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INVITED REVIEW

Biomarkers and genetic polymorphisms associated with maximal fat oxidation during physical exercise: implications for metabolic health and sports performance

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Abstract

The maximal fat oxidation rate (MFO) assessed during a graded exercise test is a remarkable physiological indicator associated with metabolic flexibility, body weight loss and endurance performance. The present review considers existing biomarkers related to MFO, highlighting the validity of maximal oxygen uptake and free fatty acid availability for predicting MFO in athletes and healthy individuals. Moreover, we emphasize the role of different key enzymes and structural proteins that regulate adipose tissue lipolysis (i.e., triacylglycerol lipase, hormone sensitive lipase, perilipin 1), fatty acid trafficking (i.e., fatty acid translocase cluster of differentiation 36) and skeletal muscle oxidative capacity (i.e., citrate synthase and mitochondrial respiratory chain complexes II–V) on MFO variation. Likewise, we discuss the association of MFO with different polymorphism on the ACE, ADRB3, AR and CD36 genes, identifying prospective studies that will help to elucidate the mechanisms behind such associations. In addition, we highlight existing evidence that contradict the paradigm of a higher MFO in women due to ovarian hormones activity and highlight current gaps regarding endocrine function and MFO relationship.

Keywords Athletes · Energy metabolism · Exercise · Gene expression · Genotype · Obesity

Abbreviations

ACE	Angiotensin-converting enzyme
AR	Androgen receptor
ATGL	Adipose triacylglycerol lipase
β-AR_3	Beta-3 adrenergic receptor
CD36	Fatty acid translocase cluster of differentiation 36
CPT1	Carnitine palmitoyltransferase 1
CRF	Cardiorespiratory fitness
CS	Citrate synthase
FATmax	Exercise intensity corresponding to maximal fat oxidation

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FFA	Free fatty acids
FFM	Fat-free mass
HIIT	High-intensity interval training
HSL	Hormone-sensitive lipase
IMTG	Intramuscular triglycerides
MFO	Maximal fat oxidation
$Mito_{VD}$	Mitochondrial volume density
OXPHOS	Mitochondrial oxidative phosphorylation capacity
VO_{2max}	Maximal oxygen uptake
VO_{2peak}	Peak oxygen uptake
VT	Ventilatory threshold

Introduction

Skeletal muscle comprises ~ 40% of total body mass and poses relevant implications for metabolic health and sports performance. A poor fat oxidation in skeletal muscle may lead to the accumulation of lipid intermediates such as diacylglycerols and ceramides which are considered "lipotoxic" and are related to insulin sensitivity (Goodpaster and Sparks 2017; Arad et al. 2020). On the other hand, increasing fat oxidation in skeletal muscle through exercise training will reduce glycogenolysis rate, delaying glycogen depletion which is related to muscle fatigue (Holloszy and Coyle 1984; Ørtenblad and Nielsen 2015; Hearris et al. 2018).

The maximal fat oxidation rate (MFO) observed at submaximal exercise intensity during an incremental-load exercise test represents the capacity of the skeletal muscle to use fatty acids as a fuel when energy demand raises by muscle contraction (Fig. 1). Since introduced by Jeukendrup and Achten (2001), many studies have analyzed the validity of this parameter as a marker of metabolic flexibility and a predictor of sports performance in endurance athletes, reporting that (1) MFO is directly related to insulin sensitivity ($r = 0.33$, $p < 0.01$) and 24-h fat oxidation ($r = 0.65$, $p < 0.01$) in healthy males (Robinson et al. 2015); (2) predicts total fat oxidation during steady-state exercise in men with obesity ($R^2 = 0.46$, $p < 0.01$) (Chávez-Guevara et al. 2021) and is positively associated with exercise fat oxidation in the postprandial state in trained males ($r = 0.83$, $p < 0.01$) (Maunder et al. 2021); (3) is related with fat mass loss induced by exercise training performed at MFO intensity (FATmax) in subjects with obesity ($r = 0.35$, $p < 0.01$) (Drapier et al. 2018); (4) it explains 12 and 14% of endurance performance on Ironman (Frandsen et al. 2017) and ultra-trail male athletes, respectively (Martinez-Navarro et al. 2020). The aforementioned evidence highlights the relevance of investigating those biological and nutritional factors as well as the fitness components that determine MFO to understand the molecular and physiological mechanisms affecting metabolic health and athletic performance.

Previous investigations reported that cardiorespiratory fitness (CRF), body fat, exercise intensity eliciting MFO (FATmax), and fasting duration before the exercise test accounted for 47% of MFO intra-individual variance in athletes (Randell et al. 2017). In a similar way, the CRF, self-reported physical activity level, fat mass, fat-free mass (FFM), biological sex, and macronutrient content of the diet determined 46% of MFO variance in healthy untrained individuals (Fletcher et al. 2017). The physiological mechanisms explaining the association of the variables mentioned above with MFO have been described by Purdom et al. (2018) while Maunder et al. (2018) and Amaro-Gahete et al. (2018) provided MFO and FATmax values for trained and untrained individuals with different nutritional status. Nevertheless, determinants of MFO in endurance athletes, people with chronic diseases and elderly adults require further analysis.

Several studies suggest that women have greater fat oxidation than men, when MFO is expressed as mg kg FFM^{-1} min^{-1} (Venables et al. 2005; Fletcher et al. 2017; Maunder et al. 2018). In these studies, females showed a lower FFM in comparison to males. Thus, when adjusting MFO relative to FFM this may result on females exhibiting a higher fat oxidation, because the regression of fat oxidation on FFM has a significant positive intercept. Nonetheless, Chrzanowski-Smith et al. (2021) reported that women exhibited a greater MFO relative to FFM in comparison to men even when both groups were matched by muscle mass. Moreover, a recent meta-analysis evidenced that sedentary women shows a higher reliance on fat oxidation during moderate intensity exercise in comparison to sedentary men (Cano et al. 2021). In this regard, several physiological mechanisms have been related with sex-based differences in fat oxidation, including (1) elevated lipolytic rate in adipose tissue; (2) elevated levels of estrogens; (3) increased proportion of type 1 muscle fibers; (4) elevated intramuscular lipids concentration. Therefore, is necessary to elucidate whether MFO is predicted by similar variables between sexes.

On the other hand, up to 50% of MFO variation remain unexplained and biomarkers representing adipose tissue metabolism and skeletal muscle oxidative capacity (i.e., lipolytic enzymes, circulating free fatty acids, mitochondrial content, hormones) could be involved in oxidative capacity processes affecting MFO (Muscella et al. 2020). In addition, Randell et al. (2017) pointed out that MFO could be also regulated by genetic and epigenetic factors, including several genetic polymorphisms associated with circulating fatty acid availability (Li et al. 2016), fat oxidation during physical exercise (Morita et al. 2009) and skeletal muscle fiber type composition (Ahmetov et al. 2012). Therefore, the present review considers several biomarkers and genetic polymorphism related to MFO, with particular emphasis on those biomarkers that have been validated as MFO predictor. Moreover, we describe the mechanisms behind such

