Impact of ageing on female metabolic flexibility: a cross-sectional pilot study in over-60 active women

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Research Article

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Abstract

Background

Ageing influences the metabolic flexibility, albeit the physical status could determine this relationship. This cross-sectional study aims to describe and analyse the metabolic flexibility/inflexibility in a group of active older women, together with the impact of ageing and physical status on their oxidation rates and maximal fat oxidation (MFO).

Methods

Fifteen volunteers (69.00 ± 6.97 years) from 24 women, completed an incremental cycling test until the second ventilatory threshold. Intensity increased 10W each 3min–15sec, starting at 30W. Gas exchange, heart rate, rate of perceived effort, pain scale and muscle power were registered, together with lactate. VO_2 and VCO_2 were considered for Fat and CHO oxidation (FATox & CHOox; Frayn's equation) at the intensities 60%, 80% and 100% from the peak of power in the test (P_{100}). Psychophysiological parameters were compared at MFO/FATmax and P_{100} , together with the main correlation analyses, with and without P_{100} and VO_2 as covariates.

Results

FATox was low at MFO (0.13 ; 95%CI $[0.09-0.17]$ $\text{g} \cdot \text{min}^{-1} \cdot \text{kg}$; 5.61 $[3.59-7.63]$ $\text{g} \cdot \text{min}^{-1} \cdot \text{kg}$ FFM), with a shifting down and leftward of a short oxidation-rate curves. CHOox and FATox were both low for a reduced power with age (77.14 ± 18.58 W & 39.29 ± 9.17 W at P_{100} and MFO respectively), pointing to metabolic inflexibility in older women despite being active. Notwithstanding, the negative correlation between age and MFO ($r = -0.54$, $p = 0.04$; $R^2 = 0.29$) disappeared when normalized with P_{100} ($r = -0.17$, $p = 0.53$), which was in turn strongly and negatively associated to age ($r = -0.85$, $p < 0.005$; $R^2 = 0.72$). P_{100} was also positive and moderately associated to MFO ($r = 0.71$, $p = 0.01$; $R^2 = 0.50$).

Conclusions

Despite the inflexibility with age, physical status (i.e., larger muscular power) suggest a key role in the preservation of the metabolic health with aging in active women.

Key Points

- Ageing supposes an impaired MFO, accompanied of a shifting down and leftward of oxidation rates curves.

- Muscle power, more than VO_2 (near the second ventilatory threshold), influences MFO in older active women, with blood lactate production preserved and displaying a moderate association to MFO.
- Larger power at higher exercise intensities is associated to better fat oxidation capacity at lower loads, pointing to a shielding effect from metabolic dysfunction.

Background

Skeletal muscle, in the basis of adaptive movement, functional capacity and physical fitness, plays a key role as a paracrine and endocrine organ (Giudice & Taylor, 2017), in addition to managing glucose metabolism and mitochondria number and function for ATP provision (Memme et al., 2021).

Notwithstanding, the muscle tissue can be deteriorated due to several reasons like chronic diseases (e.g., cancer, sepsis, etc.), chemotherapy, prolonged inactivity, or ageing (Hyatt & Powers, 2021).

More specifically, the loss of muscle quality, mass, and function in the age-related sarcopenia processes (Cruz-Jentoft & Sayer, 2019), seems to have its origins in the mitochondrial dysfunction, an abnormality in the physiological functions of these mitochondria (Brand & Nicholls, 2011). Disrupted morphology with increased fission, as well as an impoverished fusion and mitophagy with age, (Memme et al., 2021) carries into reduced mitochondrial biogenesis (de Mello et al., 2018; Jornayvaz & Shulman, 2010). Mitochondrial dysfunction results, thus, in diminished ATP production, increased mitochondrial reactive oxygen species (ROS), and the release of mitochondria-derived proapoptotic factors (Brand & Nicholls, 2011). It is also associated to the secretion of factors such as proinflammatory cytokines, proteases, and growth factors that have potent local and systemic effects such as inflammation and metastasis, becoming a hallmark of ageing (Memme et al., 2021).

