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Maximal Fat Oxidation Rate in Healthy Young Adults. Influence of Cardiorespiratory Fitness Level and Sex

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ABSTRACT

Introduction: The maximal fat oxidation (MFO) and the exercise intensity that provokes MFO (FATMAX) are inversely associated with cardiometabolic risk factors in healthy young sedentary adults. However, how both cardiorespiratory fitness (CRF) level and sex influence MFO during exercise and the FATMAX is seldom analyzed.

Objectives: This study is aimed at determining the influence of CRF and sex on MFO.

Methods: Twenty healthy young adults (i.e., 12 men and 8 women) completed a graded treadmill protocol to determine MFO, MFO relative to lean mass (MFO_{lean}), FATMAX and maximum oxygen uptake (VO_{2max}).

Results: The *k*-means cluster analysis was used to divide the sample into two different groups for CRF level (56.54 ± 2.54 and 46.94 ± 3.07 mL/kg/min, $p < 0.001$, respectively). The high-level group revealed higher MFO relative to lean mass (MFO_{lean}) (3.34 ± 1.44 and 2.73 ± 0.87 $g \cdot min^{-1} \cdot kg$, $p = 0.001$, respectively), and FATMAX in $km \cdot h^{-1}$ ($FATMAX_v$) (7.67 ± 0.90 and 7.00 ± 0.97 $km \cdot h^{-1}$, $p = 0.044$, respectively) but not for MFO (0.67 ± 0.19 and 0.71 ± 0.20 $p = 0.124$, respectively). When divided for sex, men exhibited higher values for MFO (0.76 ± 0.21 vs. 0.69 ± 0.19 $g \cdot min^{-1}$, $p = 0.039$) and FATMAXv (7.67 ± 0.96 vs. 7.30 ± 0.98 $km \cdot h^{-1}$, $p = 0.036$), while women showed higher values for MFO_{lean} (3.92 ± 1.35 vs. 2.40 ± 0.46 $g \cdot min^{-1} \cdot kg$, $p = 0.015$).

Conclusion: This study highlights the significant influence of CRF level and sex on MFO and FATMAX, offering valuable insights for tailoring exercise programs and optimizing health and performance interventions.

1 | Introduction

Physical inactivity is a major cause of early death (Lee et al. 2012). Sedentary behavior has been linked to an increased risk of obesity, type II diabetes, and cardiovascular disease (Dunstan et al. 2012). Importantly, the capacity to adapt fuel oxidation to fuel availability is called "metabolic flexibility", which may be impaired by obesity and/or type II diabetes (Galgani and Fernández-Verdejo 2021; Smith et al. 2018). Physical activity, which can improve the cardiometabolic profile in different diseases (e.g., obesity, type 2 diabetes), requires a high level of metabolic flexibility to meet its energy demands, mostly fulfilled by skeletal muscle contractions (Goodpaster and Sparks 2017). In this context, the maximal fat oxidation (MFO) during a single

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bout of exercise and the exercise intensity that provokes MFO (i.e., FATMAX; expressed as %VO_2max) are regarded as metabolic flexibility markers (Goodpaster and Sparks 2017). In this regard, MFO and FATMAX values during exercise were inversely associated with cardiometabolic risk factors in healthy young sedentary adults (Montes-de-Oca-García et al. 2021).

Closely related to MFO and FATMAX is cardiorespiratory fitness (CRF) (Montes-de-Oca-García et al. 2021). CRF is referred to as the highest value of oxygen consumption during an exercise test (i.e., VO_2max). Higher CRF levels have been related to a lower risk of all-cause mortality (Kodama et al. 2009). Metabolic flexibility and insulin sensitivity improve after regular physical activity (Rynders et al. 2018) through an increase in mitochondrial biogenesis and activity, as well as an increase in the ability of skeletal muscle for fatty acids oxidation (Goodpaster and Sparks 2017). Nevertheless, to the best of our knowledge, fuel oxidation in populations with different levels of CRF has received little attention, either in healthy individuals or with chronic metabolic diseases.

