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Maximal fat oxidation capacity is associated with cardiometabolic risk factors in healthy young adults

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Abstract

It is unknown whether resting fat oxidation (RFO), maximal fat oxidation (MFO) and FatMax (intensity at which MFO is reached) are related to cardiometabolic risk (CMR). Thus the aim of this study was to examine the association of RFO, MFO and FatMax with CMR. 81 healthy adults (n = 31 women; 22.72 ± 4.40 years) participated in this cross-sectional study. Glucose and triglycerides were analysed in plasma. Body composition, anthropometry, physical activity, blood pressure (BP) and heart rate measurements were taken. RFO and MFO were determined through indirect calorimetry. Maximal oxygen uptake (VO_2max) test was performed until exhaustion after MFO test. The CMR cluster was created from individual CMR factors: waist circumference, body fat percentage, systolic BP, diastolic BP, blood glucose and plasma triglycerides. Groups of high and low MFO and VO_2max were created. RFO was not associated with CMR (p < 0.05). FatMax, MFO and VO_2max were associated with individual CMR factors as waist circumference (R^2 = 0.144; R^2 = 0.241; R^2 = 0.285; p = 0.001; respectively) and plasma triglycerides (R^2 = 0.111; p = 0.004 and R^2 = 0.130; p = 0.002 and R^2 = 0.093; p = 0.008; respectively) and clustered CMR factors (R^2 = 0.105; p = 0.008 and R^2 = 0.162; p = 0.001 and R^2 = 0.239; p = 0.001; respectively). VO_2max was also associated with body fat percentage (R^2 = 0.105; p = 0.003) and diastolic BP (R^2 = 0.083; p = 0.01), even adjusting for sex or age (p < 0.05). Groups with high level of MFO or VO_2max obtained lower CMR (p = 0.001), even adjusting for sex or age (p < 0.01). FatMax, MFO and, especially, VO_2max are associated with CMR, regardless of age and sex. However, RFO is not associated with CMR.

Keywords: *Cardiovascular disease, cardiorespiratory fitness, obesity, exercise, metabolic syndrome, lipid metabolism*

Highlights

- The main finding of this study is that Maximal Fat Oxidation, FatMax and VO_2max, but not Resting Fat Oxidation, are associated with cardiometabolic risk.
- The present study also shows that Maximal Fat Oxidation test could be an alternative and useful new tool to estimate cardiometabolic risk.
- In agreement with previous studies, our results showed that women had higher Maximal Fat Oxidation values and lower cardiometabolic risk than men.

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Introduction

Cardiometabolic diseases are the leading cause of mortality in the world. In fact, both the prevalence and the incidence of cardiometabolic diseases are continuously increasing, being both sedentary behaviour and other unhealthy habits the main causes (Tikkanen, Gustafsson, & Ingelsson, 2018). Several studies have summarized the associations between physical fitness, fatness and cardiometabolic risk (CMR) factors from childhood to adulthood (Myers et al., 2015; Rebollo-Ramos et al., 2019; Tikkanen et al., 2018). In particular, high levels of cardiorespiratory fitness have been associated with lower risk for cardiovascular diseases (CVD) in apparently healthy, young to middle-aged adults (Swainson, Ingle, & Carroll, 2019). Indeed, cardiorespiratory fitness is considered one of the most important predictors of cardiovascular risk (Myers et al., 2015; Rebollo-Ramos et al., 2019; Tikkanen et al., 2018).

Maximal oxygen uptake (VO_2max) evaluated through a maximum exercise test constitutes a physiologic stress that may imply a greater risk to people with various diagnoses diseases than people without pathology or impairments. To avoid that, many protocols have been developed to estimate VO_2max with a submaximal exercise testing as a validated alternative. However, maximal fat oxidation (MFO) test (Achten, Gleeson, & Jeukendrup, 2002) could also provide a safe and practical method of assessing cardiometabolic fitness in both healthy adults and patients under submaximal conditions providing an alternative tool, not only for cardiometabolic prediction but also to measure metabolic flexibility, which explains insulin resistance and mechanisms governing fuel selection during exercise. Generally, the highest rates of fat oxidation can be found at low to moderate exercise intensities (i.e. range between 33% and 65% of VO_2max) and the test ends when subject reaches a Respiratory Exchange Ratio (RER) = 1.0 (Achten et al., 2002). Moreover, MFO has shown to be related to some individual CMR factors such as insulin resistance, obesity, plasma triglycerides and body weight (Cancino-Ramírez et al., 2018; Kelley, Goodpaster, Wing, & Simoneau, 2017; Robinson, Hattersley, Frost, Chambers, & Wallis, 2015; Rosenkilde, Nordby, Nielsen, Stallknecht, & Helge, 2010).

Body weight is one of the main CMR factors (Salmenniemi et al., 2004). Obesity significantly alters muscle metabolic capacities to oxidize fat during exercise, so that MFO should be lower in obese compared to lean subjects (Salmenniemi et al., 2004). In fact, it has been previously observed that obese subjects have higher MFO when aerobic capacity is taken into account, but when expressed with respect to lean mass, normal weight individuals show a greater capacity to oxidize fat during exercise (Amaro-Gahete, Sanchez-Delgado, Ara, & Ruiz, 2019). These findings suggest that obese individuals may suffer from metabolic inflexibility during exercise, which is characterized by reduced fat oxidation by the skeletal muscles under fasting conditions and in the postprandial state (Amaro-Gahete, Sanchez-Delgado, Ara, et al., 2019). In addition, it has been observed that the relative intensity at which MFO is reached (FatMax; expressed as %VO_2max) (Jeukendrup & Achten, 2001) occurs at a lower percentage of VO_2max in obese subjects (Amaro-Gahete, Sanchez-Delgado, & Ruiz, 2018). The rationale of considering FatMax in relation to health lies in the fact that higher levels of FatMax would imply a wider range of activities at which fat is the main substrate. Indeed, FatMax intensity training is recommended as a therapeutic tool for the treatment of various cardiometabolic disorders (Jiang et al., 2020). However, little is known about the association between FatMax and CMR factors.