On the one hand, the main consequence of this mitochondrial dysfunction is a limited generation of ATP by oxidative phosphorylation since this process determines the metabolic substrates alternance. Loss of muscle mass and strength go along with reduced fat oxidation (FATox), what is theoretically counteracted with greater carbohydrate oxidation (CHOox) to guarantee the energy demands (Holloszy et al., 1998). When this phenomenon appears already in basal conditions, or at very low exercise intensities, like in older adults, it is referred as metabolic inflexibility (Achten et al., 2003) as opposite to the term metabolic flexibility, coined by Kelley and colleagues (Kelley & Mandarino, 2000). Hence, older individuals who may suffer it might show a low ability to switch between energy substrates in response to changing physiological conditions, both in skeletal muscle (Calçada et al., 2014; Kelley & Mandarino, 2000; San-Millán & Brooks, 2017) and in the heart (Lesnefsky et al., 2016), in addition to poor cardiorespiratory fitness (Coen et al., 2013).

On the other hand, during physical exercise, this defect on the metabolic flexibility goes along with an increase in blood lactate (BLa) at low intensities, an indicator of limited lipid metabolism and carbohydrates dependence, at least in men (Bergman et al., 2000). However, lactate mechanisms might be also reduced in older individuals. Moreover, age is not the only parameter influencing this alternance of substrates. Also, sex (Amaro-Gahete et al., 2018), training level (Ara et al., 2011), nutrition status, fasting

time (San-Millán & Brooks, 2017) or exercise modality (Achten et al., 2003) play a key role. There is a sex variability of the oxidation rates (Amaro-Gahete et al., 2018), especially after the menopause, when the drop in oestrogen levels is accompanied by a reduced mitochondrial respiratory capacity (Frandsen et al., 2021).

In this scenario, the mechanisms involved in the metabolic inflexibility (Goodpaster & Sparks, 2017), as well as their consequences and ways of improvement, are still under research, above all in exercise conditions, and mostly in older women where research is inexistent. That's why indirect non-invasive markers with a key role in the assessment of the metabolic flexibility, such as the point of Maximal Fat Oxidation (MFO), and the FATmax -the intensity at which this point is reached, are becoming important indicators of the metabolism during physical exercise (Amaro-Gahete, Sanchez-Delgado, Alcantara, et al., 2019), despite they need further research while ageing. Lined with previous statements, the Frayn's stoichiometric equations (Frayn, 1983), complemented with Free Fat Mass (FFM) to normalise these parameters (Amaro-Gahete et al., 2018; Frandsen et al., 2021), and with the BLA outcomes -due to their inverse correlation with FATox (Jones et al., 2019)- may also cover to fill the gap and deep on these new insights of metabolic flexibility.

Therefore, the aim of this study is to describe and further understand the behaviour of metabolic flexibility/inflexibility in active older women (by means of an incremental cycling test), with the purpose of observing the impact of ageing on oxidation rates and the peak of whole-body fat oxidation produced by this population over-60. It also aims to decipher the role of the fitness level in these values. As a main hypothesis, age might impair the metabolic flexibility, reducing MFO values, being compensated by a higher CHOox, thus shifting downwards and to the left the fat oxidation curve. As a second hypothesis, unfit women will display less flexibility.

Methods

Participants

Twenty-four older women were recruited to participate in the study. As inclusion criteria: to be female over 60 years, moderately active according to International Physical Activity Questionnaire (IPAQ), and absence of any medical contraindication for physical exercise according to physical activity readiness questionnaire (PAR-Q). As exclusion criteria: diagnosed insulin resistance, the consumption of drugs (e.g., beta blockers) that limits or conditions the practice of physical exercise, and the non-compliance with any of the inclusion criteria.

Six women were discarded after the first screening, and three more failed to complete the protocol. So, table I shows descriptive data of those 15 whose data were useful to determine the metabolic flexibility.

All women received written and oral information before volunteering to participate and signed a written informed consent form of this study, approved by the Science ethical committee of University of Valencia

(H105715353921), adhered to the Principles of the Helsinki declaration.

Women were told to refrain from strenuous exercise 24 h before the test and to follow their usual diet, maintaining their macronutrient composition and energy content, except for the pre-test dinner, so as not to overly condition oxidative ration, with a meal consisting of 50% of kcal in the form of CHO. In addition, they were asked to abstain from caffeine 1.5 h and to fast at least 2 h before the test. The participants repeated the diet on both days in the study to optimise the standardisation of the test (San-Millán & Brooks, 2017). Noteworthy, they were also instructed to arrive at the laboratory well rested and were asked to travel by car or public transport.