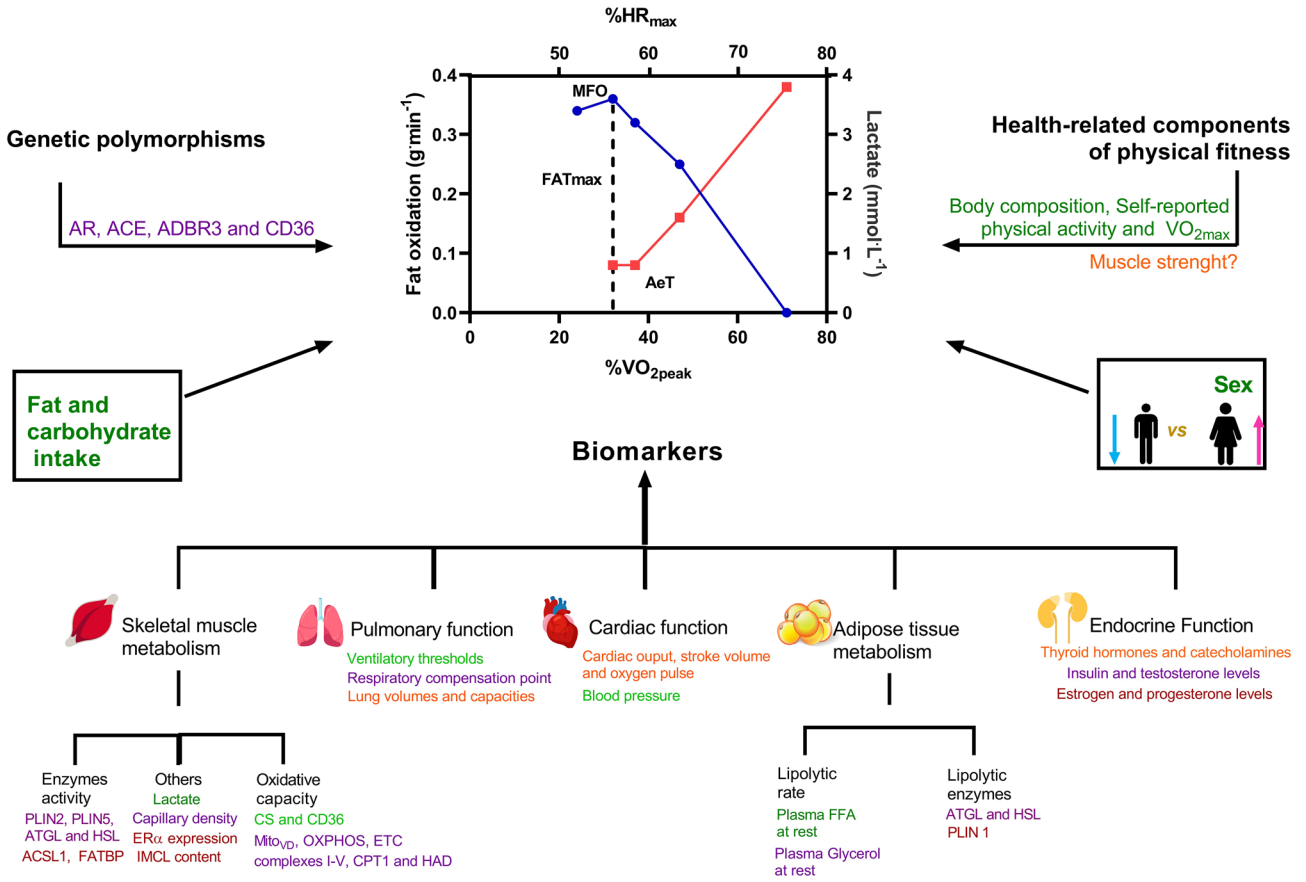

Fig. 1 Biomarkers, health-related components of physical fitness and genetic polymorphism related to maximal fat oxidation (MFO). Those biomarkers and health-related components previously validated as MFO predictors are highlighted in green color while those biomarkers that have only been correlated with MFO are remarked in purple. The biomarkers that are not associated with MFO are colored in red those biomarkers whose relationship with MFO has not been analyzed (but could be associated) are highlighted in orange. *ATGL* triacylglycerol lipase, *ACSL1* acyl-CoA synthetase, *CS* citrate synthase, *CPT1* carnitine palmitoyl transferase 1, *ERα* estrogen receptor alpha, *ECT* electron transport chain, *FATBP* fatty acid binding protein, *FATmax* exercise intensity eliciting maximal fat oxidation, *FFA* free fatty acids, *HAD* β-hydroxy-acyl-CoA-dehydrogenase, *HSL* hormone sensitive lipase, *MFO* maximal fat oxidation, *IMCL* intramuscular lipids, $Mito_{VD}$ mitochondrial volume density, *OXPHOS* mitochondrial oxidative phosphorylation capacity, *PLIN* perilipin, VO_{2max} maximal oxygen uptake. The fat oxidation and blood lactate kinetics showed in this figure correspond to Author's experimental data collected from an incremental-load exercise test performed on a patient with obesity

associations and discuss existing evidence regarding the effect of exercise training over all biomarkers and MFO. Furthermore, we identify prospective studies that will help to elucidate the molecular mechanism that may predispose to a low-fat oxidation phenotype leading to metabolic diseases and a poor physical performance.

Biomarkers associated with exercise-induced maximal fat oxidation rate

Several biomarkers regarding CRF, endocrine function, fatty acid metabolism and skeletal oxidative capacity have been related with MFO observing contrasting results depending on training status and biological sex (Table 1).

Cardiorespiratory fitness

Data from Venables et al. (2005) and Fletcher et al. (2017) showed that CRF is the main determinant of MFO variance in healthy individuals with low to moderate physical activity level (see standardized β coefficients reported in Table 1). In support of this, a positive and strong association between $VO_{2max/peak}$ and MFO has been reported in a combined group of trained/untrained individuals ($R^2 = 0.59$, $p < 0.01$) (Nordby et al. 2006) and endurance trained males ($R^2 = 0.78$, $p < 0.01$) (Amaro-Gahete et al. 2019a), with a modest association reported on female ironman athletes ($R^2 = 0.27$, $p < 0.01$) (Vest et al. 2018), sedentary young adults with overweight ($R^2 = 0.26$, $p < 0.01$) and obesity ($R^2 = 0.10$, $p < 0.05$) (Amaro-Gahete et al. 2019b), and sedentary middle-aged adults ($R^2 = 0.13$, $p < 0.01$) (Amaro-Gahete et al.

Table 1 Biomarkers and health-related fitness components associated with MFO in trained and untrained subjects

Study	Population	Exercise protocol	Analyzed biomarkers	Findings
Venables et al. (2005)	157 M (30 ± 11 years) BMI: 26 ± 4 kg m^{-2} VO_{2max}: 50.7 ± 0.7 mL kg^{-1} min^{-1} 157 W (32 ± 12 years) BMI: 25 ± 4 kg m^{-2} VO_{2max}: 41.4 ± 0.9 mL kg^{-1} min^{-1}	Treadmill; Fasted state (4 h); GXT; Frayn's equations; FATmax defined through measured valued	Self-reported physical activity level; VO_{2max}, fat mass and fat-free mass	Self-reported physical activity ($\beta = 0.15$), VO_{2max} ($\beta = 0.50$), fat-free mass ($\beta = 0.41$) and biological sex ($\beta = -0.29$) explained 34% of MFO variation
Nordby et al. (2006)	8 Untrained M (26 ± 3 years) BMI: 23.7 ± 2.5 kg m^{-2} VO_{2max}: 46.5 ± 5.1 mL kg^{-1} min^{-1} 8 Trained M (28 ± 6 years) BMI: 23.2 ± 1.1 kg m^{-2} VO_{2max}: 56.6 ± 3.6 mL kg^{-1} min^{-1}	Cycle-ergometer; Fasted state (NR); GXT; Frayn's equations; FATmax defined through a second degree polynomial regression	CS and HAD activity in skeletal muscle, lean mass and VO_{2max}	Both, lean mass and VO_{2max} were directly associated with MFO in the combined cohort ($R^2 = 0.59$, $p < 0.01$) *Authors did not report the data for each group and did not perform a multiple linear regression analysis to determine the independent association of lean mass and VO_{2max} with MFO
Stisen et al. (2006)	9 Untrained W (NR) BMI: 22.1 ± 2.4 kg m^{-2} VO_{2max}: 41.5 ± 5.1 mL kg^{-1} min^{-1} 8 Trained W (NR) BMI: 21.7 ± 1.8 kg m^{-2} VO_{2max}: 53.8 ± 3.9 mL kg^{-1} min^{-1}	Cycle-ergometer; Postprandial state (3 h); GXT; Frayn's equations; FATmax defined through a third degree polynomial regression	CS, HAD and HSL activity in skeletal muscle, intramuscular glycogen and Fiber type I	Only HAD activity was significantly correlated to MFO in the combined cohort ($r = 0.65$, $p < 0.01$) *Authors did not report neither the data for each group nor performed a multiple regression analysis
Haufe et al. (2010)	38 Sedentary M (45 ± 6 years) BMI: 35.1 ± 8.6 kg m^{-2} VO_{2max}: 26.1 ± 7.3 mL kg^{-1} min^{-1} 91 Sedentary W (42 ± 9 years) BMI: 33.9 ± 6.6 kg/m^2 VO_{2max}: 21.9 ± 8.5 mL kg^{-1} min^{-1}	Cycle-ergometer; Postprandial state (2 h); GXT; Frayn's equations; FATmax defined through a third degree polynomial regression	Body fat, IMTG, lactate threshold, fat-free mass and VO_{2max}	Only fat-free mass ($r = 0.47$, $p < 0.01$), VO_{2max} ($r = 0.59$, $p < 0.01$) and oxygen uptake at lactate threshold ($r = 0.55$, $p < 0.01$) were correlated with MFO *Authors did not perform a multiple regression analysis
Robinson et al. (2016)	56 Trained M (24 ± 7 years) BMI: 24.2 ± 2.6 kg m^{-2} VO_{2max}: 52 ± 6 mL kg^{-1} min^{-1}	Cycle-ergometer; Fasted state (10 h); GXT; Frayn's equations; FATmax defined through measured values	RFO, VO_{2max}, plasma lactate, glucose, glycerol, insulin and FFA at rest and FATmax	RFO ($r = 0.41$, $p < 0.01$), VO_{2max} ($r = 0.52$, $p < 0.01$), plasma glycerol at FATmax ($r = 0.51$, $p < 0.01$), plasma FFA ($r = 0.36$, $p < 0.01$) and insulin ($r = -0.29$, $p < 0.05$) at rest were all correlated with MFO Authors did not perform a multiple regression analysis
Fletcher et al. (2017)	150 Untrained M (24 ± 7 years) BMI: 23.9 ± 2.3 kg m^{-2} VO_{2max}: 54.4 ± 6.9 mL kg^{-1} min^{-1} 155 Untrained W (25 ± 6 years) BMI: 22.2 ± 2.2 kg m^{-2} VO_{2max}: 45.6 ± 6.6 mL kg^{-1} min^{-1}	Treadmill; Fasted state (10–12 h); GXT; Frayn's equations; FATmax defined through measured valued	Self-reported physical activity level; VO_{2max}, fat mass, fat-free mass and dietary macronutrient content	Self-reported physical activity ($\beta = 0.23$), VO_{2max} ($\beta = 0.52$), fat intake ($\beta = 0.14$) and carbohydrate intake ($\beta = -0.17$) determined 47% of MFO variation

Table 1 (continued)