Regarding fat oxidation, a recent study showed that MFO, but not resting fat oxidation (RFO), is related to cardiometabolic health (Karppinen et al., 2019). Nevertheless, no studies have been found to date in which RFO, MFO or FatMax are associated with individual and clustered CMR factors. Therefore, the main objective of this study is to determine the association between RFO, MFO and FatMax with CMR, taking into account the influence of VO_2max, in healthy young adults with different metabolic phenotype.

Material and methods

Subjects

Participants were recruited to participate in this cross-sectional study from the "NutAF" project (Rebollo-Ramos et al., 2019). A total of 115 participants met the inclusion criteria and were enrolled in the measurements. Finally, 81 healthy participants (n = 31 females) completed all the tests and were included in the present study. However, it should be noted that not all participants underwent blood tests, so the statistical analyses that included the variables of glucose and triglycerides had a smaller sample (n = 74; 28 women). All assessments were performed from January 2016 to June 2017. Written informed consent was obtained by the participants after being informed about the nature of the study, its protocol and possible risks arising from the measurements. The study was approved by the Ethical Committee of the Hospital Puerta del Mar (Cadiz, Spain), in

accordance with the Declaration of Helsinki. The inclusion criteria were: being between 18 and 45 years old, having a stable body weight (±2 kg) during the last 6 months and having a Body Mass Index (BMI) between 18.5 and 40 kg m^{-2}. On the other hand, the exclusion criteria were: having made a specific diet during the last 6 months and/or suffering any illness or injury that prevented physical exercise.

Procedure

Initially, the participants completed a questionnaire designed for this project confirming compliance with the inclusion criteria and in which they signed the written informed consent. The next day, the selected participants went to the University of Cadiz to perform the different tests proposed. On the day of measurements, fasting blood samples for biochemical determinations and laboratory tests were performed, including measurements of heart rate and blood pressure, anthropometry and body composition, physical activity, basal metabolism and MFO, FatMax and VO_2max. Participants were asked to attend the laboratory after a fasting period of at least 8 h, and they were instructed to avoid intense physical activity the day before, to maintain their usual diet and to avoid the intake of alcohol and caffeine the day before. All tests were performed in the morning (from 08:30 until 11:30), in order to avoid possible variations between participants due to the circadian rhythm (Amaro-Gahete, Jurado-Fasoli, Triviño, et al., 2019).

Heart rate and blood pressure. For the heart rate and blood pressure measurements, the participants were sitting in a chair, relaxed and with their feet on the floor. After 10 min, measurements of systolic and diastolic blood pressure and resting heart rate in the non-dominant arm were evaluated with an Omron M3 digital blood pressure monitor (HEM-7051-E). The average of three measurements, taken one minute apart, was used for analyses. During the tests, the heart rate was continuously measured with a Polar Team 2 (Polar Electro Inc., Lake Success, NY).

Blood extraction and plasma biochemical parameters. Fasting blood samples were collected in tubes containing EDTA from the antecubital vein and centrifuged to obtain plasma at 2500 rpm for 15 min at 4°C. Clotted and hemolysed samples were discarded. Plasma was stored at −80°C for subsequent analysis. At the time of the analysis, they were thawed on ice and then pipetted in duplicates of 10 µL into the microplates with 200 µL of the specific reagent from each commercial kit. In all plates, pattern curves of known concentrations of each parameter in question were added, in order to identify the absorbance data. For the analysis, the instructions of the manufacturer Spinreact (Spinreact SA, Sant Esteve d'en Bas, Gerona, Spain) were followed and, by adapting the measurements to 96-well microplates, the metabolic parameters were analysed in plasma including the levels of blood glucose (Glucose-HK Ref. 1001200) and triglycerides (TAG: Ref. 1001311). Subsequently, the microplates were introduced in a BIO-TEK PowerWave^{TM} 340 microplate reader and the absorbance readings were processed with the BIO-TEK KC Junior^{TM} program.

Anthropometry and body composition. Height was measured in a standing position, after normal expiration, using a height rod (SECA 225, range of 60–200 cm; precision of 1 mm). Waist circumference was measured using a plastic anthropometric tape (SECA 201; range of 0–205 cm; precision of 1 mm) at midpoint between the costal margin and iliac crest in the mid-axillary line in standing position at the end of a gentle expiration. The measurements were performed twice, and the average values were used for the analysis. Body weight (kg), body fat (kg and %) and fat-free mass (kg and %) were evaluated using a multi-frequency bioimpedance of 8 electrodes (TANITA-MC780MA). BMI was determined as weight (kg) divided by the square of height (m^2). The subjects wore light clothing and adopted a specific posture according to the manufacturer's instructions. Also, the participants had to urinate before the test, which should be done without any metallic object in the body that could alter the results.

Physical activity. The short IPAQ (International Physical Activity Questionnaire) form "last 7 days recall" was used to evaluate the physical activity of the participants (Craig et al., 2003). The subjects were then classified as either physically inactive or physically active according to the recommendations for 18–64 years old adults of the World Health Organization (WHO) (World Health Organization, 2011).

Basal metabolism. Oxygen uptake (VO_2) and carbon dioxide production (VCO_2) were registered in resting conditions lying on a bed in a supine position for 30 min for calculating RER (Respiratory Exchange Rario) and fat oxidation. A mask was placed on the subject's face to collect gas samples. An indirect circuit gas analyser, Jaeger MasterScreen CPX® (CareFusion, San Diego, USA) was used to register indirect calorimetry data. Calibrations were daily performed before each measurement. During the test, the gas analyser values were captured

breath-by-breathe and averaged every 20 s. For the analysis of these variables, the first 5 min of the evaluation were eliminated and a stable period of 5 min was selected with a coefficient of variation for VO_2 and VCO_2 lower than 15%. The average values of VO_2 and VCO_2 in the selected time interval were used to calculate RFO by an indirect equation proposed by Frayn (1983). Similarly, the average value of VO_2 was used to calculate the relative intensity (% VO_2max) during the rest state. The RER value was calculated as its average during the entire registration, except for the first 5 min of the test.