General design

The current pilot study, conducted between March and July 2021, followed a single centre, cross-sectional design. After a first telephone recruitment, including questions about the medical history, physical activity and health habits, the ladies came twice to the lab, on two days separately from 48 h to one week.

The first day of assessment included 10 min resting seated to register heart rate (HR) with a Polar H10 band (Polar Electro Oy, Kempele, Finland), arterial oxygen saturation ($SpO_2\%$) through the Wristox2 3100 pulse oximeter (Nonin Medical, Plymouth, Minnesota, USA) and blood pressure (BP) by the Omron M6 sphygmomanometer (HEM-7420, Omron Healthcare, Kyoto, Japan). Then, a brief interview to ascertain the participants' health status and the level of physical activity by means of PAR-Q and IPAQ questionnaires, followed by the determination of height (SECA 222, Hamburg, Germany) and body composition by bioimpedance (Tanita DC-430 MA S; Tokyo, Japan). Finally, there was a familiarisation set with the bicycle Orbea active 700 and the smart roller Saris H3 (CycleOps Hammer Direct Drive Trainer, Saris, Madison, USA). Cadence and biomechanical adjustment to the bicycle were set for the comfort of the participant in the graded test.

On the second day, the previous health controls were repeated (HR, $SpO_2\%$ and BP), followed by glycaemia baseline by means of a flash glucose monitoring system (FreeStyle Libre, Abbott Diabetes Care, Witney, UK) and baseline lactate [BLa_{pre}] (Lactate Scout, SensLab GmbH, Leipzig, Germany) before the test. Then the ladies performed the graded test on the cycle-ergometer for the determination of the respiratory exchange ratio (RER), the FATmax relative to VO_{2peak} in the test ($FATmax_{peak}$), and the VO_2 and VCO_2 peak values, together with the curve of FATox and CHOox along the test (see details below).

The exercise test

The graded tests to determine oxidation rates are characterized by long stages (Amaro-Gahete, Sanchez-Delgado, Alcantara, et al., 2019). The Smart Roller Saris and the Rouvy application (VirtualTraining, Vimperk, Czech Republic) allowed to increase 10 W every 3 min 15 sec, starting from 30 W to complete a minimum duration in the whole test. The 15 sec were added in every stage due to mechanical limitations of this population (Yamauchi et al., 2010). Intensity was continuously monitored and adjusted with the

help of an iPad tablet (Apple, Cupertino, California, USA), and rating of perceived exertion (RPE, Borg 1-10) and Visual Analogue pain Scale (VAS) were controlled every 1min 30 sec along the test.

The protocol aimed to reach the second ventilatory threshold (VT2), with at least two of the three following criteria: RER >1.1, peak of HR (HR_{peak}) >80% HR_{max} (Karapetian et al., 2008), and/or RPE >6 (Deruelle et al., 2007). Whenever a VAS>5 and/or SpO₂% <92%, the women were invited to end the test. VO₂ and VCO₂ were measured by indirect calorimetry, using the K4 B2 metabolic chart (Cosmed, Rome, Italy). The online gas analysers were carefully calibrated with an automated volume calibration and with a gas mixture recommended by the manufacturer prior to the start of each test. The last 60 s in each intensity were then retained to calculate whole-body fat oxidation rates (Amaro-Gahete, Sanchez-Delgado, Alcantara, et al., 2019), where the substrate oxidation was calculated using Frayn's equation, with the assumption that the urinary nitrogen excretion rate was negligible (Frayn, 1983):

$$FATox \text{ (g}\cdot\text{min}^{-1}\text{): } 1.67VO_2 \text{ (L}\cdot\text{min}^{-1}\text{)} - 1.67VO_2 \text{ (L}\cdot\text{min}^{-1}\text{)}$$

$$CHOox \text{ (g}\cdot\text{min}^{-1}\text{): } 4.55VCO_2 \text{ (L}\cdot\text{min}^{-1}\text{)} - 3.21VO_2 \text{ (L}\cdot\text{min}^{-1}\text{)}$$

Thereafter, FATox value at MFO was calculated, considering the FFM value (mg/min/kg FFM) as it may be more appropriate when making comparisons by sex (Amaro-Gahete et al., 2018; Frandsen et al., 2021), whereas the analysis of the CHOox ratios, since the test ended before reaching zones of maximum oxidation values of this substrate, was discarded beyond the ratios curves.

