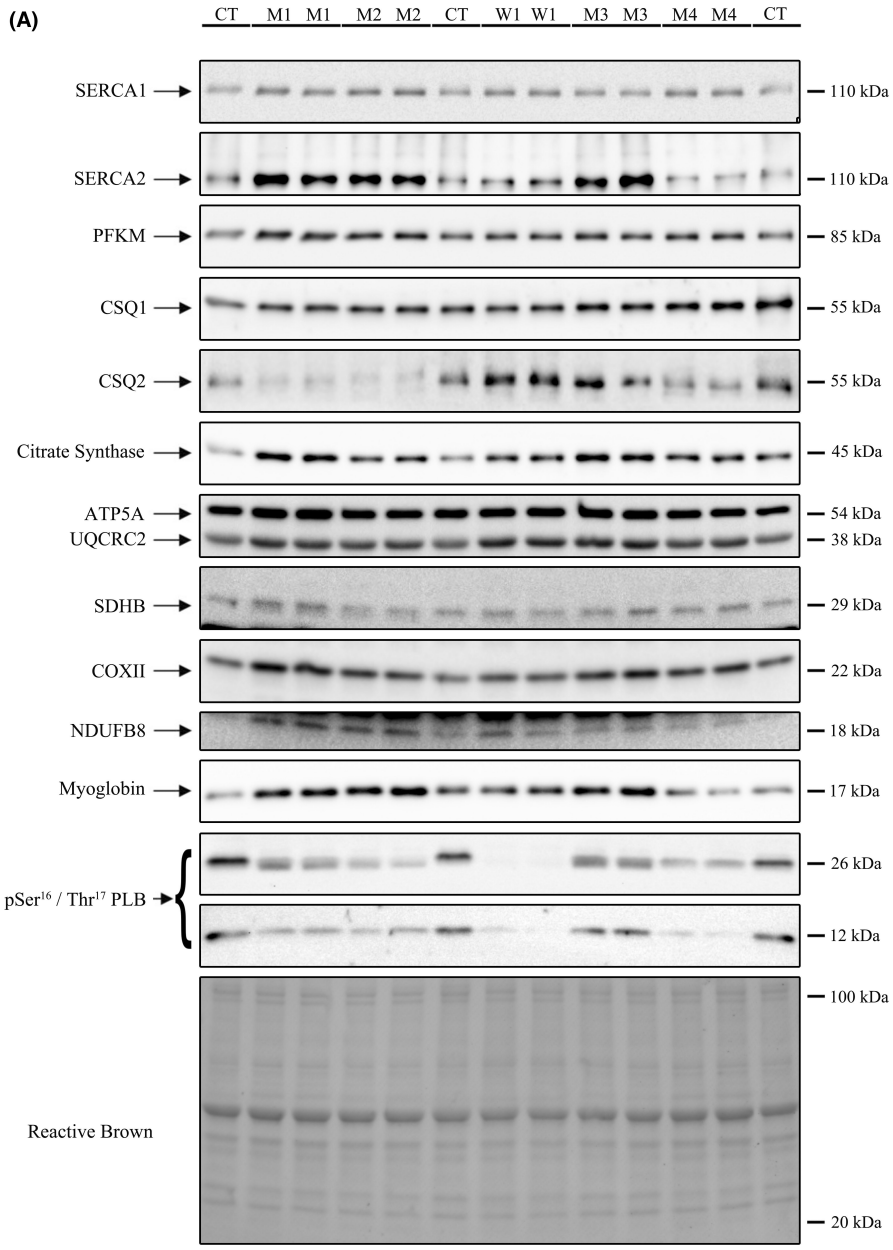


**FIGURE 3** Representative immunoblots. Protein expression levels (western Blot) and a total protein loading control staining (Reactive Brown Staining) of tested proteins from a single female (F) and four male (M) subjects. CON, control samples. Molecular weights for the proteins under examination are indicated on the right side of the blot. The images are organized as follows: SERCA1, SERCA2, phosphofructokinase 1 (PFKM), calsequestrin1 (CSQ1), calsequestrin2 (CSQ2), citrate synthase, ATP5A, UQCRC2, SDHB, COXII, NDUFB8, myoglobin, $pSer^{16}/Thr^{17}$ phospholamban, and Reactive Brown are presented in duplicate (Panel A). Total Nrf2, $pSer^{40}$ Nrf2, Keap1, Catalase, Glutathione reductase, SOD1, SOD2, and Reactive Brown are displayed in Panel B.