MFO, FatMax and VO_2max. An incremental protocol in cycloergometer (Lode Excalibur, Groningen, Netherlands) was designed from the standardized protocol (Achten et al., 2002) with two consecutive phases to determine MFO, FatMax and VO_2max. The first phase, for the determination of MFO and FatMax, consisted of 3-min steps with 15W increments in overweight/obese subjects and 30W in subjects with normal weight, with a maintained pedalling rate between 60 and 80 rpm. This differentiation between protocols depending on the weight status is based on protocols previously used in each population (Dandanell et al., 2017). This phase was interrupted when RER $\geq$ 1. After a brief pause (between 3 and 5 min), the second phase to detect VO_2max was initiated. This phase began at the load at which phase 1 ended, and continued with 1-min steps increasing at the same load rate as in phase 1, with equal cadence. This phase ended when the participant reached exhaustion. The protocol was considered maximum when the VO_2 reached a plateau, the theoretical maximal heart rate was reached and when RER $\geq$ 1.10. When the aforementioned maximality criteria were not met, the VO_2peak (maximal oxygen uptake plateau is not observed, but a peak) was used. RER, VO_2 and VCO_2 were measured by indirect calorimetry (Jaeger MasterScreen CPX®; CareFusion, San Diego, USA). To calculate the fat oxidation in the different steps of the protocol, the average values of VO_2 and VCO_2 were used in the last 60 s of each step of the test, again applying the Frayn equation (Frayn, 1983). Similarly, the average value of VO_2 was used to determine the % VO_2max reached in each step. With the values obtained from fat oxidation and % VO_2max in each step, a polynomial curve that best fits the results of the present analysis was drawn for each participant.

Statistical analysis. Parametric tests were performed, since the normal distribution of the variables was previously verified by Kolmogorov–Smirnov test. To determine the heterogeneity of the sample, a descriptive analysis with ANOVA was performed in order to establish differences between sex, BMI and level of physical activity. A CMR cluster was created from the sum of the sex-standardized values (Z-score) [(value – mean)/standard deviation] of waist circumference, body fat percentage, systolic blood pressure, diastolic blood pressure, blood glucose and plasma triglycerides. Z-score for each variable was estimated in the complete sample in order to show a representative Z-score and then the study sample was selected according to the inclusion criteria aforementioned. To verify the extent to which RFO, MFO, FatMax and VO_2max are related to both individual and clustered CMR factors, linear regression analyses were performed by including each of these variables as exposures. Specifically, for the associations between MFO and VO_2max with clustered CMR factors, four different linear regression models were performed: unadjusted (model 1), and adjusted by sex (model 2), age (model 3) and VO_2max or MFO relativized to the lean mass of legs/height^2 (model 4). VO_2max and MFO were relativized in such a way with the objective of taking into account not only the lean body mass, but also the body surface (Ponce González et al., 2017). Then, VO_2max and MFO were categorized in high and low levels by dividing the variables in tertiles and grouping them into low (tertile 1) and high levels (tertiles 2 and 3) (Amaro-Gahete et al., 2019; Yu, Yau, Ho, & Woo, 2013). To determine the differences in clustered CMR between high and low MFO and VO_2max, an ANCOVA was performed. Sex and age were used as adjustment variables. The level of significance was set at $p < 0.05$. IBM SPSS Statistics 22 program was used for the analyses.

Results

The sample was heterogeneous, since it was composed of 17 obese people (n = 8 women), 14 overweight people (n = 5 women) and 50 people with normal weight (n = 18 women). In this regard, statistically significant differences were found in physical activity (p = 0.013) and VO_2max relativized to lean mass of the legs / height^2 (p = 0.001) between the different groups of BMI. Table I describes the main characteristics of the sample and the differences between men and women. Regarding body composition, statistically significant differences were found between men and women in height (p = 0.001; higher in men), lean body mass (p = 0.001; higher in men) and body fat percentage (p = 0.001; higher in women). Moreover, significant differences were found in systolic blood pressure (p = 0.002; higher in men), blood glucose (p = 0.008; higher in men),

Table I. General characteristics of total sample and differences between men and women.

	Total (n = 81)	Men (n = 50)	Women (n = 31)	P
Age (years)	22.72 ± 4.40	22.21 ± 3.50	23.54 ± 5.55	0.186
Height (cm)	171.80 ± 8.65	176.27 ± 6.36	164.43 ± 6.68	**0.001**
Body Mass (kg)	75.99 ± 15.70	78.47 ± 14.36	71.92 ± 17.16	0.067
BMI (kg m^{-2})	25.81 ± 5.61	25.20 ± 4.02	26.81 ± 7.51	0.212
Lean Body Mass (kg)	54.17 ± 8.72	59.24 ± 6.33	45.84 ± 4.83	**0.001**
Body fat (%)	23.21 ± 9.94	18.96 ± 7.40	30.21 ± 9.70	**0.001**
Waist circumference (cm)	83.02 ± 14.48	89.41 ± 1.51	75.24 ± 1.80	**0.001***
Physical activity (min/week)	402.10 ± 244.4	429.8 ± 229.13	357.42 ± 264.91	**0.029***
Systolic Blood Pressure (mmHg)	114.20 ± 10.09	116.84 ± 8.07	109.81 ± 11.63	**0.002**
Diastolic Blood Pressure (mmHg)	68.95 ± 9.64	67.50 ± 9.01	71.36 ± 10.31	**0.001***
Plasma glucose (mg/dL)	101.01 ± 10.17	103.43 ± 9.64	97.03 ± 9.92	**0.008**
Plasma triglycerides (mg/dL)	69.86 ± 24.73	77.82 ± 3.87	63.06 ± 4.60	**0.025***
Resting Fat Oxidation (mg min^{-1})	100.71 ± 27.51	104.04 ± 29.66	95.35 ± 23.10	0.169
BM VO_2max (mL kg^{-1} of BM min^{-1})	41.08 ± 11.62	45.54 ± 10.16	33.88 ± 10.22	**0.001**
LBM VO_2max (mL kg^{-1} of LBM min^{-1})	55.93 ± 11.47	59.24 ± 10.73	50.61 ± 10.72	**0.001**
Respiratory Exchange Ratio (RER)	0.77 ± 0.05	0.78 ± 0.05	0.75 ± 0.05	**0.020**
FatMax (% VO_2max)	41.05 ± 7.08	39.69 ± 7.72	43.23 ± 5.34	**0.028**
Absolute MFO (g min^{-1})	0.37 ± 0.15	0.39 ± 0.16	0.32 ± 0.10	**0.026**
Relativized MFO (mg $(kg\ m^{-2})^{-1}$ min^{-1})	6.85 ± 2.92	6.23 ± 2.74	7.87 ± 2.97	**0.013**
Clustered CMR (Z-score)	0.47 ± 3.28	1.35 ± 0.42	−0.88 ± 0.51	**0.003***