Study	Population	Exercise protocol	Analyzed biomarkers	Findings
Frandsen et al. (2017)	61 male Ironman athlete's (35 ± 8 years) BMI: 26.3 ± 2.3 kg m^{-2} VO_{2max}: 58.7 ± 5.4 mL kg^{-1} min^{-1}	Cycle-ergometer; Fasted state (9–13 h); GXT; Frayn's equations; FATmax defines through a third degree polynomial regression	Body fat, lean body mass, VO_{2peak}, oxygen uptake at FATmax, plasma free fatty acids, glucose, glycerol and lactate at rest	Together, oxygen uptake at FATmax, fasting plasma free fatty acids and lactate accounted for 23% of the variation in MFO ($p < 0.01$)
Vest et al. (2018)	34 female Ironman athlete's (34 ± 6 years) BMI: 22.1 ± 2.0 kg m^{-2} VO_{2max}: 53.0 ± 7.8 mL kg^{-1} min^{-1}	Cycle-ergometer; Fasted state (9–13 h); GXT; Frayn's equations; FATmax defined through a second degree polynomial regression	Body fat, lean body mass, VO_{2peak}, plasma free fatty acids, estradiol, glucose, glycerol, lactate and progesterone at rest	Only VO_{2peak} showed a significant association with MFO ($R^2 = 0.27$, $p < 0.01$)
Dandanell et al. (2018)	8 Trained M (age NR) BMI: NR VO_{2max}: 71 mL kg^{-1} min^{-1} 8 Untrained M (age NR) BMI: NR VO_{2max}: 48 mL kg^{-1} min^{-1}	Cycle-ergometer; Fasted state (NR); GXT; Frayn's equations; FATmax defined through a second degree polynomial regression	Skeletal muscle CS, $Mito_{VD}$, intermyofibrillar $Mito_{VD}$, subsarcolemmal $Mito_{VD}$, IMCL, fiber type I, capillary density, OXPHOS, FAO_p and VO_{2max}	Only IMCL was not correlated with MFO when data from both groups were combined. Otherwise, only CS ($r = 0.50$, $p < 0.05$), $Mito_{VD}$ (0.50, $p < 0.05$) and VO_{2max} ($r = 0.49$, $p < 0.05$) were significantly correlated with MFO in trained individuals whilst capillary density ($r = 0.53$, $p < 0.05$), fiber type I ($r = 0.51$, $p < 0.05$) and VO_{2max} ($r = 0.63$, $p < 0.05$) were related to MFO in the untrained subjects *Authors did not perform a multiple linear regression analysis
Amaro-Gahete et al. (2019a)	12 Trained M (25 ± 4 years) BMI: 22.7 ± 2.3 kg m^{-2} VO_{2max}: 63.8 ± 9.6 mL kg^{-1} min^{-1}	Treadmill; Fasted state (7–10 h); GXT; Frayn's equations; FATmax defined through a third degree polynomial regression *Two GXT were performed on a randomized order; one in the morning and other in the evening	VO_{2max} and VT2	Both, VO_{2max} and VT2 were significantly associated with MFO in the morning ($R^2 = 0.78$, $p < 0.01$ and $R^2 = 0.55$, $p < 0.01$, respectively) and evening ($R^2 = 0.66$, $p < 0.01$ and $R^2 = 0.37$, $p < 0.05$, respectively) GXT
Amaro-Gahete et al. (2019b)	66 Sedentary lean subjects (15 M and 51 W; 25 ± 10 years) BMI: 22.2 ± 1.7 kg m^{-2} VO_{2max}: 42.7 ± 8.6 mL kg^{-1} min^{-1} 48 Sedentary overweight subjects (20 M and 28 W; 38 ± 16 years) BMI: 27.6 ± 1.3 kg m^{-2} VO_{2max}: 35.1 ± 7.6 mL kg^{-1} min^{-1} 24 Sedentary subjects with obesity (15 M and 9 W; 34 ± 15 years) BMI: 32.8 ± 2.4 kg m^{-2} VO_{2max}: 34.1 ± 5.6 mL kg^{-1} min^{-1}	Treadmill; Fasted state (5–6 h); GXT; Frayn's equations; FATmax defined through a third degree polynomial regression	VO_{2max}	VO_{2max} showed a modest association with MFO in lean individuals ($R^2 = 0.17$, $\beta = 0.005$, $p < 0.01$) as well as subjects with overweight ($R^2 = 0.26$, $\beta = 0.006$, $p < 0.01$) or obesity ($R^2 = 0.10$, $\beta = 0.006$, $p < 0.05$)

Table 1 (continued)

Study	Population	Exercise protocol	Analyzed biomarkers	Findings
Frandsen et al. (2020)	34 Recreationally active W (23 years) BMI: ~ 22 kg m^{-2} VO_{2max}: ~ 43.9 mL kg^{-1} min^{-1}	Cycle-ergometer; Fasted state (10–12 h); GXT; Frayn's equations; FATmax defined through a third degree polynomial regression *GXT was performed on three different phases of menstrual cycle (mid-follicular, late-follicular and mid-luteal)	Body fat, lean body mass, VO_{2peak}, plasma free fatty acids, estradiol, glucose, glycerol, insulin, lactate, luteinizing hormone and progesterone at rest	Neither estradiol nor progesterone was related to MFO *No information about the other biomarkers was provided
Zurbuchen et al. (2020)	11 Cyclist M (27 ± 5 yeas) BMI: 21.7 ± 0.9 kg m^{-2} VO_{2peak}: 64.9 ± 3.9 mL kg^{-1} min^{-1} 11 Trained M (29 ± 5 years) BMI: 23.9 ± 2.4 kg m^{-2} VO_{2peak}: 49.1 ± 7.4 mL kg^{-1} min^{-1}	Cycle-ergometer; Fasted state (10 h); GXT; Frayn's equations; FATmax defined through SIN model	$\Delta[HHb]_{BP}$, a_1, MAP, RCP, VT1 and VO_{2peak}	$\Delta[HHb]_{BP}$ ($r = 0.66$, $p < 0.05$), a1 ($r = -0.41$, $p < 0.05$), MAP ($r = 0.43$), RCP ($r = 0.62$, $p < 0.05$), VT ($r = 0.74$, $p < 0.01$) and VO_{2peak} ($r = 0.65$, $p < 0.01$) were directly correlated to MFO in the combined cohort *Authors did not report the data for each group nor performed a multiple regression analysis
Shaw et al. (2020)	8 Untrained M (25 ± 4 years) BMI: 25.1 ± 1.8 kg m^{-2} VO_{2peak}: 44.9 ± 5.3 mL kg^{-1} min^{-1} 7 Trained M (28 ± 7 years) BMI: 24.0 ± 2.1 kg m^{-2} VO_{2peak}: 62.6 ± 4.1 mL kg^{-1} min^{-1}	Cycle-ergometer; Fasted state (4 h); GXT; Frayn's equations; Analytical procedure for FATmax determination was not reported	Vastus lateralis %Type I, %Type IIa, %Type IIx muscle fibers, skeletal muscle capillary density, IMCL on type I and type II fibers; SDHB, UQCRC2, COXII, ATP5A, ATGL, HSL, HAD, PLIN2, PLIN5 abundance, plasma glucose, free fatty acids, insulin and triacylglycerols, and VO_{2peak}	The MFO was positively correlated with %type I fibers (0.81, $p < 0.01$), capillary density ($r = 0.61$, $p < 0.05$), SDHB ($r = 0.59, < 0.05$), UQCRC2 ($r = 0.69$, $p < 0.01$), COXII ($r = 0.77$, $p < 0.01$), ATP5A ($r = 0.59$, $p < 0.05$), ATGL ($r = 0.77$, $p < 0.01$), HSL ($r = 0.76$, $p < 0.01$), HAD ($r = 0.59$, $p < 0.05$), PLIN5 ($r = 0.68$, $p < 0.01$) and VO_{2peak} (0.73, $p < 0.01$) when combining data from both groups. Moreover, %type IIa fibers was negatively related with MFO ($r = -0.74$, $p < 0.01$) None of the measured biomarkers were correlated with MFO in the trained group whilst only type II IMTG was correlated with MFO in the untrained group Authors did not report the relationship of the different metabolites or insulin with MFO and did not perform a multiple regression analysis

Table 1 (continued)

Study	Population	Exercise protocol	Analyzed biomarkers	Findings
Frandsen et al. (2021)	11 Trained W (27 ± 4 years) BMI: 22 ± 3 kg m^{-2} VO_{2peak}: 57.4 ± 7.0 mL kg^{-1} min^{-1} 10 Trained M (31 ± 6 years) BMI: 23 ± 2 kg m^{-2} VO_{2peak}: 65.9 ± 6.1 mL kg^{-1} min^{-1}	Cycle-ergometer; 4 trials on a fasted state (NR); 4 trials on a postprandial state (10 min after a high carbohydrate meal consumption); GXT; Frayn's equations; FATmax defined through a third degree polynomial regression	Plasma free fatty acids, glycerol, glucose, lactate, β-hydroxybutyrate, and triacylglycerols concentrations were analyzed at rest and during exercise trials. Basal blood samples were analyzed for insulin, estradiol, and progesterone	[a]Plasma FFA was significantly associated with MFO in the combined cohort ($R^2 = 0.70$, $p < 0.01$) No information about the other biomarkers was reported
Chrzanowski-Smith et al. (2021)	21 Physically active M (39 ± 11 years) BMI: 24.3 ± 2.1 kg m^{-2} VO_{2peak}: 48.4 ± 6.4 mL kg^{-1} min^{-1} 15 Physically active W (41 ± 12 years) BMI: 23.0 ± 2.3 kg m^{-2} VO_{2peak}: 34.2 ± 6.4 mL kg^{-1} min^{-1}	Cycle-ergometer; Fasted state (11–13 h); GXT; Frayn's equations; FATmax defined through measured values	ESR1, ATGL, ABHD5, ACSL1, HSL and PLIN1 protein content in adipose tissue, ACSL1, ATGL, ESR1, CPT1b and FATBP content in skeletal muscle	Adipose tissue ESR1($r = 0.46$, $p < 0.05$) and HSL ($r = 0.38$, $p < 0.05$) tissue were correlated with MFO but not with MFO_{FFM} Skeletal muscle FATBP ($r = 0.44$, $p < 0.05$), ATGL ($r = 0.54$, $p < 0.01$) and CPT1b ($r = 0.52$, $p < 0.01$) were related to MFO; only ATGL ($r = 0.41$, $p < 0.05$) and CPT1b ($r = 0.45$, $p < 0.05$) were correlated with MFO_{FFM} [b]Adipose tissue ESR1 was significantly correlated with MFO in men ($r = 0.49$, $p < 0.05$) and women ($r = 0.52$, $p < 0.05$). Moreover, the relationship between MFO and HSL only reach significance when data from men and women were combined. Adipose tissue ABHD5 was correlated with MFO in men ($r = 0.68$, $p < 0.05$) but not in women Skeletal muscle ATGL ($r = 0.62$, $p < 0.05$) and CPT1b ($r = 0.74$ $p < 0.05$) were related with MFO only in women whilst correlation between MFO and FATB content only reach significance when data from men and women were combined
Maunder et al. (2021)	17 Trained M (34 ± 7 years) BMI: Not reported VO_{2peak}: 4.3 ± 0.7 L min^{-1}	Cycle-ergometer; Fasted state (11–13 h); GXT; Jeukendrup and Wallis equations; FATmax defined through a second degree polynomial regression	CS, CD36, VO_{2peak} and VT2	[a]Together, CS ($\beta = 0.59$), CD36 ($\beta = 0.27$), VO_{2peak} ($\beta = -0.03$) and VT2 ($\beta = 0.36$) explained 88% of MFO variation ($p < 0.01$)

Table 1 (continued)