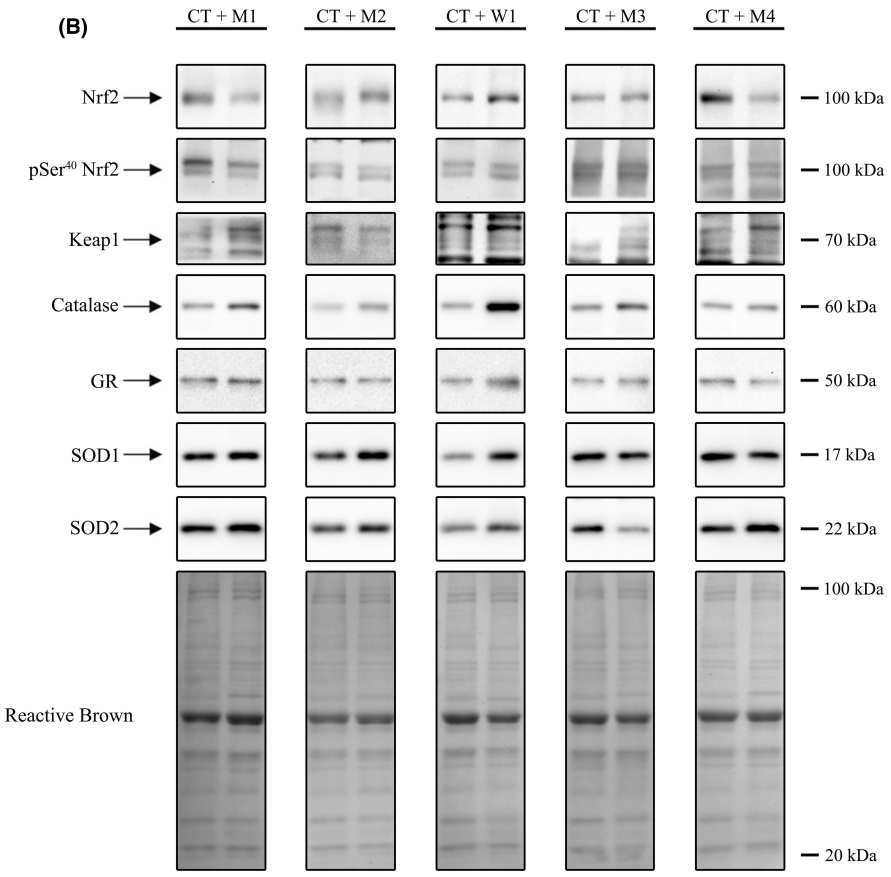


**FIGURE 3** (Continued)

and enzymes implicated in glycolytic and aerobic energy generation (PFKM, citrate synthase, OXPHOS proteins), oxygen transport and diffusion (myoglobin), RONS sensing (Nrf2/Keap1), antioxidant enzymes, and proteins implicated in $Ca^{2+}$ handling, only SDHB and Keap1 were significantly associated with sprint performance. Interestingly, once LLM was considered, no significant sex differences were observed in sprint performance, further emphasizing the dominant role of the active muscle mass for peak and mean power output in a 30-s sprint on the cycle ergometer.

## 4.1 | The active muscle mass is the main determinant of sprint performance

In agreement with our results, Kordi et al.[2] reported a significant correlation between quadriceps and hamstring muscle volume, as assessed by magnetic resonance imaging, and peak power output in 35 male cyclists specialized in sprint and endurance modalities, most of them elite athletes. Kordi et al., like us, used isokinetic tests, although their subjects performed repeated 4-s sprints with a fly start at different cadences to determine the optimal cadence, which ranged between 112 and 162 rpm, with a mean value of 131 rpm. This allowed their cyclists to achieve an average peak power output of 1260 W, with a range between 775 and 2025 W. Also in line with our results, it has been reported in 28 male track and road cyclists that *vastus lateralis* muscle volume assessed by 3D ultrasonography explained 38% of the variance in peak power output and that including MHC II % in the regression model improved the predictive capacity of the model to 65%.[1] The lower predictive value of *vastus lateralis* volume in van der Zwaard et al.[1] could potentially be attributed to the involvement of a larger number of muscles during sprint cycling.[37]