Finally, the evolution of the substrates was graphically plotted according to the intensities 60% (P_{60}), 80% (P_{80}) and 100% (P_{100}) set from each individual peak power at the end of the test. The energy substrate oxidation curve was thus short, but it allowed to analyse this heterogeneous population without leaving aside those with less physical condition.

Statistics

As a first descriptive approach, mean, standard error of the mean (SEM), confidence interval [CI 95%] and coefficient of variation (CV) were calculated, followed by the graphical analysis and the normality tests (Saphiro-Wilk). After the determination of MFO and the peak power in the test (P_{100}), t-test for paired samples were conducted to compared and further understand physiological parameters at these two key points of the test in our active older women. Finally, bivariate correlations for Age, BLa_{peak} and FATox in MFO were performed, with and without VO_{2peak} and peak power (P_{100}) as a covariate, accompanied by scatter plots and the coefficient of determination R^2 to quantify the proportion of variance in one variable explained by the other in these associations and their effect size. Cohen's d was calculated for the effect size, where it was considered small ($d=.20-.40$), medium ($d=.50-.70$) or large ($d=.80-2.0$) (Cohen, 1998); whilst R^2 was considered as small ($R^2=0.04$), medium ($R^2=0.25$) or large ($R^2= 0.64$) (Sullivan & Feinn, 2012).

All analyses were carried out using the Statistical Package for Social Sciences (SPSS, v. 25.0, IBM SPSS Statistics, IBM Corporation), and the significance level was set at <0.05.

Results

Body composition and cardiorespiratory fitness

As shown by Table 1, there was the expected heterogeneity in FFM and VO_{2peak} values. Women showed a good BMI for their age, in the lower limit of overweight (Janssen, 2007); as well as a normal-high blood pressure or pre-hypertensive scores according to the European society guidelines for hypertension (Mazón et al., 2019). Heart rate and saturation were appropriated for age and context, and noteworthy, the VO_{2peak} in the test, closed to the oxygen uptake at VT2, was lower than similar samples in other studies because of this also lower intensity in the protocol.

Table I. Subjects characteristics expressed as mean (95%confidence intervals) and Coefficient of Variation (%).

Subjects (n=15)	Mean (95% CI)	CV (%)
Age (years)	69.00 (65.14-72.85)	9.7
Weight (kg)	62.44 (56.61-68.26)	16.5
BMI (kg/m ²)	25.25 (23.87-26.63)	11.1
FFM (kg)	39.83 (36.88-42.77)	13.4
Body fat (%)	35.03 (26.17-43.89)	12.7
SBP (mmHg)	133.73 (125.05-142.41)	13.2
DBP (mmHg)	78.87 (73.46-84.28)	14
SpO ₂ (%)	97.21 (96.69-97.73)	0.8
HR _{baseline}	71.71 (65.00-78.43)	12.4

95% CI: 95% confidence intervals, BMI: Body Mass Index, FFM: Free Fat Mass, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, SpO₂: Oxygen Saturation; HR_{baseline}: Heart Rate at Baseline.

Metabolic flexibility and physiological determinants

Table II reflects a low FATox capacity at MFO, which appear at the 79.52% of the VO_{2peak} (FATmax_{peak}) and with a low increase in HR. RER was already at over the 0.85 associated to glucose predominance (Stanzione, 2020). In fact, CHOox was larger than FATox at this point of the test. Considering now P₁₀₀,

BLA_{peak} increased significantly ($p<0.05$) from BLA_{pre} (1.63 ± 1.03 to 7.34 ± 4.23 mmol·L⁻¹). Compared to MFO, all the main psychophysiological and performance parameters (i.e., HR, RPE, VAS, and RER; and W respectively) showed significant differences ($p<0.05$), but not SpO₂ (Table II). Despite being active, power at MFO was also low.