Study	Population	Exercise protocol	Analyzed biomarkers	Findings
Chávez-Guevara et al. (2021)	21 Untrained M (27 ± 11 years) BMI: 32.6 ± 2.1 kg m^{-2} VO_{2peak}: 39.9 ± 6.0 mL kg^{-1} min^{-1}	Treadmill; Fasted state (10–12 h); GXT; Frayn's equations; FATmax defined through measured values	Fat-free mass, body fat and VO_{2peak}	[a]Body fat explained 40% of MFO variation ($p < 0.01$)
Amaro-Gahete et al. (2021)	38 Sedentary M (23 ± 2 years) BMI: 27.3 ± 5.8 kg m^{-2} VO_{2peak}: 45.1 ± 8.1 mL kg^{-1} min^{-1} 81 Sedentary W (22 ± 2 years) BMI: 23.9 ± 3.7 kg m^{-2} VO_{2peak}: 39.8 ± 6.6 mL kg^{-1} min^{-1} 34 Sedentary M (54 ± 5 years) BMI: 28.8 ± 3.5 kg m^{-2} VO_{2peak}: 33.2 ± 4.4 mL kg^{-1} min^{-1} 71 Sedentary W (53 ± 5 years) BMI: 25.2 ± 3.4 kg m^{-2} VO_{2peak}: 33.2 ± 4.4 mL kg^{-1} min^{-1}	Treadmill; Fasted state (5–12 h); GXT; Frayn's equations; FATmax defined through a third degree polynomial regression	Fat mass, lean mass, visceral adipose tissue, waist circumference, plasma cholesterol, high density lipoprotein, low density lipoprotein, glucose, insulin, blood pressure, physical activity and VO_{2max}	[a]Together, waist circumference ($\beta = -0.21$), systolic blood pressure ($\beta = 0.40$) and participants sex ($\beta = 0.27$; $M = 1$; $I = 2$) determined the 19% of MFO variation in young adults ($p < 0.01$) Otherwise, only VO_{2max} ($\beta = 0.37$) was associated with MFO in middle-aged adults ($R^2 = 0.13$, $p < 0.01$)

Data are presented as mean ± SD

a_1 slope of the first phase of the muscle deoxygenation kinetics, *ABHD5* abhydrolase domain containing 5, *ATGL* adipose triglyceride lipase, *ACSL1* acyl-CoA synthetase, *ATP5A* ATP synthase subunit mitochondrial, *BLa* blood lactate concentration, *BMI* body mass index, *COXII* cytochrome c oxidase subunit 2, *CS* citrate synthase, *CPT1* carnitine palmitoyl transferase 1, *ESR1* estrogen receptor alpha, *FATBP* fatty acid binding protein, FAO_p mitochondrial fatty acid oxidation, *FFA* free fatty acids, *GXT* graded exercise test, $\Delta[HHb]_{BP}$ breaking point of muscle deoxygenation kinetics, *HAD* β-hydroxy-acyl-CoA-dehydrogenase, *HSL* hormone sensitive lipase, *IMCL* intramuscular lipids, *M* men, *MAP* maximal aerobic power, *MFO* maximal fat oxidation, MFO_{FFM} maximal fat oxidation relative to fat-free mass, $Mito_{VD}$ mitochondrial volume density, *NR* not reported, *OXPHOS* mitochondrial oxidative phosphorylation capacity, *PLIN* perilipin, *RCP* respiratory compensation point, *RFO* resting fat oxidation, *SDHB* succinate dehydrogenase, *UQCRC2* cytochrome b-c1 complex subunit 2, VO_{2peak} peak of oxygen uptake, VO_{2max} maximal oxygen uptake, *VT1* first ventilatory threshold (aerobic threshold) , *VT2* second ventilatory threshold (anaerobic threshold) , *W* women

[a]Data provided by the corresponding author

[b]Data retrieved from open access data set

2021). In the same way, a positive and significant correlation between $VO_{2max/peak}$ and MFO has been reported in trained men (*r*: 0.52–0.65) (Robinson et al. 2016; Zurbuchen et al. 2020; Chrzanowski-Smith et al. 2021), combined groups of trained/untrained individuals (*r*: 0.72–0.84) (Dandanell et al. 2018; Shaw et al. 2020) as well as sedentary subjects with obesity ($r = 0.59$, $p < 0.01$) (Haufe et al. 2010), suggesting that improving CRF would lead to an increment on MFO.

Effect of exercise training over VO_{2max} and MFO

In this regard, a concomitant increment in MFO (Δ: 0.03–0.07 g min^{-1}) and VO_{2max} was reported in adolescents and elderly adults with obesity after 8–10 weeks of exercise training at FATmax (Chávez-Guevara et al. 2020). Indeed, recent studies in young (20–40 years old) and middle-aged sedentary adults (40–65 years old) observed that changes in $VO_{2max/peak}$ induced by endurance training (3 days/week; 300 or 600 kcal day^{-1} above 70% VO_{2peak}) (Rosenkilde et al. 2015) or high intensity interval training (HIIT) (40–65 min per week at an intensity of > 95% VO_{2max}) (Amaro-Gahete et al. 2020) predicted MFO increments, demonstrating that enhancing CRF improves fat metabolism in untrained subjects. Up to date, the chronic effect of an exercise training intervention over MFO in athletic population remains unexplored. Nonetheless, Achten and Jeukendrup (2003a) reported that MFO was 16% higher in endurance trained men with high *vs.* low VO_{2max} (71.9 ± 6.1 vs. 58.6 ± 5.2 mL kg^{-1} min^{-1}). In the same way, a significant mean difference in MFO levels (~ 0.18 g min^{-1}) was observed between long-distance male runners with high vs. low VO_{2max} (68.4 ± 4.5 vs. 58.6 ± 5.4 mL kg^{-1} min^{-1}) (Lima-Silva et al. 2010), suggesting that an increment on CRF induced by exercise training would augment MFO in endurance athletes, a hypothesis that needs to be supported by further studies.

Of note, all the above-mentioned studies evaluating the increment of MFO and VO_{2max} induced by exercise training employed an intervention period ≤ 12 weeks. Further studies are needed to elucidate whether CRF and fat metabolism keep improving after 12 weeks of physical exercise. Otherwise, findings from previous randomized clinical trials suggest that MFO and VO_{2max} increments depend of exercise training characteristics (i.e., intensity, volume). In young men with obesity, a large increment on MFO (Δ: 0.08 g min^{-1}; $d = 1.49$) and VO_{2max} (Δ: 2.8 mL kg^{-1} min^{-1}; $d = 0.82$) was observed after 2 weeks of HIIT (8 exercise sessions speeded over 14 days; 10 × 60-s cycling intervals at workload eliciting ~ 90% HRmax interspersed with 60-s recovery at 50 W) while only a low increment on MFO (Δ: 0.03 g min^{-1}; $d = 0.32$) and VO_{2max} (Δ: 1.8 mL kg^{-1} min^{-1}; $d = 0.27$) was observed after steady-state exercise training at FATmax (8 exercise sessions speeded over 14 days; 40–50 min day^{-1}) (Lanzi et al. 2015). In addition, both MFO (Δ16%, $p < 0.05$) and VO_{2max} (Δ14%, $p < 0.05$) increased significantly after 3 weeks of high intensity continuous training (5 days/week; 2 sessions day^{-1}; 70% VO_{2max}) in adolescents with obesity but remain unchanged in patients who trained at FATmax (5 days/week; 2 sessions day^{-1}) (Lazzer et al. 2011). Thus, in the short-term, training at high intensity seems to be more effective at improving MFO in comparison to exercising at FATmax. On the other hand, high-volume endurance training (3 days/week; 600 kcal day^{-1} above 70% VO_{2peak}) resulted on a larger increment on MFO in comparison to low-volume endurance training (3 days/week; 600 kcal day^{-1} above 70% VO_{2peak}) in sedentary men with overweight (Δ0.14 vs. 0.09 g min^{-1}) (Rosenkilde et al. 2015). Moreover, in healthy men, the MFO and FATmax are higher during treadmill running in comparison to stationary cycling (Achten et al. 2003), elliptical and rowing exercises (Filipovic et al. 2021). Thus, the effect of exercise training on MFO may depend on exercise intensity, type and volume. Further clinical trials are need to stablish the optimal training characteristics for improving MFO in athletes, sedentary lean individuals and patients with chronic diseases.

Despite a clear relationship is observed between VO_{2max} and MFO, the mechanisms behind such association are not fully understood. The VO_{2max} represents the capacity of an organism for transport O_2 from the environment to the body tissues for energy production in the mitochondrion through oxygen-dependent pathways, including fat oxidation. (Bassett and Howley 2000; Levine 2008). In this regard, the VO_{2max} is determined by lungs capacity and volume, cardiovascular function, microcirculation, and skeletal muscle oxidative capacity (Wagner 1996; Levine 2008). Therefore, all the aforementioned factors might be implicated on the association between MFO and VO_{2max}.

Biomarkers of skeletal muscle oxidative capacity associated with MFO

The VO_{2max} is positively associated with several biomarkers of skeletal muscle oxidative capacity, including mitochondrial volume density ($Mito_{VD}$) and oxidative phosphorylation capacity (OXPHOS) (Lundby and Jacobs 2016), capillary density (Hendrickse and Degens 2019) and type 1 muscle fibers abundance (Foster et al. 1978). Recent studies have evaluated the association of MFO with all the above-mentioned biomarkers providing valuable insights about the mechanism that regulate fat oxidation capacity.

Mitochondrial volume density and oxidative phosphorylation capacity

Dandanell et al. (2018) investigated the relationship of $Mito_{VD}$ and OXPHOS with MFO, observing a moderate

correlation between these variables ($r = 0.56$ and $r = 0.52$ for $Mito_{VD}$ and OXPHOS respectively) when combining data from endurance trained and untrained men. In the same study, mitochondrial fatty acid oxidation measured in vitro turned to be positively related with MFO ($r = 0.59$) suggesting that exercise training improves skeletal muscle fat oxidation capacity by enhancing mitochondrial quantity and OXPHOS.