Fat oxidation rates increase from low to moderate exercise intensities and decline at higher intensities (Brooks and Mercier 1994; Jeukendrup 2002). Strong positive correlations have been previously reported between MFO and VO_2max during an incremental graded treadmill FATMAX test (r = 0.72) (Robinson et al. 2015) in young healthy men, and between fat oxidation and VO_2max during a running interval session (r = 0.86) in well-trained and recreationally trained runners (Hetlelid et al. 2015). Croci and colleagues compared MFO and FATMAX between normal-weight and overweight physically active men with similar CRF (Croci et al. 2014). They found that FATMAX and MFO for both groups (i.e., normal-weight and overweight groups) were similar, suggesting that CRF may be a more critical determinant of fat oxidation during exercise than body composition (Croci et al. 2014).

Sex differences in MFO have also been documented. Venables and colleagues reported that MFO during exercise occurred at a FATMAX of 48% ± 1% VO_2max, with women exhibiting significantly higher values (52% ± 1% VO_2max) compared to men (45% ± 1% VO_2max). This observation is supported by a later study (Frandsen et al. 2021), whose authors noted higher MFO rates in young women than in men, although this sex-related difference diminishes after the age of 45 due to declining estrogen concentrations, independent of CRF. Such findings suggest that CRF levels and sex-specific physiological differences may jointly influence fat oxidation during exercise. Young, physically active individuals are less likely to display metabolic and physiological responses to exercise to be confounded by the effects of age-related changes or chronic conditions, allowing thus the establishment of baseline relationships between CRF, MFO, and FATMAX.

Given these gaps in understanding, this study aims to evaluate how the different CRF levels influence MFO during exercise and FATMAX. Additionally, sex-related differences in fat oxidation are examined, considering prior evidence that women may cope better with fatigue (Frandsen et al. 2021). It is hypothesized that: (i) CRF level influences MFO and FATMAX, and (ii) women present higher MFO values than men when considering body composition. Understanding these relationships could provide valuable insights into tailoring health interventions. For instance, recognizing the role of CRF and sex differences in MFO may help design personalized exercise programs to optimize fat utilization and improve metabolic health, particularly in populations at risk for cardiometabolic diseases.

2 | Materials and Methods

2.1 | Participants

Twenty sports sciences students (i.e., 12 men [Body weight: 80 ± 9 kg, Lean mass: 22% ± 5%] and 8 women [Body weight: 60 ± 8 kg, Lean mass: 26% ± 3%]) voluntarily participated in this study. All participants met the following inclusion criteria: (i) older than 18 and younger than 30 years old, (ii) free from injuries within the 6 months before data collection, and (iii) physically active according to the guidelines of the American College of Sports Medicine (Thompson et al. 2013).

After receiving information on the study procedures, every participant signed an informed consent. Ethics approval was obtained from the Committee of the University of Granada, and the study was performed following the ethical standards of the Declaration of Helsinki.

2.2 | Procedures

2.2.1 | Experimental Design

A crossover study design was utilized to assess whether MFO and FATMAX are influenced by CRF level and the individual's sex. Participants completed a single-session protocol.

2.2.2 | Body Composition

Body mass (in kg) and both lean and fat mass percentages (in %), which directly influence metabolic flexibility and fat oxidation during exercise, were obtained by bioimpedance analysis (Inbody 230, Inbody, Seoul, Korea) (Ling et al. 2011). Participants were encouraged to follow the American Society of Exercise Physiologists' recommendations before the testing session (Heyward, 2001).