Table II. Psychophysiological parameters at Maximal Fat Oxidation (MFO) and maximal power once over VT₂ (P₁₀₀).

	MFO (n=14)	SEM	P ₁₀₀ (n=15)	SEM
HR (bpm)	99.50 (92.17-106.83)	3.40	135.46 (125.00-145.92)	4.88
%HR _{max}	62.38 (57.36-67.40)	2.32	84.12 (77.57-90.87)	3.03
VO ₂ (ml/kg/min)	11.77 (9.04-14.52)	1.27	16.34 (11.67-21.01)	2.18
SpO ₂ (%)	96.85 (95.98-97.73)	0.40	95.64 (94.49-96.79)	0.49
RPE	1.5 (0.60-2.40)	0.42	5.26 (4.52-6.00)	0.34
VAS	0.93 (0.20-1.66)	0.34	2.73 (1.16-4.30)	0.73
FATox (g·min ⁻¹)	0.13 (0.09-0.17)	0.19	0.00 (0.00-0.01)	0.00
FATox (g·min ⁻¹ ·kg FFM)	5.61 (3.59-7.63)	0.93	0.17 (0.00-0.37)	0.89
CHOox (g·min ⁻¹)	0.64 (0.46-0.84)	0.84	1.88 (1.35-2.41)	0.24
CHOox (g·min ⁻¹ ·kg FFM)	25.35 (17.68-33.01)	2.32	75.57 (53.93-97.21)	3.03
RER	0.89 (0.86-0.93)	0.17	1.19 (1.05-1.32)	0.06
Power (W)	39.29 (33.99-44.58)	2.45	77.14 (66.42-87.87)	4.96

Data are expressed as Mean (95% CI). SD: Standard Deviation; SEM: Standard Error of Mean; 95%CI: 95% Confidence Interval; MFO: Maximal Fat Oxidation; P_{100%}: Point of 100% of Power Output; HR: Heart Rate; %HR_{max}: Percentage of maximal Heart Rate according to Tanaka et al. (2001); VO₂: Oxygen Consumption; SpO₂: Oxygen Saturation; RPE: Rate Perceived Exertion; VAS: Visual Analogue Scale of pain; FATox: Fatty Acid Oxidation; CHOox: Carbohydrate Oxidation; RER: Respiratory Exchange Ratio; W: watts.

The three points of the ratio oxidation curve (P₆₀; P₈₀ and P₁₀₀, at mean power outputs of 46.00 ± 10.80 , 61.33 ± 14.40 , and 76.67 ± 17.99 W, Figure 1) reflected limited capacity to use both, FATox and CHOox, despite being active.

*** Insert figure 1 near here***

Figure 1. FATox and CHOox mean rates as a function of graded exercise (power).

Caption: FATox: Fat Oxidation, CHOox Carbohydrate Oxidation.

Regarding the associations (Figure 2), an a priori analysis showed that age was large and negatively associated to P_{100} ($r=-0.85$, $p<0.005$), and moderate and negatively associated to BLa_{peak} ($r=-0.66$, $p<0.01$), whilst it showed no association with VO_{2peak} ($r=0.24$, $p=0.38$). P_{100} and VO_{2peak} appeared to be independent ($r=0.25$, $p=0.37$) but they did associate large and significantly when considering age as a covariate ($r=0.89$, $p<0.005$).

Of outer most importance, the participants showed a significative, medium size and negative correlation between age and MFO ($r=-0.54$, $p=0.04$), which increased when this partial correlation was performed with VO_{2peak} as a control variable ($r=-0.65$, $p=0.01$). Instead, MFO-age association turned non-significant just as soon as the partial correlation was performed with P_{100} as covariate ($r=-0.17$, $p=0.53$), which was in turn large and positively associated to MFO ($r=0.71$, $p=0.01$). BLa_{peak} also showed a positive and moderated association with fat oxidation at MFO ($r=0.52$, $p=0.04$).

To conclude, the coefficients of determination R^2 (Figure 2) confirmed the big effect size in the association between age and P_{100} ($R^2=0.72$); which decreased to medium between MFO and age ($R^2=0.29$). P_{100} correlations with BLa_{peak} and MFO were also moderate ($R^2=0.36$ and $R^2=0.50$ respectively).