The enzymatic activity of citrate synthase (CS) and the protein content of the mitochondrial respiratory chain complexes are considered reliable biomarkers of $Mito_{VD}$ and OXPHOS in skeletal muscle (Larsen et al. 2012) and its relationship with MFO has been analyzed in combined cohorts of trained/untrained individuals (Nordby et al. 2006; Stisen et al. 2006; Dandanell et al. 2018; Shaw et al. 2020). These studies noted a higher CS activity in trained vs. untrained subjects (35–44% higher) but only Dandanell et al. (2018) found a strong and significant relationship between CS and MFO ($r = 0.67$, $p < 0.01$) which may be explained due to a larger difference on VO_{2max} and CS activity among trained and untrained groups (see Table 1). In support of these data, the study by Maunder et al. (2021) observed that CS activity was more determinant than VO_{2max} for predicting MFO in young trained men (see standardized beta coefficients reported in Table 1). Moreover, Shaw et al. (2020) found a significant relationship between MFO and mitochondrial respiratory chain complexes II, III, IV and V when combining the data from trained and untrained individuals (Table 1), supporting the hypothesis that increasing skeletal muscle oxidative capacity would lead to a higher MFO.

The augment of MFO was positively correlated with the increment of muscle CS activity ($r = 0.63$, $p < 0.05$) and OXPHOS ($r = 0.78$, $p < 0.01$) after 10 weeks of exercise training at FATmax in subjects with type 2 diabetes (Bordenave et al. 2008). Moreover, Rosenkilde et al. (2015) showed that changes in CS activity and mitochondrial complexes chain complexes II, III, IV, and V induced by 12 weeks of endurance exercise training were related with MFO augment in young sedentary overweight men. Up to date, there is no evidence of a concomitant increment on $Mito_{VD}$ and MFO in athletic population.

Skeletal muscle contraction promotes mitochondrial biogenesis through activation of the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α) that recruits and co-regulates several transcription factors (i.e., nuclear respiratory factors 1 and 2, peroxisome proliferator-activated receptors, estrogen related receptor α) controlling the expression of mitochondrial proteins, including mitofusin 1 and 2 which regulate mitochondrial fusion and fission, respectively (Egan and Zierath 2013). In the same way, activation of PGC1-α upregulates the expression of several genes encoding CS and other proteins that regulate fatty acid trafficking and oxidation (see below) (Egan and Zierath 2013). Thus, future investigations should analyze the association between PGC1-α and MFO to elucidate molecular mechanisms implicated on metabolic health and sports performance. Interestingly, exercise intensity is positively related to exercise-induced increment of PGC1-α mRNA levels ($r = 0.38$) (Granata et al. 2018a) whilst training volume is directly related to $Mito_{VD}$ changes induced by physical exercise (Granata et al. 2018b). Therefore, future studies are needed to stablish training recommendations for optimizing skeletal muscle oxidative capacity and enhancing MFO.

In muscle fibers, the mitochondria are mainly distributed on intermyofibrillar cell compartment (Lundby and Jacobs 2016). Nonetheless, the relative increment of subsarcolemmal $Mito_{VD}$ is larger than in intermyofibrillar mitochondria after 6 weeks of high intensity endurance training (~ 72% VO_{2max}) (Suter et al. 1995). This may optimize oxygen and fatty acid diffusion to skeletal muscle mitochondria improving fat oxidation capacity. Indeed, subsarcolemmal $Mito_{VD}$ showed a larger correlation with MFO ($r = 0.62$, $p < 0.01$) in comparison to intermyofibrillar $Mito_{VD}$ ($r = 0.37$, $p < 0.01$) (Dandanell et al. 2018). Whether an increment on subsarcolemmal $Mito_{VD}$ influences muscle oxygenation kinetics and fatty acid uptake elevating MFO remains to be elucidated.

In addition, endurance training also improves intrinsic mitochondrial function by enhancing the surface of the mitochondrial inner membrane that in turn is positively related to VO_{2max} in both sedentary and trained individuals (Nielsen et al. 2017). The increment of mitochondrial cristae might lead to the assembly of electron transport chain supercomplexes enhancing mitochondrial respiratory capacity and ATP synthesis (Greggio et al. 2017). As before mentioned, a significant relationship between MFO and mitochondrial respiratory chain complexes II, III, IV and V was observed in lean individuals (Shaw et al. 2020). Nevertheless, whether an increment on electron transport chain supercomplexes could elevate MFO requires further investigation.

Exercise training improves $Mito_{VD}$, mitochondrial respiration and insulin sensitivity in patients with obesity and type 2 diabetes, suggesting a link between skeletal muscle oxidative capacity and metabolic health (Genders et al. 2020). The mechanism behind such association is not fully understood but it might be possible that a higher fat oxidation induced by enhanced $Mito_{VD}$ and OXPHOS could attenuate the accumulation of bioactive lipids like diacylglycerols and ceramides which are related to insulin resistance (Perreault et al. 2018). In this regard, alongside with increased MFO and CS activity, Rosenkilde et al. (2015) also reported a significant reduction on fasting insulin levels (− 30%) after 12 weeks of endurance training in sedentary men with overweight. On the contrary, 10 weeks of exercise training at FATmax did not reduce insulin or glucose circulating levels

in patients with diabetes despite a significant augment in MFO and CS activity (Bordenave et al. 2008). In the two aforementioned studies, changes of intramuscular lipids induced by exercise training were not measured. Therefore, future investigations are needed to elucidate whether an increment on MFO is associated with changes in skeletal muscle oxidative capacity, intramuscular lipid intermediates and insulin sensitivity.

In addition to increased $Mito_{VD}$ and OXPHOS, the abundance and activity of some glycolytic enzymes (e.g., lactate dehydrogenase) are reduced with exercise training attenuating muscle glycogenolysis which might delay glycogen depletion improving endurance performance (Holloszy and Coyle 1984; Egan and Zierath 2013; Knuiman et al. 2015; Hearris et al. 2018). Further studies should analyze the modifications in MFO and glycogen utilization induced by exercise training in athletes.

Capillary density

The MFO was positively correlated with muscle capillary density (*r*: 0.53–0.61) in young men (Dandanell et al. 2018; Shaw et al. 2020). Capillaries are blood vessels that deliver oxygen and nutrients to skeletal muscle fibers, being the number of capillaries per muscle fiber increased with endurance training throughout a process named angiogenesis (Egan et al. 2013; Hendrickse and Degens 2019). Up to date, whether an increment on capillary density induced by exercise training leads to a higher oxygen and fatty acid delivery to skeletal muscle elevating MFO remains undefined.

Fiber type distribution

Recent studies performed on healthy men, reported a positive correlation between MFO and %type I muscle fibers (*r*: 0.51–0.81) (Dandanell et al. 2018; Shaw et al. 2020). This relationship could be explained due to a higher capillary density, larger $Mito_{VD}$ and a higher activity of CS and mitochondrial respiratory chain complexes on type 1 muscle fibers in comparison to type 2 fibers (Egan and Zierath 2013). In addition, the abundance of transport proteins involved on fatty acid trafficking [i.e., fatty acid binding protein and fatty acid translocase cluster of differentiation 36 (CD36)] and the enzymatic activity of 3-hydroxyacyl-CoA dehydrogenase that regulates oxidation of fatty acids into the mitochondrion is also higher in type 1 muscle fibers (Vistisen et al. 2004; Egan and Zierath 2013).

To the best of our knowledge, the concomitant changes on %type I muscle fibers and MFO have not been analyzed neither in sedentary or trained individuals. Nevertheless, it is widely reported that skeletal muscle undergoes a remodeling process depending on exercise training characteristics. Several studies have shown that sprint, power and plyometric training could elicit a transition of type I muscle fibers to type IIa (Plotkin et al. 2021). Otherwise, endurance training promotes the shift of muscle fibers type IIa/IIx towards type I muscle fibers (Plotkin et al. 2021). The increment of type 1 muscle fibers might be of particular interest for long-distance runners in whom %type 1 muscle fibers is directly related to endurance performance (Foster et al. 1978). Moreover, patients with metabolic syndrome might benefit from increasing %type 1 muscle fibers because a diminished proportion of these muscle fibers is observed in this population when compared to lean subjects (Stuart et al. 2013; Albers et al. 2015). Further research is needed to elucidate whether an increment on MFO and the proportion of type 1 fibers improves insulin sensitivity and endurance performance in patients with diabetes and athletes respectively.

Cardiovascular function

A recent study evaluating the relationship between fat oxidation and muscle deoxygenation kinetics—assessed by the variations in deoxygenated hemo- and myoglobin concentrations—reported that the breakpoint and slope of the muscle deoxygenation kinetics correlated with MFO ($r = 0.66$, $p < 0.05$; $r = -0.41, < 0.05$, respectively) in a combined group of physically active individuals and well-trained cyclist (Zurbuchen et al. 2020). Therefore, the greater the capacity for maintaining a balance between oxygen delivery and extraction throughout a larger range of exercise intensities, the greater the reliance on aerobic metabolism and fat oxidation for ATP production.

The cardiovascular system is responsible for oxygen transport from the lungs to the different bodily tissues (Wagner 1996; Hoppeler and Weibel 2000; Levine 2008), thereby, cardiovascular function might be associated with exercise fat oxidation. In this regard, Skattebo et al. (2022) reported a higher MFO relative to muscle mass during one-leg cycling in comparison with two-leg cycling (+ 54%) being such differences associated with an elevated mass-specific oxygen delivery and a higher increment on cardiac output in response to one-leg cycling. These data suggest that enhancing oxygen delivery to skeletal muscle by increasing cardiac output may elevate MFO.

An increment of cardiac output occurs in response to exercise training due to a larger stroke volume, derived from an increment of left ventricle chamber dimension and also an increment in end-diastolic filling pressure (Hoppeler and Weibel 2000; Levine 2008). Recently, Vaccari et al. (2020) reported a concomitant increment of cardiac output, stroke volume, VO_{2max} and fat oxidation rates at 60–80% VO_{2max} after 3 months of HIIT in patients with obesity (34 exercise sessions; 3-min sprint intervals at ~ 100% VO_{2peak} interspersed by 90 s of walking at ~ 50% VO_{2peak}). Nonetheless, in the same study, MFO did not increase in response to

exercise training. Noteworthy, despite a large increment on cardiac output was reported by the previous study, authors did not evaluate modifications of muscle oxygenation kinetics. Therefore, further studies are needed to investigate the association among MFO, oxygen delivery to skeletal muscle and biomarkers of cardiovascular function (Fig. 1).