2.2.3 | Graded Exercise Test

A graded exercise test was carried out on a treadmill (WOODWAY Pro XL, Woodway Inc., Waukesha, WI, USA) to assess CRF. Participants ran for 10 min at a comfortable self-selected velocity, followed by three 30-s bouts at a moderate to high self-selected speed to finish the warm-up. After warming up, participants were instrumented with a portable metabolic analyzer (PNOE, ENDO Medical, Palo Alto, CA) (Tsekouras et al. 2019). The metabolic cart was calibrated according to the manufacturer's instructions with ambient calibration. The graded exercise protocol was designed to obtain CRF and metabolic responses to exercise such as MFO, FATMAX, and VO_2max within the same testing session (Amaro-Gahete et al. 2019). Participants started at 5 km/h, and velocity was increased by 1 km/h every 3 min

TABLE 1 | A comparison between cardiorespiratory fitness level groups in terms of VO_2max for the metabolic variables obtained during the incremental test (mean ± SD).

	LLG (*n* = 11)	HLG (*n* = 9)	*p*	ES (d)
VO_{2max} (mL/kg/min)	46.94 ± 3.07	56.54 ± 2.54	**< 0.001**	3.23
MFO ($g \cdot min^{-1}$)	0.67 ± 0.19	0.71 ± 0.20	0.124	0.20
MFO_{lean} ($g \cdot min^{-1} \cdot kg$)	2.73 ± 0.87	3.34 ± 1.44	**0.001**	0.50
$FATMAX_v$ ($km \cdot h^{-1}$)	7.00 ± 0.97	7.67 ± 0.90	**0.044**	0.68
FATMAX (%VO_{2max})	56.98 ± 7.64	54.28 ± 7.90	0.739	0.33

Abbreviations: FATMAX, percentage of the VO_2max that elicits MFO; $FATMAX_V$, velocity at which exercise intensity elicits MFO; HLG, high-level group; LLG, low-level group; MFO, maximal fat oxidation rate; MFO_{lean}, maximal fat oxidation rate corrected by lean mass; VO_2max, maximum oxygen uptake.
Note: Bold indicates $p < 0.05$.

until the respiratory gas exchange rate was ≥ 1.0. Then, velocity remained constant while gradient increased 2% every minute until volitional exhaustion. This ensured that the data on fat oxidation are obtained across a range of intensities, which is essential for analyzing the relationship between CRF levels and fat oxidation during exercise.

2.2.4 | Maximum Oxygen Uptake and MFO Determination

VO2max was determined as the highest 30-s rolling mean value. Mean values of O_2 and CO_2 for the last 60 s in each 3-min step were considered for calculations. Total fat oxidation rates were calculated using Frayn's equation (Frayn 1983), assuming that urinary nitrogen excretion rate was negligible. Values for fat oxidation, calculated for every participant, were shown graphically as a function of exercise intensity (FATMAX, in %VO_2max), and a second polynomial curve with intersection in (0,0) was constructed to determine MFO ($g \cdot min^{-1}$), MFO relative to lean mass (MFOlean), and FATMAX individually (Amaro-Gahete et al. 2019). Regarding same absolute exercise intensities, values for fat oxidation were also depicted as a function of running velocity (FATMAXv, in $km \cdot h^{-1}$), and a second polynomial curve without intersection in (0,0) was constructed. Environmental conditions remained constant during testing trials (i.e., ~21°C temperature and ~60% humidity), and perspiration was minimized using a fan placed in front of the treadmill during the entire protocol. Participants were asked to avoid vigorous activity 48 h before testing and to be in at least a 4-h fasting state. Women were evaluated during their luteal phase, determined by the calendar-based counting method (Chidi-Ogbolu and Baar 2019).