Despite the fact that in the present investigation the participants were not cyclists and performed the test from a stopped start, the mean $W_{peak}$ value reached by our male participants was just 22% lower than achieved by the sprint cyclists studied by Kordi et al.[2] Although our subjects would be expected to reach $W_{peak}$ at an optimal cadence close to 100–110 rpms,[38,39] the difference in $W_{peak}$ between 80 and 100 rpms is below 4% and between 80 and 120 rpm is 1.6% in elite cyclists,[40] meaning that setting the cadence at 80 rpm for most of our subjects, should have barely affected the interpretation of our results.[41] The latter also agrees with the fact that the percentage of MHC II is associated with peak power output

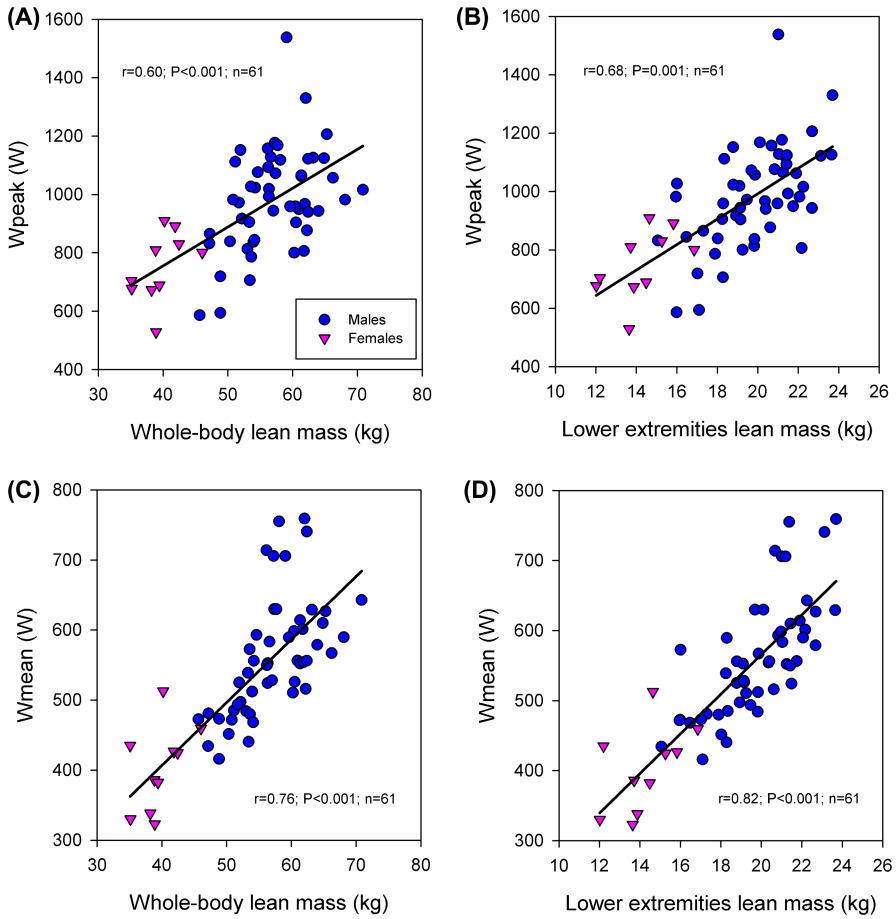


**FIGURE 4** Relationships between instantaneous peak power output ($W_{peak}$) and whole-body lean mass (A), and lower extremities lean mass (B), as well as the relationship between mean power output and whole-body (C) and lower extremities lean mass (D).

in 74-year-old men having a mean MHC II percentage of 26%, despite an optimal pedaling velocity of 90 rpm.[5] However, additional experiments would be required to determine whether setting the isokinetic pedaling rate to 100 or 110 rpm would enhance the predictive value of the percentage of MHC II for $W_{peak}$. Other factors, such as differences in training background, lean body mass, and the type of cycle ergometer, could explain our subject's lower mean power output compared to those reported in elite cyclists.[2,40]