*** Insert Figure 2 near here***

Figure 2. Coefficients of determination (R^2) between key parameters in the test (Charts A-D: MFO associations).

Caption: MFO: Maximal Fat Oxidation, BLa_{peak} : Blood Lactate Peak, VO_{2peak} : Oxygen Consumption at peak intensity of test.

Discussion

In line with our main hypothesis, we found an impaired MFO, accompanied of a shifting down and leftward of the oxidation rates curves. Both, CHOox and FATox, were low for a reduced power with age, which points a possible metabolic inflexibility with ageing in women despite of being active. To the best of our knowledge this is the first study to analyze women over 60, providing insight into the presence of metabolic abnormalities in this segment of the population, as well as knowledge to understand their responses to physical exercise.

As a second finding, aligned with our second hypothesis, preserving power to reach higher intensities in exercise suggests ensuring a better ability to oxidize fat in the lower loads (or in an upside-down reading, the better capacity to use both fuel sources may help to preserve muscle power). Moreover, although power decreases significantly the older the women, higher neuromuscular capacity, and not the higher cardiorespiratory fitness, seems to be responsible of a slightly better metabolic flexibility, with the ability to produce lactate also pointing to be key in this response to graded exercise.

The metabolic inflexibility with ageing has been previously described (Calçada et al., 2014), confirming the specific weight of ageing in the impaired ability to combine energy substrates (Fethney, 2010). Despite the unknown mechanisms of the origins of these metabolic behaviors (Goodpaster & Sparks, 2017), our data confirm a notably impaired fat oxidation [0.13 (0.09 - 0.17 $\text{g}\cdot\text{min}^{-1}$)] even compared to other longitudinal studies on populations with limited oxidative capacity, i.e., middle age obese [0.36 (0.31 - 0.40 $\text{g}\cdot\text{min}^{-1}$)] or sedentary [0.24 (0.22 - 0.25 $\text{g}\cdot\text{min}^{-1}$)] women (Amaro-Gahete et al., 2018). CHOox rates do not compensate this low fuel provision leading to an early cessation of exercise. Age-related changes in the metabolic status, but also in the skeletal muscle, may account for it.

On the one hand, there is the lower respiratory capacity of skeletal muscle due to a lower capacity of mitochondrial oxidative enzymes (25-40%) in older people (Brunner et al., 2007), as well as the drop in percentage of type I fibres or mitochondrial density (Holloszy et al., 1998). The acute response to exercise is conditioned by the abundance and function of these mitochondria, becoming an indirect method for understanding their functioning and oxidative capacity in different populations (San-Millán & Brooks, 2017b). The skill to alternate between carbohydrate and lipid metabolism in response to graded exercise would be thus affected in over-60 active women. Noteworthy, previous studies already showed limited PGC-1 α mRNA expression with age (Holloszy et al., 1998), as well as the ability of increasing PGC-1 α in the elderly with exercise (Cobley et al., 2012), what might explain the large heterogeneity in our sample, but also the strong influence of power in our results (as discussed below).

On the other hand, the body composition may also influence metabolic flexibility. Previous studies have observed a superior lipid metabolism, both for a higher intramyocellular lipid content that leads to the increased fatty acids availability in obese fitness population (Ara et al., 2011), or for an increase in the protein cluster of differentiation 36 (CD36) (Bonen et al., 2004; Sogaard et al., 2019). These authors suggest that this marker could be associated with increased free fatty acids uptake in older adults. However, the women in our sample are not only with better body composition, but also older. Our data show lower body mass index values as well as fat mass respect to the study of Sogaard et al., (2019) (BMI; 25.5 vs 30.06 kg/m^2 ; 35.03 vs 39.10 kg respectively), while regarding the FFM the differences observed were far superior (39.83 vs 51.8 kg).

In this sense, already Amaro-Gahete et al. (2019) highlighted the relevance of normalizing FATox through FFM, as performed in our study. The ageing process is in turn associated to increased sarcopenia (Cruz-Jentoft & Sayer, 2019) and impoverishment of FFM, affecting body composition. Albeit we consider the FFM in FATox estimation, following the scarce recent studies such as Frandsen et al. (2020), FATox rates

were still low in our sample: 5.61 (3.59-7.63) mg/min/kgFFM vs. 7.3 (6.2–8.4) mg/min/kgFFM in a group of middle-aged sedentary people and 7.6 (6.4–8.8) mg/min/kgFFM in a group of middle-aged trained people in Frandsen et al. (2020). Therefore, the worsening with age is maintained despite normalizing FFM.