2.3 | Statistical Analysis

Descriptive statistics are represented as mean (SD), as well as percentages (%). Tests of normal distribution and homogeneity (Kolmogorov–Smirnov and Levene's tests, respectively) were conducted before analysis. Mean comparisons (i.e., *t*-tests) were performed between sexes for all variables (i.e., body composition and metabolic variables). A *k*-means cluster analysis was performed according to the VO_2max relative to body mass (mL/kg/min), allowing the possibility to split the group into two different CRF level groups: (i) low-level group (LLG, *n* = 11, six men and five women) with a VO_2max of 46.9 ± 3.1 mL/kg/min, and (ii) high-level group (HLG, *n* = 9, six men and three women) with a VO_2max of 56.5 ± 2.5 mL/kg/min. The authors considered sex as a covariate for the comparisons between level groups (analysis of covariance, ANCOVA). Chi-squared test was implemented to analyze between-group differences in sex. The Hedges g effect size (ES) was also calculated to determine the magnitude of the differences, interpreted as follows: Trivial (< 0.2), small (0.2–0.59), moderate (0.60–1.19), large (1.20–2.0), and extremely large (> 2.0) (Hopkins et al. 2009). The level of significance was set at an alpha level of 0.05. Data analysis was performed using the SPSS (version 21, SPSS Inc. Chicago, IL).

TABLE 2 | Sex comparison for the metabolic variables obtained during the incremental test (mean ± SD).

	Men (*n* = 12)	Women (*n* = 8)	*p*	ES
MFO ($g \cdot min^{-1}$)	0.76 ± 0.21	0.58 ± 0.10	**0.039**	0.98
MFO_{lean} ($g \cdot min^{-1} \cdot kg$)	2.40 ± 0.46	3.92 ± 1.35	**0.015**	1.59
$FATMAX_v$ ($km \cdot h^{-1}$)	7.67 ± 0.96	6.75 ± 0.75	**0.036**.	1.00
VO_{2max} (mL/kg/min)	52.48 ± 5.74	49.42 ± 5.27	0.243	0.53
FATMAX (%VO_{2max})	55.31 ± 8.99	56.44 ± 5.65	0.757	0.14

Abbreviations: FATMAX, percentage of the VO_2max that elicits MFO; $FATMAX_V$, velocity at which exercise intensity elicits MFO; MFO, maximal fat oxidation rate; MFO_{lean}, maximal fat oxidation rate corrected by lean mass; VO_2max, maximum oxygen uptake.
Note: Bold indicates $p < 0.05$.

3 | Results

3.1 | Cardiorespiratory Fitness

When splitting our sample into two different groups for CRF level comparison (LLG vs. HLG), the HLG showed higher values (all $p < 0.05$) than the LLG for VO_{2max} (56.54 ± 2.54 and 46.94 ± 3.07 mL/kg/min, $p < 0.001$, respectively), MFO_{lean}