Conceptually, peak power output requires an optimal combination of force and muscle contraction velocity,[42] represented by the muscle size and the percentage of fast-twitch fibers. Our regression model attributes a markedly larger impact to the muscle mass, represented by the lean mass of the lower extremities. This is explained by the fact that the muscle cross-sectional area (or volume) and maximal isometric force are closely related, as well as cross-sectional area and muscle volume (or mass) in males, females, adults, elderly, and children.[43–48] The speed of muscle contraction is determined by myosin heavy chain composition.[49,50] Individual muscle fibers tested in vitro show remarkably different maximal contraction speeds,[51] with the fibers expressing MHC II being the fastest.[49] In agreement, the present results indicate that myosin heavy chain composition contributes to explaining differences in $W_{peak}$ in humans, but its relative contribution is small (2%) compared to that of the muscle mass. This finding should, however, be interpreted cautiously because the tests were performed under isokinetic conditions at a fixed pedaling rate of 80 rpm, which may be more favorable for type 1 than type 2 fibers in humans.[5,42,52] However, our results match the $W_{peak}$ measured in our laboratory 15 years ago using isoinertial Wingate tests in subjects of similar characteristics, which was 50.4 and 50.5 $W \cdot kg\ LLM^{-1}$ in 123 males and 32 females, respectively.[4]

Previous studies have also reported a bivariate correlation between lean mass and mean power output during Wingate tests.[3,4] However, none of the previous studies carried out a comprehensive assessment of additional muscle phenotypic characteristics that could contribute to explain the human variability in mean and peak power output during sprint exercise. In the present investigation, we show for the first time that SDHB and Keap1, in addition to MHC II %, are relevant determinants of peak power output, as analyzed in the following sections.

**TABLE 3** Predictive models for peak power output during sprint exercise (Wingate test).

| Predictor | Estimate | SE | 95% confidence interval | | t | p | Stand. estimate | 95% confidence interval | | Model | Model fit measures | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Lower | Upper | | | | Lower | Upper | | R | $R^2$ | p |
| Intercept | 41.39 | 209.5 | −380.84 | 463.62 | 0.198 | 0.844 | | | | | | | |
| LLM (kg) | 42.7 | 10 | 22.55 | 62.84 | 4.272 | <0.001 | 0.647 | 0.3416 | 0.9518 | 1 | 0.689 | 0.475 | <0.001 |
| MHC II (%) | 4.14 | 1.41 | 1.29 | 6.99 | 2.93 | 0.005 | 0.304 | 0.0948 | 0.5127 | 2 | 0.720 | 0.518 | <0.001 |
| Keap1 (a.u.) | −137.99 | 49.15 | −237.04 | −38.94 | −2.808 | 0.007 | −0.266 | −0.4576 | −0.0752 | 3 | 0.749 | 0.562 | <0.001 |
| Log SDHB (a.u.) | 196.82 | 79.55 | 36.49 | 357.15 | 2.474 | 0.017 | 0.25 | 0.0464 | 0.4538 | 4 | 0.790 | 0.624 | <0.001 |
| Sex (1 = male; 2 = female) | 65.07 | 72.86 | −81.76 | 211.91 | 0.893 | 0.377 | 0.326 | −0.41 | 1.0626 | 5 | 0.794 | 0.631 | <0.001 |

*Note*: Power is predicted in watts.

Abbreviations: Keap1, kelch-like ECH-associated protein 1; LLM, lower extremities lean mass; MHC II, myosin heavy chain II percentage; SDHB, succinate dehydrogenase [ubiquinone] iron–sulfur subunit.

**TABLE 4** Predictive models for mean power output during sprint exercise (Wingate test).

| Predictor | Estimate | SE | 95% confidence interval | | t | p | Stand. estimate | 95% confidence interval | | Model | Model fit measures | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Lower | Upper | | | | Lower | Upper | | R | $R^2$ | p |
| Intercept | −247 | 142.53 | −532.4 | 38.4 | −1.733 | 0.089 | | | | | | | |
| LLM (kg) | 27.2 | 3.63 | 19.93 | 34.5 | 7.487 | <0.001 | 0.79 | 0.57842 | 1.001 | 1 | 0.821 | 0.675 | <0.001 |
| Log MHC II (%) | 150.9 | 73.44 | 3.8 | 297.9 | 2.054 | 0.045 | 0.169 | 0.00425 | 0.334 | 2 | 0.834 | 0.696 | <0.001 |
| Sex (1 = male; 2 = female) | 12.9 | 29.16 | −45.48 | 71.3 | 0.443 | 0.66 | 0.129 | −0.45463 | 0.713 | 3 | 0.835 | 0.697 | <0.001 |

*Note*: Power is predicted in watts.