In this scenario, the age-related fall of MFO through the graded test would require the early involvement of carbohydrates. However, the shifting down and leftward of the CHOox rates curve highlights the big difficulty to get additional fuel metabolism over certain intensities (i.e., the metabolic inflexibility), since the glycolytic pathway might be also punished because of the greater loss of muscle mass in type II fibers (Brunner et al., 2007).

Of outermost importance, this phenomenon suggests being counterbalanced by training, as shown by the strong association between P_{100} and MFO in our active women, as well as the moderate influence of BLA in MFO (P_{100} $r=0.71$, $p=0.01$; BLA_{peak} $r=0.52$, $p=0.04$ respectively). Figure 2 confirms that power could be explaining up to the 50% of the variability in MFO; and up to the 36% of the variability in BLA. This latter (BLA) would in turn explained the 23% of the variability in MFO, which is a moderate association. Even more, the negative association between MFO and age disappeared when considering P_{100} as a covariate, since this latter was the one who was really affected by ageing ($r=-0.85$, $p<0.01$, $R^2=0.72$), unlike the VO_{2peak} associations. According to San-Millán & Brooks (2017), exercise lowers circulating lactate by increasing lactate clearance, thus increasing lipid oxidation, and reducing CHOox. This behavior could be preserved in the stronger women over-60, at least in the very first stages of the graded test, in addition to the benefits of exercise through the PGC-1 α participation, leading to mitochondrial biogenesis (Cobley et al., 2012; San-Millán & Brooks, 2017).

Therefore, our data indicate that muscle power is severely affected by age in older women, even being active, confirming their need of power training, both for neuromuscular and cardiovascular (i.e., metabolic) health. Peak of power falls with age due to the deterioration of neural function and the drop in the number of motor units (Yamauchi et al., 2010), despite no changes in the muscle cross-sectional area (Frontera et al., 2000). However, those women who have preserved the ability to produce larger muscle power in our pilot study, have also maintained larger fat and CHOox rates, adding new reasons to increase power exercise training with age. Muscle power becomes thus an important indicator from a metabolic perspective and confirms its importance in active ageing strategies. In line with Cadore & Izquierdo (2018) statements, this parameter is of paramount importance, since the larger the power, the better the physical cardiorespiratory fitness and the better the metabolic flexibility in our older female population.

Concerning other performance key parameters like the FATmax, unlike MFO, we found significantly higher values compared to the obese and sedentary women in the cited previous studies (Amaro-Gahete et al., 2019) [79.52 (66.40-92.64 % VO_{2peak}) vs. 43.3 (28.28-48.21) and 46.1 (42.17-50.02) % VO_{2peak} respectively]. This phenomenon settles down in a low and anticipated VO_{2peak} because of the test was planned to reach an intensity close to $RER\approx 1$ in a large-stages test, and this intensity was achieved early

on in this older population. Besides the nature of the test, our results reflect once more a limited behaviour across the intensity spectrum. The higher FATmax must be therefore contextualized and considered just a FATmax_{peak} in the test, more than the elderly women' FATmax (VO_{2max} %).

In summary, we found metabolic inflexibility reflected by low FATox and CHOox rates and declined MFO values in women over-60 despite being active. MFO was influenced by power and lactate production (both peripheral factors of women's motor performance and health) and not by age and VO_{2peak} in the test. According to the scarce literature, these results are conditioned by body composition, the test duration, and the intensity at which we achieve the respiratory exchange ratio. Up to our knowledge, this is the first study to confirm the influence of the status of training (i.e., muscle power and lactate in the test), variables that might revert all the above.