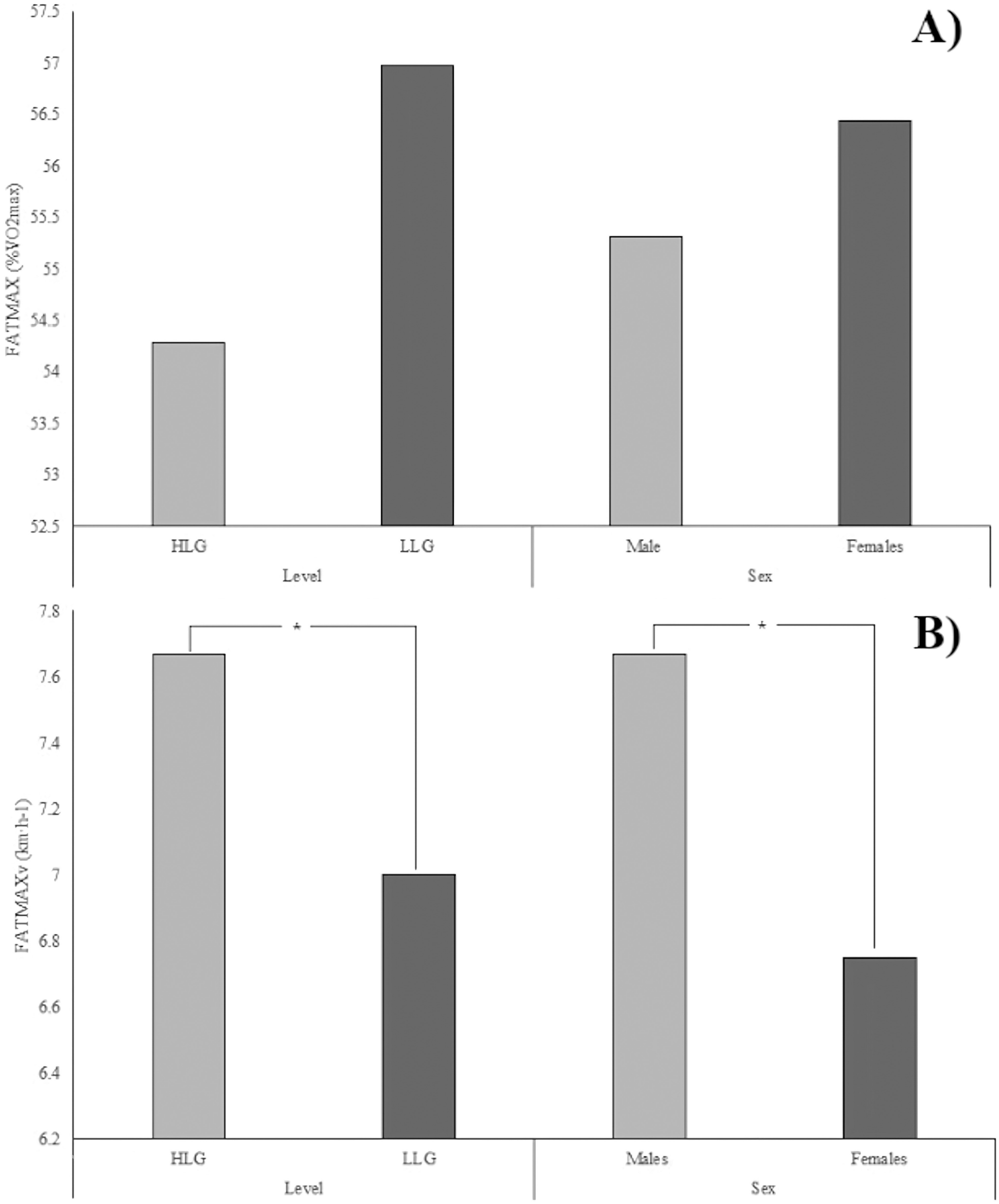

FIGURE 1 | Sex and level comparison for the FATMAX variables obtained during the incremental test. $FATMAX_V$, velocity at which exercise intensity elicits maximal fat oxidation rate; FATMAX, percentage of the maximum oxygen uptake that elicits maximal fat oxidation rate; HLG, high-level group; LLG, low-level group.

(3.34 ± 1.44 and $2.73 \pm 0.87\,g \cdot min^{-1} \cdot kg$, $p = 0.001$, respectively), and $FATMAX_V$ (7.67 ± 0.90 and $7.00 \pm 0.97\,km \cdot h^{-1}$, $p = 0.044$, respectively) (Table 1). No significant differences between LLG and HLG were found ($p > 0.05$) for MFO and FATMAX. In terms of CRF level, the Chi-squared test also revealed no significant between-group differences in sex ($p = 0.582$).

3.2 | Sex Comparison

Men exhibited higher values for MFO (0.76 ± 0.21 vs. $0.69 \pm 0.19\,g \cdot min^{-1}$, $p = 0.039$) and $FATMAX_V$ (7.67 ± 0.96 vs. $7.30 \pm 0.98\,km \cdot h^{-1}$, $p = 0.036$), while women showed higher values for MFO_{lean} (3.92 ± 1.35 vs. $2.40 \pm 0.46\,g \cdot min^{-1} \cdot kg$, $p = 0.015$) (Table 2). No significant differences were obtained for VO_{2max} (Women: 49.42 ± 5.27 mL/kg/min; Men: 52.48 ± 5.74 mL/kg/min, $p = 0.243$) and FATMAX (Women: $56.44\% \pm 5.65\%$ VO_{2max}; Men: $55.31\% \pm 8.99\%$ VO_{2max}, $p = 0.757$) (Table 2).