Statistically significant differences ($p < 0.05$) appear in bold. (*) Indicates that differences were only statistically significant after adjusting for age and VO_2max relativized to body mass. Values are expressed as mean ± standard deviation.
Abbreviations: BMI, Body Mass Index; BM, Body mass; LBM, Lean body mass; VO_2max, Maximal oxygen uptake; FatMax, Relative intensity at which MFO is reached (% VO_2max); MFO, Maximal Fat Oxidation; CMR, Cardiometabolic risk.

VO_2max relativized to body mass (p = 0.001; higher in men), VO_2max relativized to lean body mass (p = 0.001; higher in men), RER (p = 0.020; higher in men) and FatMax (p = 0.028; greater in women). The differences in MFO by sex are also observable in Table I. Men obtained higher values (p = 0.026) in absolute MFO. However, women obtained significantly higher values in MFO when it was relativized to lean mass of the legs/height^2 (p = 0.013). In addition, after adjusting for age and VO_2max relativized to body mass, women obtained a lower value in waist circumference (p = 0.001), plasma triglycerides (p = 0.025), physical activity (p = 0.029) and clustered CMR (p = 0.003) and a higher value in diastolic blood pressure (p = 0.001).

Table II shows the results of the linear regression models for MFO and VO_2max (both relativized to lean mass of the legs/height^2) with clustered CMR factors after adjusting by sex, age and VO_2max or MFO (both relativized to lean mass of the legs/height^2). Figure 1 shows the associations between MFO and VO_2max (both relativized to lean mass of the legs/height^2) with individual and clustered CMR factors, obtained from linear regression analysis.

RFO was not associated with any individual or clustered CMR factors ($p > 0.05$). The results of RFO are included as supplementary material (see Table S1). FatMax was associated with waist circumference (R^2 = 0.144; β = −0.380; p = 0.001), plasma triglycerides (R^2 = 0.111; β = −0.334; p = 0.004) and clustered CMR factors (R^2 = 0.105; β = −0.324; p = 0.008). MFO was associated with waist circumference (R^2 = 0.241; β = −0.491; p = 0.001), plasma triglycerides (R^2 = 0.130; β = −0.360; p = 0.002) and clustered CMR factors (R^2 = 0.162; β = −0.403; p = 0.001). VO_2max was associated with waist circumference (R^2 = 0.285; β = −0.534; p = 0.001), percentage of body fat (R^2 = 0.105; β = −0.324; p = 0.003), diastolic blood pressure (R^2 = 0.083; β = −0.288; p = 0.01), plasma triglycerides (R^2 = 0.093; β = −0.305; p = 0.008) and clustered CMR factors (R^2 = 0.239; β = −0.489; p = 0.001). These associations did not materially change when sex and age were included in the model.

Table III shows the differences in clustered CMR according to the groups created by levels of high or low MFO and VO_2max. The group of low MFO had greater values of clustered CMR factors compared to the group with high MFO (p = 0.001; 1st tertile vs. 2nd + 3rd tertile jointly), even when it was adjusted by sex and age jointly (p = 0.009). Regarding VO_2max, a significant difference between high and low VO_2max groups (p = 0.001; 1st tertile vs. 2nd + 3rd tertile jointly) that was maintained even when adjusting by sex and age jointly (p = 0.008). In addition, it is observed that the difference in CMR was greater within VO_2max groups (Low VO_2max: 2.78; High VO_2max: −0.813) compared to MFO groups (Low MFO: 2.45; High MFO: −0.493).

Table II. Associations between maximal fat oxidation (MFO) and maximal oxygen uptake (VO_2max) with clustered CMR factors.

	MFO			VO_2max		
	β	R^2	*P*	β	R^2	*P*
Model 1	−0.403	0.162	**0.001**	−0.489	0.239	**0.001**
Model 2	−0.401	0.162	**0.001**	−0.485	0.245	**0.001**
Model 3	−0.248	0.350	**0.024**	−0.413	0.244	**0.011**
Model 4	−0.104	0.244	0.510	−0.304	0.369	**0.008**

Model 1: Unadjusted.
Model 2: Adjusted for sex.
Model 3: Adjusted for age.
Model 4: Adjusted for VO_2max or MFO relativized to lean mass of the legs/height^2.
Abbreviations: MFO, Maximal fat oxidation relativized to lean mass of the legs / height^2; VO_2max, Maximal oxygen uptake relativized to lean mass of the legs / height^2; β, Standardized coefficient; R^2, Coefficient of determination; *P*, *p* value.
Note: Statistically significant results ($p < 0.05$) appear in bold.