Pulmonary function

Ventilatory threshold

A moderate association of the first and second ventilatory thresholds (VT) with MFO has been reported by several studies in trained men (R^2: 0.46–0.55) (Amaro-Gahete et al. 2019a, b; Zurbuchen et al. 2020; Maunder et al. 2021). The VT are positively related with muscle oxygenation kinetics (Zurbuchen et al. 2020) and performance in long-distance runners. Moreover, the VT increase with exercise training in sedentary men and women, with a higher improvement when exercise training is performed above the VT intensity (Gaskill et al. 2001).

The second VT indicates an increment on pulmonary ventilation that results from accumulation of lactate in the blood and skeletal muscle (Binder et al. 2008). The accumulation of lactate in skeletal muscle sarcoplasm induces a reduction of muscle pH that decrease the activity of carnitine palmitoyl transferase 1 (CPT1), thus reducing the entry of long-chain fatty acids into the mitochondrial matrix (Starritt et al. 2000; Achten and Jeukendrup 2004). Indeed, blood lactate levels are inversely associated with fat oxidation rates during a graded exercise test (San-Millán and Brooks 2018). Exercise training improves lactate clearance in skeletal muscle fibers by increasing the density of monocarboxylate transporters (MCT1 and MCT4) in the sarcolemma (Thomas et al. 2012). This adaptation delays the reduction of sarcoplasmic pH and the inhibition of CPT1 allowing a higher fat oxidation. San-Millán and Brooks (2018) reported that lactate threshold and MFO occurred at a higher workload in endurance trained athletes in comparison with patients that suffer from metabolic syndrome. Nevertheless, whether a parallel increment on lactate threshold, VT and MFO in obtained after exercise training remains to be elucidated.

Lung capacities and volumes

Pulmonary function is a critical determinant of pulmonary ventilation, oxygen uptake and VO_{2max} (Wagner 1996). Several biomarkers of lung volumes (e.g., tidal volume) are positively related to physical activity and seem to be increased with endurance training (Lutfi 2017). Moreover, Durmic et al. (2017) suggest that lungs capacities also increase with exercise training because vital capacity and forced expiratory volume were higher in endurance trained individuals vs. sedentary controls. The adaptations of lungs capacities might be related to exercise intensity, type and volume because vital capacity and maximal expiratory volume are notably higher in water polo athletes when compared against basketball, handball and soccer players (Durmic et al. 2015). Although the mechanism behind the increment of lung volumes and capacities are not fully understood, Durmic et al. (2017) proposed that endurance exercise may change airways resistance conduction, alveolar expansion and lung elasticity. In addition, exercise training increases the strength of respiratory muscles contributing to a higher pulmonary ventilation (Hackett 2020). Further studies need to evaluate whether lung capacities and volumes correlate with muscle oxygenation kinetics and MFO.

Fatty acid metabolism

The contribution of fatty acid delivered from adipose tissue and intramuscular triglycerides (IMTG) to energy expenditure is determined by exercise intensity, being the plasma free fatty acids (FFA) the main energy source during low to moderate exercise intensity while IMTG increases its contribution as workload progressively augment during a graded exercise test (Hargreaves and Spriet 2020). The FATmax is commonly observed between 45 and 65% VO_{2max} (Purdom et al. 2018) which represent a moderate exercise intensity (Mann et al. 2013). Nevertheless, because the FATmax and the aerobic threshold occur at a similar intensity in both sedentary (Peric and Nikolovski 2020) and trained individuals (Nikolovski et al. 2021), MFO is located at a low exercise intensity (MacIntosh et al. 2021). Thus, FFA would be the main fuel used for ATP production and its concentration might be associated with MFO.

Adipose tissue lipolysis

In this sense, Robinson et al. (2016) reported a low correlation between FFA concentration at FATmax and MFO in trained men ($r = 0.27$, $p < 0.05$). Such correlation disappeared when plasma FFA at rest was included as a covariate. Indeed, the FFA increment from rest to FATmax was not correlated with the differences between fat oxidation at rest and MFO. To the best of our knowledge, only this study has analyzed the association between FFA concentration at FATmax and MFO, therefore, further research is necessary to stablish a robust conclusion. Interestingly, sedentary men with obesity exhibited higher FFA availability at FATmax and higher absolute MFO during an incremental-load exercise in comparison to lean subjects (Lanzi et al. 2014) which has been related with a larger fat mass in patients with obesity (Amaro-Gahete et al. 2019b). Moreover, women with low abdominal to lower body fat mass ratio exhibited a

higher MFO and FATmax in comparison to women with high abdominal to lower body fat mass ratio despite no differences in FFM and total fat mass (Isacco et al. 2014). The last finding could be explained by lower insulin levels and higher FFA concentration during moderate intensity exercise in women with high abdominal to lower body fat mass ratio (Isacco et al. 2013). Thereby, the relationship between FFA and MFO could be influenced by nutritional status and fat mass distribution, a hypothesis that also require further research.

The data from Robinson et al. (2016) suggest that fatty acid availability prior exercise seems to be more determinant than lipolytic response to this stimulus. In this sense, Frandsen et al. (2017) showed that overnight fasting (9–13 h) plasma FFA at rest was independently associated with MFO explaining 6% of its variation in male ironman athletes, although such association was not observed in female ironman athletes ($R^2 = 0.07$, ns) in whom VO_{2peak} was the only biomarker associated with MFO (Vest et al. 2018). The discrepancy across these studies suggests that MFO is determined by different physiological mechanisms across male and female ironman athletes which require further analysis. On the other hand, Frandsen et al. (2021) showed that increasing FFA throughout prolonged fasting (> 22 h) and repeated exercise sessions resulted on a higher MFO in endurance athletes. In the same study, a linear regression analysis showed that an increase of 500 µmol L in plasma FFA would augment MFO by 4.5 mg kg lean body mass^{-1} min^{-1} in men and by 5.5 mg kg lean body mass^{-1} min^{-1} in women ($R^2 = 0.70$, $p < 0.01$), highlighting the association between FFA availability and MFO. Future investigations are needed to corroborate whether a higher FFA availability led to higher fatty acid uptake and oxidation in skeletal muscle.

Triacylglycerol hydrolysis (lipolysis) is a sequential process involving (1) several enzymes called lipases and (2) different regulatory proteins located in the surface of lipid droplets named perilipins. The enzymes triacylglycerol lipase (ATGL) and hormone sensitive lipase (HSL) catalyze the hydrolysis of the first and second ester bonds producing diacylglycerol and monoacylglycerol, respectively, while perilipin 1 facilitates the access of lipases into the lipid droplet (Tsiloulis and Watt 2015). Both, HSL and perilipin phosphorylation increases in response to β-adrenergic stimulation which augment during physical exercise (Jordy and Kiens 2014; Tsiloulis and Watt 2015). A pilot study in healthy individuals showed that MFO was positively related with HSL ($r = 0.38$, $p < 0.05$) and perilipin 1 ($r = 0.71$, $p < 0.01$) abundance in adipose tissue (Chrzanowski-Smith et al. 2021). The higher expression of these proteins may elevate FFA availability inducing a higher MFO. Nevertheless, this study did not measure the activity or phosphorylation status of these proteins. Moreover, in the same study, ex vivo adipose tissue lipolysis rates and MFO were not associated ($r = -0.38$, $p > 0.05$). Several studies have informed an increment on adipose tissue HSL, ATGL and perilipin abundance after endurance training in rodents (Tsiloulis and Watt 2015). Moreover, aerobic exercise training seems to improve beta-adrenergic induced lipolysis in the sub-cutaneous adipose tissue of patients with obesity by decreasing the anti-lipolytic activity of alpha-2 adrenergic receptors (Laurens et al. 2020). Nonetheless, several studies report that FFA concentration at rest and during exercise does not increase with exercise training (Horowitz 2003; Tsiloulis and Watt 2015). Indeed, MFO increment after exercise training at FATmax (Bordenave et al. 2008), moderate intensity exercise (Rosenkilde et al. 2015) and high intensity interval training (Lanzi et al. 2015) occurred without a concomitant augment of circulating FFA at rest. Hence, despite increasing FFA availability is associated with an increment on MFO (Frandsen et al. 2021) more research is guaranteed to identify novel strategies that promote adipose tissue lipolysis in patients with obesity and athletes, to reduce body fat and increase the reliance on fatty acids for ATP production.

Intramuscular triglycerides

As above mentioned, MFO is located at a low exercise intensity when IMTG shows a modest contribution to energy expenditure. Nevertheless, skeletal muscle HSL ($r = 0.75$, $p < 0.01$), ATGL (r: 0.54–0.76) and perilipin 5 ($r = 0.67$, $p < 0.01$) content were positively related to MFO in healthy individuals (Shaw et al. 2020; Chrzanowski-Smith et al. 2021), suggesting that elevating IMTG breakdown would increase MFO. Of note, intramuscular lipids content was not related to MFO in patients with obesity (Haufe et al. 2010) or trained subjects (Dandanell et al. 2018). Hence, the relevance of IMTG utilization on MFO remains to be elucidated.

Previous studies, reported that perilipin 2 and 5 expression is upregulated by resistance exercise and endurance training in sedentary men, resulting on increased IMTG hydrolysis during physical exercise (Shaw et al. 2012; Shepherd et al. 2013, 2014). In addition, lipid droplets proximity to mitochondria in skeletal muscle increases with exercise training due to mobilization of lipid droplets from subsarcolemal region to intramyofibrillar cell compartment as well as elevated $Mito_{VD}$ and OXPHOS in both athletes and patients with obesity (Gemmink et al. 2020). These changes led to increased IMTG utilization during exercise which is related to improvements on insulin sensitivity in patients with obesity (Sheperd et al. 2014; Gemmink et al. 2020) and exercise performance on athletes (Hearris et al. 2018). Therefore, future studies are needed to investigate whether a simultaneous increase on IMTG breakdown and oxidation is associated to MFO.