To conclude, the authors acknowledge the presence of several limitations. Among these, most of the women in the sample were Nordic Walking practitioners, so they were not currently familiarized with the bicycle in the higher intensities. This may have conditioned the end of the test, however, a first familiarization session and the VAS and RPE scales helped to ensure that women felt comfortable and secure enough to increase intensities up to VT2. On the other hand, the sample may be somehow low (due to COVID19-pandemic limitations), fact that limits the analysis and conclusions which can be drawn from this research, as well as the transferability of these results. Noteworthy, this is a pilot study, and the sample is very representative of these active women over-60, since Nordic Walking is a widespread sport modality in these ages. Finally, lack of invasive procedures in the study does prevent us from outlining the mechanism behind these findings. Future studies will therefore need to explore the explanation of these phenomena using biopsies or blood samples. Moreover, a more detailed analysis of efficiency in both pathways to complement these findings, as well as further analysis of the premature and advanced glycolytic RER, could also shed light on exercise responses with ageing. In this aspect, it is worth highlighting the absence of maximum values of VO₂, power or lactate in the protocol, as this work focused on intensities close to VT2.

Conclusion

The present research shows a remarkable metabolic inflexibility in older women during an incremental test up to the second ventilatory threshold. Furthermore, despite the attenuation of the fitness level (i.e., muscle power) with age, it was observed that it is a relevant variable for metabolic flexibility, as well as the body composition.

Contrary to expectations, we observed not only a worsening of fat metabolism, but also of carbohydrate metabolism, which resulted in a lower total energy production, despite that this fact is exacerbated by the early RER due to the protocol.

Practical Applications

Based on the findings of the current study, we believe that emphasis should be placed on increasing physical fitness in this population of older women to reverse the effects of age on metabolic flexibility; especially power training, with the intention of improving this parameter could be key, given the correlation of power with both age and MFO.

On the other hand, targeted work at the bioenergetic level, combining higher intensities (i.e., larger power and lactate production) with the lower ones (for fat oxidation) with the intention of general adaptations at the mitochondrial level, would be just as beneficial in this population, so that mitochondrial dysfunction could be combated by the formation of new organelles and their better functioning.

Novelty Statement

This study is the first to investigate metabolic flexibility in women over 60 and show the influence of power and lactate on whole-body maximal fat oxidation rates. In addition, we have observed a premature RER which could be one of the limiting peculiarities of this population.

Abbreviations

BLa: Blood Lactate; BMI: Body Mass Index; BP: Blood Pressure; CHOox: Carbohydrate Oxidation; DBP: Diastolic Blood Pressure; FATmax: the intensity at which MFO point is reached; FATox; FFM: Free Fatty Mass; HR: Heart Rate; IPAQ: International Physical Activity Questionnaire; MFO: Maximal Fat Oxidation; PAR-Q: Physical Activity Readiness Questionnaire; RER: Respiratory Exchange Ratio; RPE: Rate Perceived Effort; SBP: Systolic Blood Pressure; SpO₂: oxygen saturation; VAS: Visual Analogue Scale of Pain; VT₂: Ventilatory Threshold II.

Declarations

Ethics Approval and Consent to Participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards approved for the University of Valencia (H105715353921).

Consent for publication

Not applicable.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are not publicly available due to the conditions of the ethical approval provided by the Valencia University Human Research Ethics

Committee. Notwithstanding, their anonymous data and analysis are available from the corresponding author on reasonable request.

Competing Interests

Jordi Monferrer-Marín, Ainoa Roldán, Pablo Monteagudo, Iván Chulvi-Medrano and Cristina Blasco-Lafarga declare no potential conflict of interests.

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Authors' Contributions

Experiments in this study were conducted in Performance's lab in University of Valencia. Conception and design of the experiments was undertaken by J.M-M., A.R., P.M. and C.B-L. Data collection was undertaken by J.M-M., A.R., P.M., I.C-M. and C.B-L, whilst assembly, analysis, and interpretation of data was undertaken by J.M-M., A.R., and C.B-L. Drafting the article or revising it critically for important intellectual content was undertaken by J.M-M and C.B-L. All authors have contributed to review and improved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Figures

Figure 1

FATox and CHOox mean rates as a function of graded exercise (power).

Caption: FATox: Fat Oxidation, CHOox Carbohydrate Oxidation.

Figure 2

Coefficients of determination (R^2) between key parameters in the test (Charts A-D: MFO associations).

Caption: MFO: Maximal Fat Oxidation, BLA_{peak}: Blood Lactate Peak, VO_{2peak}: Oxygen Consumption at peak intensity of test.