Discussion

Associations between MFO, VO_2max and RFO with CMR

The main finding of the present study was that participants who had higher MFO or VO_2max presented lower CMR. In the association of VO_2max with clustered CMR factors, R^2 was 0.239, which means that VO_2max accounts for 23.9% of the variation in CMR. Moreover, in the association of MFO with clustered CMR factors, R^2 was 0.162, which means that MFO accounts for 16.2% of the variation in CMR. Despite that VO_2max showed a stronger association with CMR, probably because MFO is a variable that depends largely on VO_2max (Randell et al., 2017), we have shown that MFO could also be a complementary test to the validated submaximal VO_2max testing, which may add additional useful information for the creation of effective exercise interventions aimed at preventing CMR and metabolic inflexibility. MFO was obtained at 42% of VO_2max in our study, which, similarly to submaximal VO_2max testing, highly reduces the concomitant risk associated to a maximal exercise test, since people without known health problems can exhibit unexpected responses (e.g. cardiac dysrhythmias) and this incidence increases with advancing age (Noonan & Dean, 2000). Moreover, these tests may increase safety and minimize undue strain, both essential factors in the assessment of CMR through exercise testing. In fact, previous findings suggest that reducing maximum RER until 0.95 would allow to apply smaller workload increments without affecting the MFO estimation and without increasing the test duration, which would allow more fat oxidation values around Fatmax, increasing the accuracy of the MFO estimation (Amaro-Gahete, Sanchez-Delgado, Helge, & Ruiz, 2019). Regarding RFO, no association was found between RFO and the individual and clustered CMR factors, so that

Table III. Differences in clustered cardiometabolic risk (CMR) factors depending on the different groups of maximal fat oxidation (MFO) and maximal oxygen uptake (VO2max) levels.

	MFO groups		VO_2max groups	
	1st vs. (2nd + 3rd) tertiles		1st vs. (2nd + 3rd) tertiles	
	Mean	*P*	Mean	*P*
Model 1	Low MFO: 2.45	**0.001**	Low VO_2max: 2.78	**0.001**
Model 2	High MFO: −0.493	**0.009**	High VO_2max: −0.813	**0.008**

Model 1: Unadjusted.
Model 2: Adjusted for sex and age.
Abbreviations: MFO, Maximal fat oxidation; VO_2max, Maximal oxygen uptake *P*, *p* value.
Note: Both MFO and VO_2max were previously relativized to lean mass of the legs / height^2. Statistically significant results ($p < 0.05$) appear in bold.

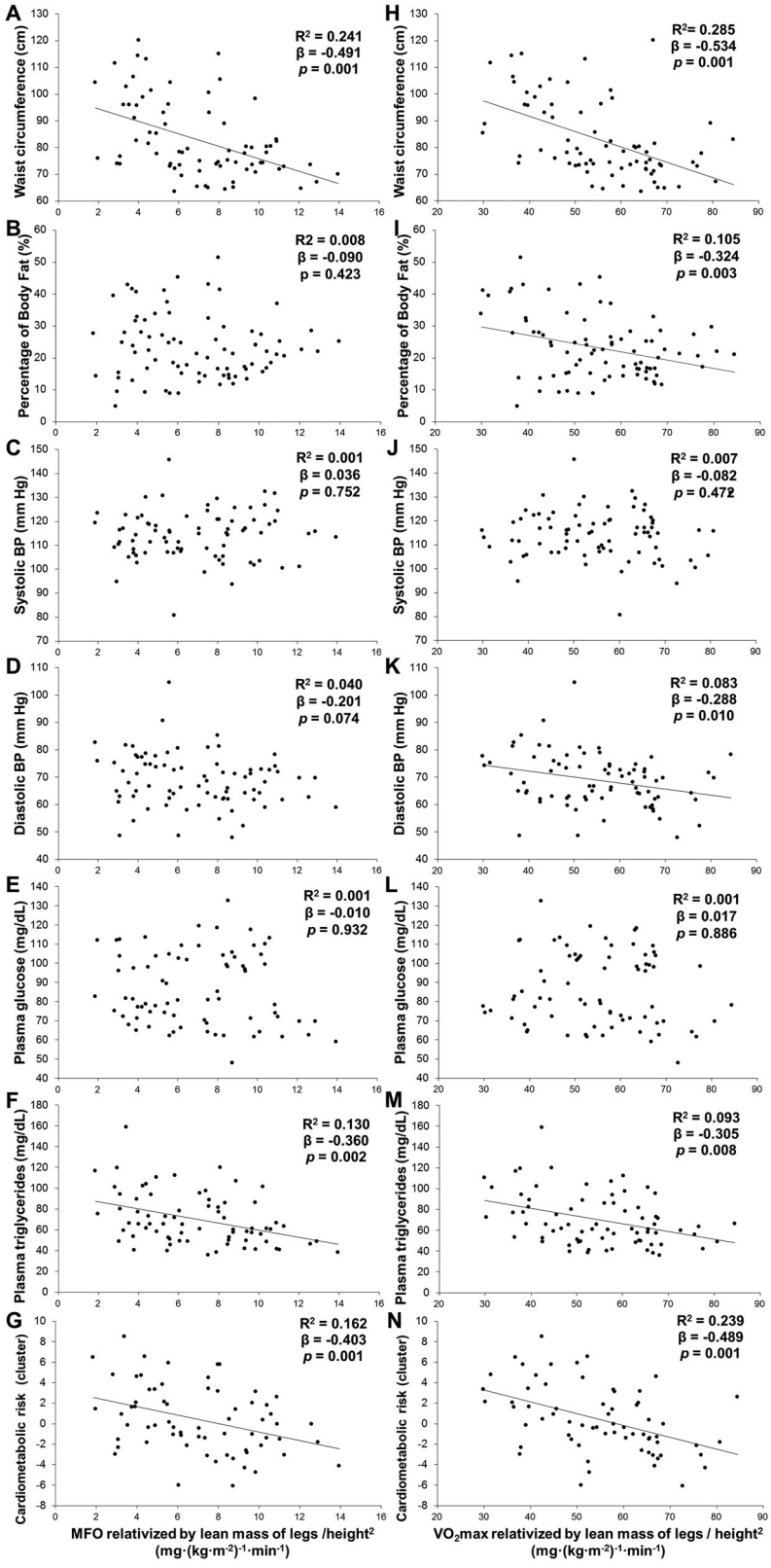

Figure 1. Association between individual and clustered cardiometabolic risk (CMR) factors with maximal fat oxidation (MFO) and maximal oxygen uptake (VO_2max). Left panel shows the association of MFO relativized to lean mass of legs/height^2 with waist circumference (A), body fat percentage (B), systolic BP (C), diastolic BP (D), blood glucose (E), plasma triglycerides (F) and clustered cardiometabolic risk (CMR) factors (G). Right panel shows the association of VO_2max relativized to lean mass of legs/height^2 with waist circumference (H), body fat percentage (I), systolic blood pressure (J), diastolic blood pressure (K), blood glucose (L), plasma triglycerides (M) and clustered cardiometabolic risk (CMR) factors (N). Abbreviations: β, Standardized coefficient; R^2, Coefficient of determination; P, p value; BP, blood pressure. The statistical analysis performed was linear regression.