Abbreviations: LLM, lower extremities lean mass; MHC II, myosin heavy chain II percentage.

### 4.2 | A high percentage of MHC II is positively associated with peak and mean power output during sprint exercise

A higher percentage of MHC II, which predominates in fast-twitch muscle fibers (FT), allows for faster shortening speed and greater peak power generation[5–9] due to the higher ATPase activity of MHC IIa and IIx compared to MHC I.[50,53] Although FT fibers are less efficient than slow-twitch (ST) fibers,[54] under rested unfatigued conditions when ATP availability and resynthesis rate are not limiting, a higher MHC II % contributes to determining peak power and mean power output. However, the present investigation indicates that a larger muscle mass is more critical than a higher proportion of MHC II. This is supported by the similar peak and mean power output normalized to the LLM in males and females despite the higher expression of MHC II in the former. Besides, muscle maximal shortening speed is barely changed with training, meaning that the improvements in sprint performance elicited by training rely mainly on ameliorating muscle force.[9] Similarly, muscle force has been identified as the main factor explaining differences in sprint performance between young and elderly subjects.[55,56]

### 4.3 | The protein expression level of the mitochondrial enzyme SDHB in skeletal muscle is positively associated with peak power output

Mitochondrial complex II (succinate-ubiquinone oxidoreductase [SDH]) is formed by four subunits encoded in the nuclear genome (SDHA, SDHB, SDHC, and SDHD). Genetic suppression of the SDHB subunit is associated with increased basal cytosolic oxidant stress and has been shown to facilitate mitochondrial ROS production.[57] A similar mechanism may operate in human skeletal muscle, where a lower expression level of SDHB may facilitate ROS production already in basal conditions and even more during exercise. On the contrary, a higher expression of SDHB may attenuate succinate accumulation,[57] and so more during high-intensity exercise.[58] Succinate accumulation has been shown to elicit mitochondrial ROS production.[59,60] More recently, it has been shown that increased expression of SDHA leads to the accumulation of fumarate,[61] which evokes succinylation of Keap1 in cysteines C151 and C288, resulting in disruption of the interaction between Keap1 and Nrf2, promoting Nrf2 signaling.[62,63]

Thus, the fact that SDHB expression is positively associated with peak power output may indicate that a higher capacity of skeletal muscle to quench free radicals, oxidants, and electrophiles during high-intensity exercise may be crucial for maximizing power output during sprint exercise. In agreement with this hypothesis, it has been shown that ROS can contribute to fatigue by reducing sarcoplasmic $Ca^{2+}$ release and troponin $Ca^{2+}$ sensitivity, among other potential mechanisms.[11,64,65] Likewise, the basal SOD2 protein expression in vastus lateralis, a mitochondrial antioxidant enzyme, is associated with $VO_2$max in humans, further supporting a role for antioxidant enzymes in energy metabolism in skeletal muscle.[66]

Although SDHB is an enzyme involved in oxidative phosphorylation and, therefore, intervenes in establishing the rate of aerobic ATP resynthesis, aerobic energy supply does not limit peak power output during sprint exercise in humans.[15,67,68]

### 4.4 | Why do subjects with increased peak power output have lower Keap1 resting levels?

The present study demonstrates for the first time that the basal expression level of Keap1 protein in human skeletal muscle is a negative predictor of peak power output. Previous research in transgenic mice unable to express Keap1 in their skeletal muscles has shown that Keap1 ablation in skeletal muscle is associated with enhanced endurance performance, although this effect was observed only in female rodents.[69] These animals possess constitutively elevated Nrf2 protein levels, which promote phenotypic changes in muscle fibers, facilitating aerobic exercise.[69] Sprint performance relies primarily on substrate-level phosphorylation, which has two main components: the phosphagen and glycolytic metabolic pathways. Peak power output requires a fast rate of ATP resynthesis from phosphocreatine and glycolysis, both being higher in FT than ST fibers.[13,70] Genetic ablation of Keap1 in skeletal muscle is associated with enhanced expression of antioxidant enzymes, which could allow a more efficient counteraction of RONS produced in response to the extremely high glycolytic rates attained during the first seconds of a maximal sprint in human skeletal muscle.[13,15,24,71]