Fatty acid trafficking and oxidation in skeletal muscle

The abundance of different proteins regulating fatty acid trafficking [i.e., carnitine palmitoyltransferase 1 (CPT-1), fatty acid binding protein and fatty acid translocase cluster of differentiation 36 (CD36)] and oxidation (3-hydroxyacyl-CoA dehydrogenase) in skeletal muscle has been also associated with MFO variations in different cohorts of trained/untrained individuals (Stisen et al. 2006; Shaw et al. 2020), trained men (Maunder et al. 2021) and physically active men and women (Chrzanowski-Smith et al. 2021). From the aforementioned proteins, only CD36 has been validated as MFO predictor explaining 11% of MFO variation in trained men (Maunder et al. 2021). The CD36 mediates long-chain fatty acids transports across the sarcolemma and mitochondrial double membrane where it colocalizes with CPT1 (Smith et al. 2012). Thus, long-chain fatty acids mobilization across sarcolemma and mitochondria seems to be an important determinant of MFO, a finding that need to be corroborated in other populations. A previous study in untrained women, reported an increment on both skeletal muscle mitochondria CD36 content (+ 51%) and fat oxidation (+ 68%) during moderate intensity exercise (65% VO_{2peak}) after 6 weeks of high intensity interval training (3 days/week; 4-min cycling bouts at 90% VO_{2peak}) (Talanian et al. 2010). In the same way, colocalization of CD36 with CPT-1 in the mitochondria increased by 25% after an endurance training intervention (3 days/week; 35–45 min day^{-1} at 70–85% HRmax) in women with obesity which turned to be strongly associated with the increment of fat oxidation rate at rest ($R^2 = 0.85$, $p < 0.01$) (Schenk and Horowitz 2006). Likewise, expression levels of CD36 and CPT1 in skeletal muscle increased after 12 weeks of endurance training (5 days/week; 60 min day^{-1} at 85%HRmax) in elderly adults (Mulya et al. 2017). In the last study, the increment of muscle CPT1 was positively correlated with a resting fat oxidation augment ($r = 0.45$, $p = 0.05$). The aforementioned evidence suggest that exercise training could enhance MFO by promoting fatty acid uptake across the sarcolemma and mitochondrial membranes. Nevertheless, future investigation is needed to corroborate such hypothesis. Otherwise, the CD36 also mediates fatty acid release from adipose tissue (Pepino et al. 2014). It would be interesting that future studies investigate whether CD36 abundance in adipose tissue and skeletal muscle is associated with FFA rate of appearance/disappearance affecting MFO.

The fat oxidation rate at rest is positively correlated with MFO in trained men ($r = 0.47$, $p < 0.01$) (Robinson et al. 2016) and is downregulated by high glucose and insulin concentrations (Melzer 2011). Insulin suppresses adipose tissue lipolysis by stimulating the activity of cellular phosphodiesterase-3 (Horowitz 2003) and reduces skeletal muscle long-chain fatty acids oxidation and long-chain acylcarnitine concentration showing a decrement on the functional activity of CPT1 (Rasmussen et al. 2002; Sidossis et al. 1996). A previous study by Achten and Jeukendrup (2003b) reported a decline on MFO (~ 28%) when 75 g of glucose was consumed 45 min prior exercise testing in moderately trained individuals. This evidence suggests that circulating glucose, insulin and lactate—a metabolite representing skeletal muscle glycolytic flux—may be associated with MFO. In this regard, Amaro-Gahete et al. (2021) did not found any relationship between MFO, plasma glucose and insulin concentration in sedentary young and middle-aged adults (Amaro-Gahete et al. 2021). Furthermore, plasma glucose and lactate were not associated with MFO in trained men (Robinson et al. 2016). On the contrary, plasma lactate levels at rest were negatively associated with MFO ($R^2 = 0.12$, $p < 0.01$) in male ironman athletes (Frandsen et al. 2017), although similar lactate concentrations were noted in comparison to the participants from the study of Robinson et al. (2016) (0.8 ± 0.3 vs. 0.8 ± 0.2 mmol L^{-1}, respectively). Thus, the influence of glycolytic flux at rest and MFO remains controversial and future investigations including individuals with obesity and type II diabetes mellitus who exhibit higher circulating levels of lactate, glucose and insulin will help to elucidate the aforementioned mechanism.

Endocrine function

Physical exercise induces lipolysis throughout the action of several hormones (i.e., adrenaline, atrial natriuretic peptide, cortisol, glucagon and growth hormone) that regulate adipose tissue and skeletal muscle HSL and ATGL activity (Muscella et al. 2020). Nonetheless, whether the activity, circulating concentration of these hormones and the level of expression of their respective cellular receptors are associated with MFO remain unclear. Furthermore, previous studies have demonstrated that thyroid hormones regulate fatty acid oxidation in the skeletal muscle through p43 thyroid receptor alpha isoform which increases AMP-activated protein kinase phosphorylation and mitochondrial associated protein activity (enzymes that regulate CD36 translocation to sarcolemma and mitochondrial fatty acid oxidation) (Sayre and Lechleiter 2012). Thus, future studies should determine whether there is a direct association between thyroid hormones and MFO (Fig. 1).

On the other hand, female ovarian hormones (i.e., estradiol and progesterone) have been associated with higher MFO in women vs. men (Venables et al. 2005; Purdom et al. 2018; Maunder et al. 2018). In particular, estrogens could promote fatty acid oxidation through binding the

estrogen receptor alpha in the sarcoplasm that dimerizes and subsequently is translocated to the nucleus promoting the transcription of Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, Nuclear Respiratory Factor and additional genes involved in mitochondrial biogenesis and long-chain fatty acids oxidation (Ventura-Clapier et al. 2019). Nonetheless, circulating estradiol concentration did not exhibit a correlation with MFO neither in recreationally active or ironman female athletes (Vest et al. 2018; Frandsen et al. 2020). Moreover, skeletal muscle estradiol receptor alpha, CPT1, HSL and ATGL content were not different between physically active men and women in spite the higher MFO reported in the female participants' cohort (Chrzanowski-Smith et al. 2021). Whereby, a higher skeletal muscle lipolysis and oxidative capacity induced by female sexual hormones seems not to be the physiological mechanism explaining MFO differences between sexes and further mechanisms should be explored. Interestingly, a recent study by Ponce González et al. (2017) reported a positive relationship between free testosterone levels and MFO in young men ($r = 0.45$, $p < 0.05$), which might be explained by the anabolic effect of testosterone that favor a higher muscle mass and a subsequent upregulation of genes involved in mitochondrial biogenesis and fatty acid metabolism via androgen receptor activity (Usui et al. 2014; Kelly and Jones 2015). Therefore, current evidence indicates that male but not female sexual hormones are related to MFO.

Genetic polymorphisms

As before discussed, association of different biomarkers with MFO are inconsistent among studies investigating participants with a similar physical fitness, with most of these biomarkers explaining less than 40% of MFO variation (Table 1). This evidence points out that genetics may play an important role on determining an organism capacity for increasing fat oxidation in response to physical exercise. Indeed, recent studies have reported a significant association of ACE, ADRB3, AR and CD36 genotype with MFO (Table 2) providing valuable evidence that enhance the comprehension of the molecular pathways regulating exercise metabolism.

The ACE gene encodes the angiotensin-converting enzyme (ACE) which converts angiotensin I to angiotensin II increasing the vasoconstriction activity of this hormone that raise total peripheral resistance and blood pressure (Vasudeva et al. 2020). An intronic variant consisting of the deletion (D) of a 216 bp segment in intron 16 of the ACE gene has been associated with higher circulating levels of angiotensin II (Rigat et al. 1990), elevated blood pressure (Ma et al. 2018) and reduced CRF (Bueno et al. 2016). Thereby, the DD genotype is considered an independent cardiovascular risk factor (Yuan et al. 2017). A recent study by Montes-de-Oca-Garcia et al. (2021) reported that MFO was 29% higher in female carriers of the DD genotype in comparison to carriers of the reference allele (II). Interestingly, the opposite was found in male participants were MFO was superior in male carriers of the DD genotype (15% higher), demonstrating a sex–genotype interaction regarding MFO whose explanation require further analysis. The DD genotype has been associated with higher circulating levels of the angiotensin II hormone (Rigat et al. 1990) which stimulates vasoconstriction and may reduce fatty acid and oxygen uptake by skeletal muscle. In fact, male carriers of the DD genotype exhibit a reduced capillary density on skeletal muscle (Valdivieso et al. 2017) which may explain a low MFO. However, further studies evaluating the association of circulating ACE concentration with FFA rate of appearance/disappearance, skeletal muscle deoxygenation kinetics and MFO are needed for elucidating the mechanisms behind the association of ACE genotype with MFO.

On the other hand, a missense mutation in the ADRB3 gene—resulting on the interchange of a tryptophan (W) for an arginine (R) in the position 64 of the Beta-3 adrenergic receptor (β-AR_3)—was associated with a lower energy expenditure at FATmax in healthy adolescents (~ 14% lower), being considered a consequence of lower fat oxidation rates (Correa de Jesus et al. 2018). The β-AR_3 is a G-coupled protein located on adipocytes plasma membrane that regulate adipose tissue lipolysis by triggering HSL activation after binding of adrenaline (Tsiloulis and Watt 2015; Schena and Caplan 2019). The W > R substitution is located on a cytoplasmic topological domain that may negatively affect the receptor sensitivity reducing lipolysis and FFA availability during physical exercise which has been directly associated with MFO. Nonetheless, a previous study by Gómez-Gómez et al. (2014) did not observe a lower lipolytic rate in male carriers of the R allele during a steady-state exercise session at ~ 62% VO_{2max}. Thus, evidence of a lower FFA availability and MFO in carriers of the W64R polymorphism is needed. Moreover, as both W64R polymorphism and MFO are associated with body mass index and obesity (Amaro-Gahete et al. 2019a, b; Schena and Caplan 2019; Chávez-Guevara et al. 2021) analyze the interaction of β-AR_3 and MFO in this population would be especially relevant.