The Figures 1 and 2 reinforce the presentation of the aforementioned results, including the between-level and between-sex comparisons.

4 | Discussion

This study aimed to assess the influence of CRF level (i.e., VO_2max) on MFO (absolute and relative to lean body mass) and FATMAX (expressed as $\%VO_2max$ and km/h) as well as to evaluate the differences between physically active young men and

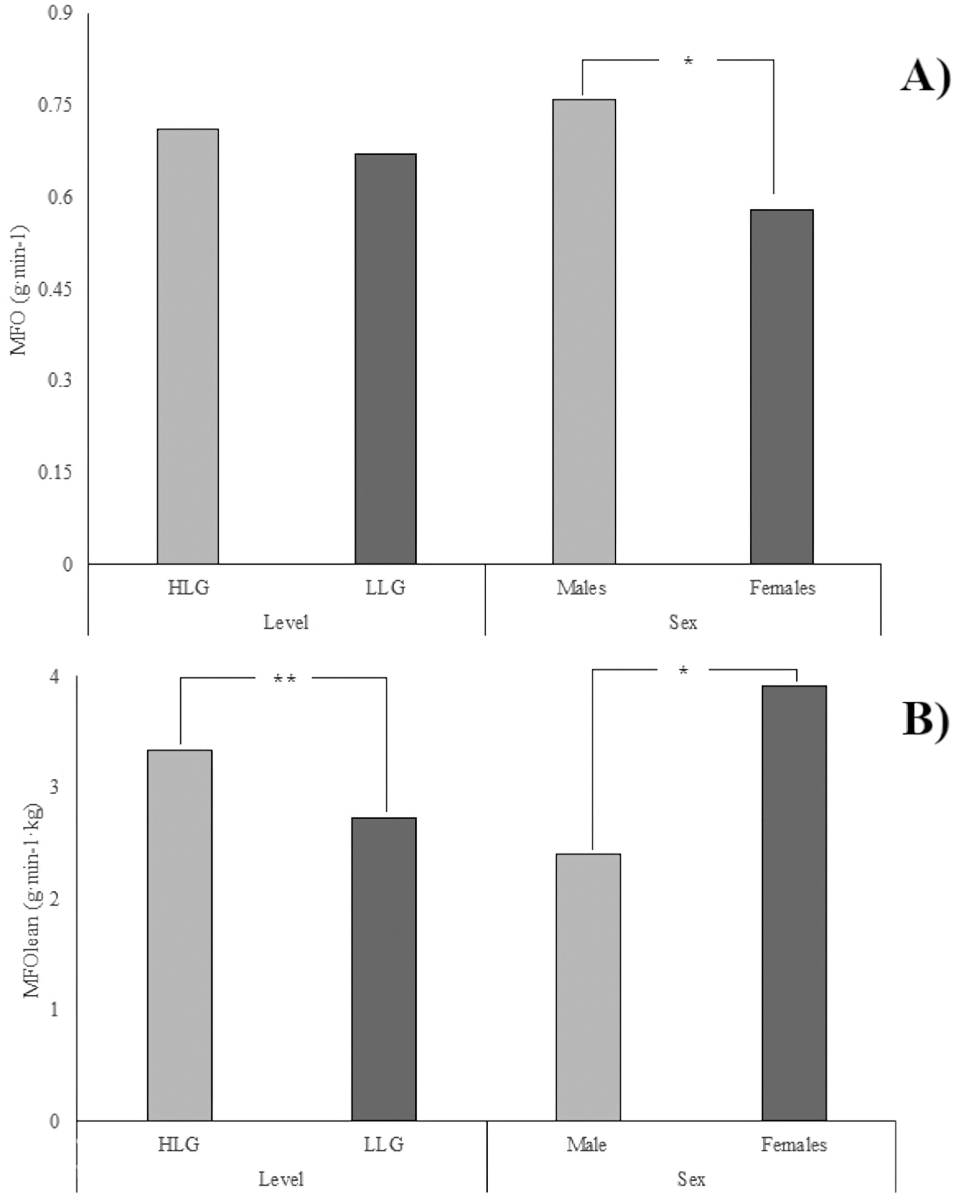

FIGURE 2 | Sex and level comparison for the maximal fat oxidation rate variables obtained during the incremental test. HLG, high-level group; LLG, low-level group; MFO, maximal fat oxidation rate; MFO_{lean}, maximal fat oxidation rate corrected by lean mass.

women regarding fat oxidation. It has been demonstrated that although men exhibited significantly higher values for MFO, women had higher MFOlean. Furthermore, while the CRF level influenced MFOlean and FATMAXv, it had no effect on MFO or FATMAX.

The findings regarding CRF, when grouping the sample into two levels (i.e., LLG vs. HLG; $p<0.05$; $VO_2max = 46.94 \pm 3.07$ and 56.54 ± 2.54 mL/kg/min, respectively), are consistent with Venables, Achten, and Jeukendrup (2005), who stated that fat oxidation rates rely on CRF. In our study, the HLG exhibited significantly higher values for both MFOlean (3.34 ± 1.44 g·min^{-1}·kg^{-1}) and FATMAXv (7.67 ± 0.90 km/h), reinforcing the notion that the higher the fitness level, the greater the ability to oxidize fat. Interestingly, Robinson et al. (2015) determined that increasing MFO during exercise also impacts resting fat oxidation. Although not measured in the present study, resting metabolic rate contributes up to 75% of daily energy expenditure (Ravussin et al. 1986), which could be a key factor in weight management.