Keap1 cysteines may be covalently modified by several endogenous metabolites.[62,63,72,73] For example, by methylglyoxal, an electrophile that results from the elimination of triose phosphate, which accumulates in conditions with high glycolytic flux, as occurs during sprint in normoxia[13,71] and sprint[15] or moderate-intensity exercise in hypoxia.[74] Methylglyoxal is highly reactive and could non-enzymatically cross-link and dimerize Keap1 molecules via a methyl imidazole-based

linkage between cysteine (C151) and arginine (R15 or R135) residues, facilitating Nrf2 signaling.[72] More recently, it has been shown that accumulation of glyceraldehyde 3-p elicits S-lactoylation of several cysteines in Keap1 by mechanisms that have yet to be elucidated.[73] Since glyceraldehyde 3-p readily accumulates during sprint exercise,[13] it could contribute to Nrf2 signaling by impeding the inhibitory action of Keap1 through its S-lactoylation.

Although a definitive answer to the question cannot be provided, low levels of Keap1 may permit higher expression of antioxidant and pentose phosphate pathway proteins,[73] which prevent the negative effects of oxidants and electrophiles produced during the high glycolytic rates associated with maximal sprint performance.[13,71] Moreover, with lower levels of Keap1, more newly synthesized Nrf2 will escape inhibition by a less abundant Keap1, facilitating the Nrf2 response to acute exercise.[75] Additionally, reduced basal levels of Keap1 could also facilitate the adaptative response to training.

## 4.5 | Perspectives

The lower levels of Keap1 at the start of exercise should facilitate Nrf2 activation, which elicits adaptations in skeletal muscle, preventing oxidative and electrophilic damage and accumulation of potentially toxic glycolytic intermediaries like methylglyoxal in subsequent exercises demanding high glycolytic rates. Thus, keeping low levels of Keap1 may be necessary for a physiological adaptation to sprint (or high-intensity exercise) in humans. Previous studies show that Keap1 protein is reduced in human skeletal muscle immediately after high-intensity exercise,[75] although Keap1 pre-exercise levels are recovered shortly after exercise.[75] It remains to be elucidated whether sprint exercise training reduces resting Keap1 protein expression level in human skeletal muscle. Additional experiments are required to determine whether Keap1 dimerization and Keap1 S-lactoylation occur during sprint exercise in humans and how the production of these Keap1 derivatives is modified by training, aging, disease and if they display sexual dimorphism in humans.

Additional experiments manipulating the expression of SDHB would be required to clarify how changes in the expression of this protein could influence sprint performance.

In summary, we have found that the lean mass of the lower extremities and the percentage of type II fibers are the main variables predicting cycling sprint performance. As a novelty, we have shown that SDHB and Keap1 are also associated with sprint performance in humans. Overall, Keap1 emerges as a potential sensor of the glycolytic rate in skeletal muscle to mediate specific adaptations critical for sprint performance.

## AUTHOR CONTRIBUTIONS

The contributions of the authors are as follows: conception and design of the study: VGA, MMR, and JALC; Collection, analysis, and interpretation of data: ALL co-authors; Drafted the manuscript: VGA, MMR, and JALC; Critically evaluated and contributed to the manuscript: ALL co-authors. All authors have approved the final version of the manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

## DATA AVAILABILITY STATEMENT

Deidentified participant data are available from the senior author (ORCID: 0000–0002–9215-6234) on reasonable request for research purposes.

## ORCID

*Victor Galvan-Alvarez* https://orcid.org/0000-0002-1272-3471
*Angel Gallego-Selles* https://orcid.org/0000-0002-8068-9586
*Miriam Martinez-Canton* https://orcid.org/0000-0002-2925-3429
*Ismael Perez-Suarez* https://orcid.org/0000-0002-7969-0061
*Eduardo Garcia-Gonzalez* https://orcid.org/0000-0002-0872-9889
*Marcos Martin-Rincon* https://orcid.org/0000-0002-3685-2331
*Jose A. L. Calbet* https://orcid.org/0000-0002-9215-6234

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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