the results of previous studies in this regard are corroborated (Karppinen et al., 2019). Despite that, RFO was associated with MFO but not with VO_2max in our study (Data not shown), as previously reported (Karppinen et al., 2019).

In agreement, Cancino-Ramírez et al. (2018) showed that VO_2max and MFO are protective factors for insulin resistance and, therefore, CMR in sedentary women with overweight or obesity. However, VO_2max was more strongly associated with CMR, suggesting that VO_2max is a better predictor compared to MFO. Kelley et al. (Kelley et al., 2017) showed that triglyceride accumulation in skeletal muscle in obesity derives from reduced capacity for fat oxidation and that inflexibility in regulating fat oxidation is related to insulin resistance, obesity and weight loss. Higher levels of both RFO and MFO capacity during exercise have been associated with lower metabolic disease risk in overweight sedentary men (Rosenkilde et al., 2010). Robinson et al. (2015) presented that MFO is positively associated with fat oxidation in 24 h and insulin sensitivity in healthy young men. Hence, improvement in MFO through exercise intervention may be a useful tool to prevent cardiometabolic diseases.

Association between FatMax and CMR

Likewise, FatMax was associated with both individual (waist circumference and plasma triglycerides) and clustered CMR factors in our study, being the first study that show this association. In agreement with previous data (Amaro-Gahete et al., 2018), FatMax was higher in women compared to men in our study, who had also higher protection against CVD (Maas & Appelman, 2010). Moreover, it has been shown that FatMax was positively associated with the level of physical activity and VO_2max in a large cohort of healthy adults (Nordby, Saltin, & Helge, 2006; Venables, Achten, & Jeukendrup, 2005). Both factors were inversely associated with CMR in previous studies (Brambilla, Pozzobon, & Pietrobelli, 2011; Myers et al., 2015; Rebollo-Ramos et al., 2019; Tikkanen et al., 2018). Therefore, FatMax could be useful as an indicator of metabolic health and could be considered a new and important marker of cardiometabolic status.

Differences in MFO and CMR by sex

Furthermore, women obtained higher levels of MFO relativized to lean mass of the legs than men in our study results. In agreement, Randell et al. showed that women had a lower MFO in absolute terms, but when values were relativized to lean body mass, women had a higher MFO than men. Other previous studies also confirm the findings of this study regarding the increased fat oxidation capacity of women (Chenevière, Borrani, Sangsue, Gojanovic, & Malatesta, 2011; Dasilva et al., 2011). The higher MFO that occur in women could protect against CVD during adult age compared to men. In fact, the risk of heart disease in women is less common in premenopausal women compared to age-matched men and the development of CVD appears 7–10 years later compared to men (Maas & Appelman, 2010). This difference could be explained by the higher circulating levels of estrogens in women, which are related to increased lipolytic activity and greater activation of AMPK (AMP-activated protein kinase) (Oosthuyse & Bosch, 2012). In concordance, our results showed that women had lower individual and clustered CMR factors than men after adjusting for age and VO_2max relativized to body mass.

MFO and VO_2max indexes

Since lipid metabolism and oxygen uptake during cycling exercise in our study could be affected by body size and quantity of muscle mass in legs (Heusner, 1985), both MFO and VO_2max were relativized to lean mass of the legs / height^2 as previously reported (Ponce González et al., 2017; Shepherd, Ng, Sommer, & Heymsfield, 2017; Ponce-González et al., 2016). It could minimize the MFO and VO_2max values dispersion due to differences in body composition parameters which is common between men and women. There is evidence that shows that lean mass, which is influenced by body size, is a key factor in fat oxidation (Romijn, Coyle, Sidossis, Rosenblatt, & Wolfe, 2017). In this sense, MFO was higher in women compared to men in our study only when it was relativized to lean mass of the legs/height^2. Moreover, age was also included in the adjustment, since it has been related to the decline in muscle mass, MFO and VO_2max (Laukkanen et al., 2016; Liu et al., 2017).

Limitations and future lines of research

The main limitation of this cross-sectional study is the composition of the sample, since they were healthy young adults without any mild or serious cardiovascular complications, hence the associations of CMR score with MFO or VO_2max could be hampered. Moreover, there are other factors that were not considered in the current study and that could affect MFO capacity, such as dietary patterns (Compher, Frankenfield, Keim, & Roth-Yousey, 2006). Another limitation was the measurement of body composition

by bioimpedance instead of using another more precise method like DXA (Shepherd et al., 2017).

Therefore, future studies including a sample with higher heterogeneity in CMR parameters, and MFO and VO_2max values are warranted. Likewise, it would also be interesting to carry out future studies in which the sample is made up of participants with CVD, since our study is based only on healthy people. In addition, descriptive (cross-sectional and follow-up studies) and, above all, experimental designs should be carried out to correctly determine the causation of the studied associations. Finally, future studies using more direct parameters of substrate metabolism, such as mitochondrial respiration, the activity of oxidative enzymes or the presence or phosphorylation of key proteins in fat oxidation, in addition to indirect calorimetry, would be of great interest.