The AR gene encodes the androgen receptor protein (AR) that function as a steroid-hormone activated transcription factor that could upregulate lipolysis and fat oxidation in skeletal muscle (Kelly and Jones 2015). This gene contains 2 polymorphic trinucleotide repeat segments that encode polyglutamine (CAG) and polyglycine (GGN) tracts in the N-terminal transactivation domain of its protein that may affect AR sensitivity. Previous studies reported that MFO was ~ 35% higher in male carriers of the CAG_L and GGN_L

Table 2 Genetic polymorphisms associated with MFO on trained and untrained subjects

Study	Population	Exercise protocol	Genetic polymorphism	Findings
Jayewardene et al. (2014)	15 men and 7 women (18–30 years) Physically active BMI: NR VO_{2max}: NR	Cycle-ergometer; Fasted state (10 h); GXT	Gene: CD36 Intron variant (SNP) Alleles: T > C Global MAF: 0.51	Carriers of the CC genotype exhibited a higher MFO (NR) in comparison to individuals with TT genotype
Ponce González et al. (2016)	90 men Physically active CAG_S BMI: 23.2 ± 3.6 kg m^{-2} VO_{2max}: 42.6 ± 7.7 mL kg^{-1} min^{-1} CAG_L BMI: 27.8 ± 5.4 kg m^{-2} VO_{2max}: 44.4 ± 9.3 mL kg^{-1} min^{-1} GGN_S BMI: 24.9 ± 4.8 kg m^{-2} VO_{2max}: 43.5 ± 8.4 mL kg^{-1} min^{-1} GGN_L BMI: 26.3 ± 5.4 kg m^{-2} VO_{2max}: 43.4 ± 8.9 mL kg^{-1} min^{-1}	Cycle-ergometer; Fasted state (NR); GXT; Frayn's stoichiometric equations	Gene: AR Exon variant: repeated polymorphism $GAG_s \leq 21$ repeats $CAG_L > 21$ repeats $GGN_s \leq 23$ repeats $GGN_L \geq 24$ repeats	Subjects with the CAG_L exhibited a higher MFO in comparison to individuals with the CAGs genotype (242.6 ± 100.6 mg min^{-1} vs. 332.8 ± 117.1 mg min^{-1}, $p < 0.05$). This difference prevailed even after accounting for VO_{2max} as covariate No difference in MFO was observed between GGN_L and GGN_s carriers
Ponce González et al. (2017)	38 men Physically active CAG_S BMI: NR VO_{2max}: 42.4 ± 7.1 mL kg^{-1} min^{-1} CAG_L BMI: NR VO_{2max}: 46.6 ± 8.7 mL kg^{-1} min^{-1} GGN_S BMI: NR VO_{2max}: 40.6 ± 7.2 mL kg^{-1} min^{-1} GGN_L BMI: NR VO_{2max}: 43.7 ± 6.5 mL kg^{-1} min^{-1}	Cycle-ergometer; Fasted state (NR); GXT; Frayn's stoichiometric equations	Gene: AR Exon variant: repeated polymorphism $GAG_s \leq 23$ repeats $CAG_L \geq 24$ repeats $GGN_s \leq 22$ repeats $GGN_L \geq 25$ repeats	Subjects with the CAG_L genotype showed a higher MFO in comparison to individuals with the CAG_s genotype (362.2 ± 160.9 mg min^{-1} vs. 267.0 ± 104.6 mg min^{-1}, $p < 0.05$) Carriers of the GGN_L genotype showed a higher MFO in comparison to individuals with the GGNs genotype (375.2 ± 149.8 mg min^{-1} vs. 276.6 ± 93.7 mg min^{-1}, $p < 0.05$) *Differences between groups disappear after accounting for VO_{2max} as a covariate
Correa de Jesus et al. (2018)	33 healthy boys and 39 healthy girls (11–17 years) Trp64Trp BMI: 20.9 (11.06) kg m^{-2} VO_{2max}: 43.9 ± 8.2 mL kg^{-1} min^{-1} Trp64Arg + Arg64Arg BMI: 21.7 (12.38) kg m^{-2} VO_{2max}: 41.0 ± 8.0 mL kg^{-1} min^{-1}	Treadmill running; GXT; Energy expenditure at FATmax was defined according to Lusk's table based on the respiratory exchange ratio	Gene: ADRB3 Missense variant (SNP) Alleles: A > G Global MAF: 0.07	Carriers of the Arg64 allele exhibited a lower energy expenditure at FATmax in comparison with carriers of the Trp64 genotype (6.75 kcal min^{-1} vs. 7.78 kcal min^{-1}, $p < 0.05$)

Table 2 (continued)

Study	Population	Exercise protocol	Genetic polymorphism	Findings
Montes-de-Oca-García et al. (2021)	46 healthy M (22 ± 4 years) and 28 healthy W (23 ± 5 years) DD BMI: 27.6 ± 7.3 kg m^{-2} VO_{2max}: 37.3 ± 13.1 mL kg^{-1} min^{-1} ID + II BMI: 24.9 ± 4.4 kg m^{-2} VO_{2max}: 42.6 ± 11.0 mL kg^{-1} min^{-1}	Cycle-ergometer; Fasted state (> 8 h); GXT; Frayn's stoichiometric equations; FATmax was defined through a polynomial regression	Gene: ACE Intron variant: insertion/deletion of a 216 bp fragment in intron 16	No differences in MFO across genotypes was observed in the combined group A sex x genotype interaction was observed for MFO_{FFM}. Carriers of DD polymorphism showed a higher MFO_{FFM} in males (8.4 ± 3.0 mg kg FFM min^{-1} vs. 6.5 ± 2.9 mg kg FFM min^{-1}) whilst carriers of the ID/II polymorphism exhibited a higher MFO_{FFM} in women (7.6 ± 2.6 mg kg FFM min^{-1} vs. 6.6 ± 2.3 mg kg FFM min^{-1}) ($p < 0.05$, $\dot{\eta}_p^2 = 0.092$)

Data are presented as mean ± SD

BMI body mass index, *GXT* graded exercise test, *M* men, *MFO* maximal fat oxidation, MFO_{FFM} maximal fat oxidation relative to fat-free mass, *SNP* single nucleotide polymorphism, VO_{2peak} peak of oxygen uptake, VO_{2max} maximal oxygen uptake, *W* women

genotypes in comparison with carriers of the CAG_S and GGN_S genotypes (see genotype classification described in Table 2) (Ponce-González et al. 2016; Ponce González et al. 2017). These results are interesting but controversial since the GAG_L genotype was associated with a lower AR sensitivity (Chamberlain et al. 1994) that may lead to a reduced fat oxidation. Indeed, a recent study reported that AR inhibition attenuated skeletal muscle CPT1 and exercise fat oxidation increment on mice after 4 weeks of treadmill running (Kim et al. 2019). Therefore, it seems plausible that if a lower AR activity is associated with GAG_L and GGN_L genotypes, carriers of these genotypes should exhibit a lower MFO.

Interestingly, a single nucleotide polymorphism (T > C) in the upstream promoter region of the CD36 gene (intron 1B, − 3489 bp relative to the translation start site), was associated with higher levels of MFO in healthy individuals (Jayerwardene et al. 2014). This genetic variant has been also associated with a lower prevalence of type 2 diabetes in patients with obesity (Corpeleijn et al. 2006), supporting the association between MFO and insulin sensitivity (Robinson et al. 2016). Indeed, the T > C polymorphism may affect the transcription of CD36 gene (Corpeleijn et al. 2006). Because the CD36 abundance in skeletal muscle has been positively associated with MFO (Maunder et al. 2021), future studies performing skeletal muscle biopsies are required to determinate whether CT/CC genotypes exhibit a higher content of FAT/CD36 on skeletal muscle, and whether these hypothetical greater values are associated to elevated MFO. Moreover, the study by Jayewardene et al. (2014) showed a poor statistical power ($n = 22$; $n \leq 9$ subjects per group) and they have to combine data from men and women together, matching participants by CRF. Thus, future investigations with a large statistical power and an appropriate control of those biological determinant of MFO are necessary to corroborate whether the CD36 genotypes partially explain MFO variance.

As noted, only a few number of studies have analyzed the association of genetic polymorphism with MFO, focusing on healthy subjects with low to moderate physical activity. Future studies investigating the interplay between the above-mentioned polymorphism, MFO and body fat reduction induced by exercise training in patients with obesity are needed. Besides, different polymorphism on the CD36 (rs2232169), SLC6A14 (rs2011162) and PCSK1 (rs6235) genes have been associated with a reduced fat oxidation at rest in European adults with obesity (Corpeleijn et al. 2010). Consequently, these may also be associated with MFO.

Conclusions and future directions

In conclusion, there are several biomarkers, health-related components of physical fitness and genetic polymorphism associated with MFO (Fig. 1). Up to date, only $VO_{2max/peak}$ has been validated as MFO predictor in athletes and sedentary individuals while plasma FFA, circulating lactate concentrations and the protein content of CS and CD36 in skeletal muscle show a modest association with MFO in trained subjects. Although numerous manuscripts have been recently published about the present topic, there is still a lot of "gaps" regarding the physiological mechanism that regulate exercise-induced fat oxidation. Noteworthy, the majority of the here discussed studies focuses on evaluating the relationship of novel biomarkers with MFO rather than analyzing their contribution to prediction models reported for athletes (Randell et al. 2017) and healthy individuals (Venables et al. 2005; Fletcher et al. 2017). Mainly, this occurs due to a low statistical power, the independent evaluation of men and women and the lack of physical activity level and dietary macronutrient content assessments. Besides, most of the studies focus on healthy young adults with a scarce information regarding elderly adults and people with chronic diseases. Nonetheless, this work set the basis for future large-scale metabolomics and genomics studies that will contribute to the progress of exercise metabolism field. Indeed, further research about the association of MFO with different transcription factors (e.g., Peroxisome proliferator-activated receptor alpha, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, Nuclear respiratory factor) regulating mitochondrial biogenesis and fatty acid metabolism (Egan and Zierath 2013) will provide a further understanding about the molecular mechanisms influencing metabolic health and athletic performance. Moreover, investigating different microRNAs related to CPT1 (i.e., miR-1) (Rodrigues et al. 2021), CD36 (i.e., miR-145, miR-20a-5p, miR-29a) (Ding et al. 2019; Lin et al. 2019; Wang et al. 2020) and ACE (i.e., miR-143) (Vasudeva et al. 2019) might help to identify epigenetic mechanisms controlling MFO. Recent studies reported that physical exercise could regulate the expression of the above-mentioned microRNAs (Fernández-Sanjurjo et al. 2018; Silva et al. 2020); therefore, these might be implicated on metabolic adaptations induced by exercise training.

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Declarations

Conflict of interest All authors declare that they have no conflict of interest.

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