When analyzing differences in fat oxidation between men and women, our findings support the initial hypothesis that women present a higher capacity to oxidize fat than men. Although men exhibited higher MFO values compared to women (0.76 ± 0.21 vs. 0.58 ± 0.10 g·min^{-1}; $p = 0.039$), when MFO was reported relative to lean mass (i.e., MFOlean), women exhibited higher values than men (3.92 ± 1.35 vs. 2.40 ± 0.46 g·min^{-1}·kg^{-1}, respectively; $p = 0.015$). This is consistent with previous research (Frandsen et al. 2021), which also found that women had higher MFOlean values than men (10.96 ± 0.30 vs. 8.73 ± 0.25 mg/min/kg lean body mass, $p < 0.001$). Differences between studies might be due to variations in the methods used (i.e., treadmill protocol vs. treadmill + cycloergometer protocol), as these affect the results (Amaro-Gahete et al. 2019). Our findings also align with Venables, Achten, and Jeukendrup (2005) regarding FATMAX, where men exhibited lower FATMAX values than women (55.31 ± 8.99 vs. 56.44% ± 5.65% VO_2max, $p = 0.757$), although this difference was not statistically significant in our study. Differences in participant characteristics (age: 21 ± 1.86 years; VO_2max: 51.26 ± 5.63 mL/kg/min) and statistical analyses may explain these discrepancies (Amaro-Gahete et al. 2019). Notably, women reported significantly lower FATMAXv values than men (6.75 ± 0.75 vs. 7.67 ± 0.96 km/h, $p = 0.036$), suggesting that women oxidize more fat relative to lean mass at lower velocities.