Practical applications

The data obtained with MFO test allows us (i) to determine the capacity of a person to oxidize fat as an important data to identify metabolic inflexibility, lipid oxidation impairment and diminished lipolysis; (ii) MFO data could be useful as new tool to estimate CMR factors similar than VO_2max; (iii) VO_2max could be also assessed with a submaximal test, but MFO test provides an important information according to fat oxidation capacity as we reflected in (i) point; and (iv) according to the results of this test we can design effective body weight loss strategies by optimizing exercise interventions based on the FatMax of each person, which is also determined during MFO test. Nevertheless, the applicability of MFO as a CMR indicator in this population needs to be confirmed in future studies.

Conclusion

In conclusion, MFO and FatMax, but not RFO, are associated with CMR. Moreover, MFO is associated with CMR regardless of age and sex. However, the association between VO_2max and CMR was stronger than with MFO. In fact, VO_2max was associated with CMR regardless of age, sex and MFO. Likewise, this study once again demonstrates that MFO is only higher in men than in women in terms of absolute values, since when the values are relativized to lean mass, MFO is higher in women. However, despite the fact that the results obtained in this study offer important information about MFO and its association with CMR, more studies are needed in order to deepen in the research of MFO and its relationship with health.

Author contributions

All authors played a role in the content and writing of the manuscript. JGPG was the principal investigator and contributed the original idea for the study. All authors had input into the study design and conduct of study; AMOG, APB, JCP, DVD, MR y JGPG collected the data; AMOG, AP, EOD, MCG and JRFS performed data analysis; AMOG, AP, EOD, FAG, MR, MCG and JGPG interpretation of the data, and writing of the manuscript. Each one of the authors has read and becomes responsible for any of the aspects included in the manuscript.

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No potential conflict of interest was reported by the author(s).

Supplemental data

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References

Achten, J., Gleeson, M., & Jeukendrup, A. (2002). Determination of the exercise intensity that elicits maximal fat oxidation. *Medicine & Science in Sports & Exercise*, *34*(1), 92–97.

Amaro-Gahete, F. J., Jurado-Fasoli, L., Triviño, A. R., Sanchez-Delgado, G., Helge, J. W., & Ruiz, J. R. (2019). Diurnal variation of maximal fat-oxidation rate in trained male athletes. *International Journal of Sports Physiology and Performance*, *14* (8), 1140–1146.

Amaro-Gahete, F. J., Sanchez-Delgado, G., Alcantara, J. M. A., Martinez-Tellez, B., Acosta, F. M., Helge, J. W., et al. (2019). Impact of data analysis methods for maximal fat oxidation estimation during exercise in sedentary adults: Data analysis maximal fat oxidation. *European Journal of Sport Science*, *19*(9), 1230–1239.

Amaro-Gahete, F. J., Sanchez-Delgado, G., Ara, I., & Ruiz, J. R. (2019). Cardiorespiratory fitness may influence metabolic inflexibility during exercise in obese persons. *Journal of Clinical Endocrinology and Metabolism*, *104*(12), 5780–5790.

Amaro-Gahete, F. J., Sanchez-Delgado, G., Helge, J. W., & Ruiz, J. R. (2019). Optimizing maximal fat oxidation assessment by a treadmill-based graded exercise protocol: When should the test end? *Frontiers in Physiology*, *10*, 909.

Amaro-Gahete, F. J., Sanchez-Delgado, G., & Ruiz, J. R. (2018). Commentary: Contextualising maximal fat oxidation during exercise: Determinants and normative values. *Frontiers in Physiology*, *9*, 1460.

Brambilla, P., Pozzobon, G., & Pietrobelli, A. (2011). Physical activity as the main therapeutic tool for metabolic syndrome in childhood. *International Journal of Obesity*, *35*(1), 16–28.

Cancino-Ramírez, J., Soto-Sánchez, J., Zbinden-Foncea, H., Moreno González, M., Leyton-Dinamarca, B., & González-Rojas, L. (2018). Cardiorespiratory fitness and fat oxidation during exercise as protective factors of insulin resistance in sedentary women who are overweight or obese. *Nutricion Hospitalaria*, *35*(2), 312–317.

Chenevière, X., Borrani, F., Sangsue, D., Gojanovic, B., & Malatesta, D. (2011). Gender differences in whole-body fat oxidation kinetics during exercise. *Applied Physiology, Nutrition and Metabolism*, *36*(1), 88–95.

Compher, C., Frankenfield, D., Keim, N., & Roth-Yousey, L. (2006). Best practice methods to apply to measurement of resting metabolic rate in adults: A systematic review. *Journal of the American Dietetic Association*, *106*(6), 881–903.

Craig, C. L., Marshall, A. L., Sjöström, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., et al. (2003). International physical activity questionnaire: 12-Country reliability and validity. *Medicine & Science in Sports & Exercise*, *35*(8), 1381–1395.

Dandanell, S., Praest, C. B., Søndergård, S. D., Skovborg, C., Dela, F., Larsen, S., et al. (2017). Determination of the exercise intensity that elicits maximal fat oxidation in individuals with obesity. *Applied Physiology, Nutrition and Metabolism*, *42*(4), 405–412.

Dasilva, S. G., Guidetti, L., Buzzachera, C. F., Elsangedy, H. M., Krinski, K., De Campos, W., et al. (2011). Gender-based differences in substrate use during exercise at a self-selected pace. *Journal of Strength and Conditioning Research*, *25*(9), 2544–2551.

Frayn, K. N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology*, *55* (2), 628–634.

Heusner, A. A. (1985). Body size and energy metabolism. *Annual Review of Nutrition*, *5*(1), 267–293.

Jeukendrup, A. E., & Achten, J. (2001). Fatmax: A new concept to optimize fat oxidation during exercise? *European Journal of Sport Science*, *1*(5), 1–5.

Jiang, Y., Tan, S., Wang, Z., Guo, Z., Li, Q., & Wang, J. (2020). Aerobic exercise training at maximal fat oxidation intensity improves body composition, glycemic control, and physical capacity in older people with type 2 diabetes. *Journal of Exercise Science and Fitness*, *18*(1), 7–13.