The findings of this study have significant practical applications for designing personalized exercise programs, optimizing health interventions, and enhancing athletic performance. Improving CRF through structured physical activity can boost fat oxidation, making it particularly beneficial for individuals at risk of cardiometabolic diseases. The observed sex differences in fat oxidation suggest that women, especially premenopausal, may benefit from moderate-intensity exercises tailored to their physiology. For athletes, training to enhance CRF could optimize energy utilization and endurance. In clinical and rehabilitation settings, assessing CRF and FATMAX can guide recovery-focused exercise plans, while for older adults, improving CRF may counteract age-related metabolic declines. These insights also support public health initiatives aimed at tailoring exercise recommendations to individual needs, promoting overall metabolic health and well-being across diverse populations.

Despite the valuable insights provided by this study, several limitations must be considered. The sample size could not be enough to detect small differences between groups. Also, other variables as fatigue, familiarization with the protocol or other external factors as daily intake may conditioned the results. Although the calendar-based method might not be ideal for identifying menstrual phases (Chidi-Ogbolu and Baar 2019), all female participants were eumenorrheic. Despite this method's limitations, all women participated during the luteal phase of their menstrual cycle, which is relevant because the estrogen-progesterone ratio in this phase can affect fat oxidation (i.e., estrogen stimulates fat oxidation, while progesterone hinders it) (Elliott-Sale et al. 2021). Thus, sex differences in fat use during exercise may be under control in this study. Another limitation is that fat intake was not controlled, and individual variation in MFO may be influenced by fat consumption during exercise (Fletcher et al. 2017). Additionally, the findings are based only on healthy, young, physically active adults, what might limit their generalizability to other populations (e.g., elderly, obesity, type 2 diabetes). Nevertheless, young, physically active individuals are less likely to exhibit exercise-induced metabolic and physiological responses influenced by age-related changes or chronic conditions, enabling the identification of baseline relationships between CRF, MFO, and FATMAX. Future studies should investigate the relationship between CRF, MFO, and FATMAX in broader populations (e.g., older adults, individuals with obesity) and examine how menstrual phases, hormonal variations, and resting metabolic rate influence fat oxidation. These insights could refine personalized exercise recommendations and metabolic health strategies.

5 | Conclusion

When MFO was reported relative to lean mass, it seems that both CRF level and sex influence fat oxidation rates. It has been shown here that the higher the CRF level, the higher the rate of fat oxidation. Additionally, our study suggests that women can oxidate higher amounts of fat when MFO is reported relative to lean mass. The findings of this study underscore the significant role of CRF level and sex in determining MFO during exercise and its associated exercise intensity (FATMAX). These insights have practical implications for designing personalized exercise programs, optimizing health interventions, and enhancing athletic performance.

Author Contributions

Felipe García-Pinillos, Diego Jaén-Carrillo: conceptualization. **Felipe García-Pinillos, Abel Plaza-Florido, Diego Jaén-Carrillo:** methodology. **Santiago A. Ruiz-Alias, Felipe García-Pinillos:** software. **Abel Plaza-Florido, Felipe García-Pinillos:** validation. **Diego Jaén-Carrillo, Santiago A. Ruiz-Alias:** formal analysis. **Diego Jaén-Carrillo, Iván Fernández-Navarrete, Leonardo Riquelme-Sebastián, Santiago A. Ruiz-Alias:** investigation. **Felipe García-Pinillos:** resources. **Felipe García-Pinillos, Iván Fernández-Navarrete, Leonardo Riquelme-Sebastián, Abel Plaza-Florido:** data curation. **Diego Jaén-Carrillo, Iván Fernández-Navarrete, Leonardo Riquelme-Sebastián:** writing – original draft preparation. **Felipe García-Pinillos, Abel Plaza-Florido, Iván Fernández-Navarrete, Leonardo Riquelme-Sebastián, Santiago A. Ruiz-Alias:** writing – review and editing. **Diego Jaén-Carrillo:** visualization. **Felipe García-Pinillos, Abel Plaza-Florido:** supervision. **Felipe García-Pinillos:** project administration. **Santiago A. Ruiz-Alias:** funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Research data are not shared.

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