Karppinen, J. E., Rottensteiner, M., Wiklund, P., Hämäläinen, K., Laakkonen, E. K., Kaprio, J., et al. (2019). Fat oxidation at rest and during exercise in male monozygotic twins. *European Journal of Applied Physiology*, *119*(11–12), 2711–2722.

Kelley, D. E., Goodpaster, B., Wing, R. R., & Simoneau, J.-A. (2017). Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *Am J Physiol Metab*, *277*(6), E1130–E1141.

Laukkanen, J. A., Zaccardi, F., Khan, H., Kurl, S., Jae, S. Y., & Rauramaa, R. (2016). Long-term change in cardiorespiratory fitness and all-cause mortality. *Mayo Clinic Proceedings*, *91*(9), 1183–1188.

Liu, P., Hao, Q., Hai, S., Wang, H., Cao, L., & Dong, B. (2017). Sarcopenia as a predictor of all-cause mortality among community-dwelling older people: A systematic review and meta-analysis. *Maturitas*, *103*, 16–22.

Maas, A. H., & Appelman, Y. E. (2010). Gender differences in coronary heart disease. *Netherlands Hear Journal*, *18*(12), 598–603.

Myers, J., McAuley, P., Lavie, C. J., Despres, J. P., Arena, R., & Kokkinos, P. (2015). Physical activity and cardiorespiratory fitness as major markers of cardiovascular risk: Their independent and interwoven importance to health status. *Progress in Cardiovascular Diseases*, *57*(4), 306–314.

Noonan, V., & Dean, E. (2000). Submaximal exercise testing: Clinical application and interpretation. *Physical Theraphy.*, *80* (8), 782–807.

Nordby, P., Saltin, B., & Helge, J. W. (2006). Whole-body fat oxidation determined by graded exercise and indirect calorimetry: A role for muscle oxidative capacity? *Scandinavian Journal of Medicine & Science in Sports*, *16*(3), 209–214.

Oosthuyse, T., & Bosch, A. N. (2012). Oestrogen's regulation of fat metabolism during exercise and gender specific effects. *Current Opinion in Pharmacology*, *12*(3), 363–371.

Ponce-González, J. G., Rodríguez-Garcia, L., Losa-Reyna, J., Guadalupe- Grau, A., Rodriguez-Gonzalez, F. G., Díaz-Chico, B. N., & Calbet, J. A. L. (2016). Androgen receptor gene polymorphism influence fat accumulation: A longitudinal study from adolescence to adult age. *Scandinavian Journal of Medicine & Sciencein Sports*, *26*(11), 1313–1320.

Ponce González, J. G., Guadalupe-Grau, A., Rodríguez-González, F. G., Torres-Peralta, R., Morales-Alamo, D., Rodríguez-García, L., et al. (2017). Androgen receptor gene polymorphisms and maximal fat oxidation in men. A longitudinal study. *Nutricion Hospitalaria*, *34*(5), 1089–1098.

Randell, R. K., Rollo, I., Roberts, T. J., Dalrymple, K. J., Jeukendrup, A. E., & Carter, J. M. (2017). Maximal fat oxidation rates in an Athletic population. *Medicine & Science in Sports & Exercise*, *49*(1), 133–140.

Rebollo-Ramos, M., Velázquez-Díaz, D., Corral-Pérez, J., Barany-Ruiz, A., Pérez-Bey, A., Fernández-Ponce, C., et al. (2019). Aerobic fitness, Mediterranean diet and cardiometabolic risk factors in adults. *Endocrinol Diabetes y Nutr*, *67*(2), 113–121.

Robinson, S. L., Hattersley, J., Frost, G. S., Chambers, E. S., & Wallis, G. A. (2015). Maximal fat oxidation during exercise is positively associated with 24-hour fat oxidation and insulin sensitivity in young, healthy men. *Journal of Applied Physiology*, *118* (11), 1415–1422.

Romijn, J. A., Coyle, E. F., Sidossis, L. S., Rosenblatt, J., & Wolfe, R. R. (2017). Substrate metabolism during different exercise intensities in endurance-trained women. *Journal of Applied Physiology*, *88*(5), 1707–1714.

Rosenkilde, M., Nordby, P., Nielsen, L. B., Stallknecht, B. M., & Helge, J. W. (2010). Fat oxidation at rest predicts peak fat oxidation during exercise and metabolic phenotype in overweight men. *International Journal of Obesity*, *34*(5), 871–877.

Salmenniemi, U., Ruotsalainen, E., Pihlajamäki, J., Vauhkonen, I., Kainulainen, S., Punnonen, K., et al. (2004). Multiple abnormalities in glucose and energy metabolism and coordinated changes in levels of adiponectin, cytokines, and adhesion molecules in subjects with metabolic syndrome. *Circulation*, *110*(25), 3842–3848.

Shepherd, J. A., Ng, B. K., Sommer, M. J., & Heymsfield, S. B. (2017). Body composition by DXA. *Bone*, *104*, 101–105.

Swainson, M. G., Ingle, L., & Carroll, S. (2019). Cardiorespiratory fitness as a predictor of short-term and lifetime estimated cardiovascular disease risk. *Scand J Med Sci Sport*, *29*(9), 1402–1413.

Tikkanen, E., Gustafsson, S., & Ingelsson, E. (2018). Associations of fitness, physical activity, strength, and genetic risk with cardiovascular disease: Longitudinal analyses in the UK biobank study. *Circulation*, *137*(24), 2583–2591.

Venables, M. C., Achten, J., & Jeukendrup, A. E. (2005). Determinants of fat oxidation during exercise in healthy men and women: A cross-sectional study. *Journal of Applied Physiology*, *98*(1), 160–167.

World Health Organization. (2011). *Global recommendations on physical activity for health*. World Health Organization.

Yu, R., Yau, F., Ho, S. C., & Woo, J. (2013). Associations of cardiorespiratory fitness, physical activity, and obesity with metabolic syndrome in Hong Kong Chinese midlife women. *BMC Public Health*, *13*(1), 614.