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Associations between heart rate variability and maximal fat oxidation in two different cohorts of healthy sedentary adults

Anabel González-Acedo [a,1], Abel Plaza-Florido [b,*,1], Francisco José Amaro-Gahete [b,c], Jerzy Sacha [d,e], Juan M.A. Alcantara [b]

[a] *Biomedical Group (BIO277), Department of Nursing, Faculty of Health Sciences, University of Granada (Spain), Avda. Ilustración, 60, 18016, Spain*
[b] *PROFITH "PROmoting FITness and Health Through Physical Activity" Research Group, Sport and Health University Research Institute (iMUDS), Department of Physical and Sports Education, Faculty of Sport Sciences, University of Granada, Spain*
[c] *EFFECTS-262 Research Group, Department of Physiology, School of Medicine, University of Granada, Spain*
[d] *Faculty of Physical Education and Physiotherapy, Opole University of Technology, Opole, Poland*
[e] *Department of Cardiology, University Hospital in Opole, University of Opole, Opole, Poland*

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Abstract *Background and aims:* Resting heart rate variability (HRV) and maximal fat oxidation (MFO) during exercise are both considered as a noninvasive biomarkers for early detection of cardiovascular risk factors. Thus, this study aimed to analyze the relationship between resting HRV parameters and MFO during exercise, and the intensity of exercise that elicit MFO (Fat_{max}) in healthy sedentary adults.

Methods and results: A total of 103 healthy young adults (22.2 ± 2.3 years old, 67% female; from the ACTIBATE cohort) and 67 healthy middle-aged adults (53.1 ± 5.0 years old, 52% female; from the FIT-AGEING cohort) were included in this cross-sectional study. HRV was assessed using a Polar RS800CX heart rate monitor, while MFO and Fat_{max} were determined during a graded exercise treadmill test using indirect calorimetry. No significant associations were observed for healthy young adults (standardized β coefficients ranged from −0.063 to 0.094, and all $P \geq 0.347$) and for middle-aged adults (standardized β coefficients ranged from −0.234 to 0.090, and all $P \geq 0.056$). Nevertheless, only a weak association was observed between one HRV parameter in time-domain (the percentage of R-R intervals that shows a difference higher than 50 ms [pNN50]) and MFO in the cohort of middle-aged adults (β coefficient = −0.279, and $P = 0.033$).

Conclusion: The results of this study suggest that resting HRV parameters are not associated with MFO and Fat_{max} during exercise in two independent cohorts of healthy sedentary young and middle-aged adults, respectively.

* Corresponding author. Department of Physical and Sports Education, Faculty of Sports Science, University of Granada, Granada, Spain.
E-mail addresses: anabelglez@ugr.es (A. González-Acedo), abeladrian@ugr.es (A. Plaza-Florido), amarof@ugr.es (F.J. Amaro-Gahete), sacha@op.pl (J. Sacha), alcantarajma@ugr.es (J.M.A. Alcantara).
[1] These authors have made an equal contribution.

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1. Introduction

Cardiovascular diseases (CVD) are one of the leading causes of mortality worldwide [1,2]. Individuals with lower values on vagal-related heart rate variability (HRV) parameters while resting – i.e., a low variation on the time interval between consecutive R peaks recorded by an electrocardiograph – present a higher risk of developing CVD [3–5]. It has been proved that low values on vagal-related HRV parameters during resting conditions predict the first adverse cardiovascular event in apparently healthy adults without CVD [6]. Besides, vagal-related HRV parameters are also inversely associated with CVD risk factors (hereinafter, we refer to *metabolic syndrome markers*) in healthy sedentary adults [7,8].

On the other hand, the highest value of oxygen consumption during an exercise test (VO_{2max}) is also widely used to predict CVD risk and mortality in adults [9]. In fact, studies showed that resting vagal-related HRV parameters were positively associated with VO_{2max} in healthy young adults [10,11]. Moreover, both the maximal fat oxidation (MFO) during exercise and the intensity of exercise that elicits MFO (i.e., $Fat_{max;}$ expressed as a percentage of $VO2_{max}$), have been considered as biomarkers related to metabolic flexibility [12]. There is previous evidence postulating that the capacity to adapt fuel oxidation to fuel availability (i.e., metabolic flexibility) may be affected by various pathophysiological conditions (e.g., obesity, impaired glucose tolerance, or type 2 diabetes), all of them being associated with altered levels of metabolic syndrome markers and an increased CVD risk [13,14]. Moreover, metabolic syndrome markers (measured during resting conditions) have been negatively associated with both MFO and Fat_{max} during exercise in healthy sedentary young adults [15]. However, another recent study did not report significant associations of resting metabolic syndrome markers with MFO and Fat_{max} during exercise in healthy sedentary young and middle-aged adults [16]. To the best of our knowledge, no previous study has reported the associations of resting vagal-related HRV parameters (as a noninvasive indicator of metabolic syndrome markers) with MFO and Fat_{max} during exercise in healthy sedentary adults of different ages.

The vagus nerve (the main nerve of the parasympathetic nervous system) can release acetylcholine, decreasing the production of pro-inflammatory cytokines by immune cells, which has been described as the cholinergic/anti-inflammatory pathway [17]. Inflammation contributes to insulin resistance and induces the alteration of metabolic syndrome markers [18]. Higher values on vagal-related HRV parameters during resting are related to a better inflammatory profile and metabolic syndrome markers [19,20]. Besides, an optimal function of the autonomic nervous system during exercise (the predominance of sympathetic activity over vagal activity) is needed to mobilize lipids from fat depots and induce fat oxidation [21] (being a higher MFO during exercise related to a healthier CVD risk profile [15]). Therefore, it could be hypothesized that those individuals presenting a "healthier" cardiac autonomic nervous system activity during resting (i.e., higher values on resting vagal-related HRV parameters) will show higher MFO and Fat_{max} values during exercise.

The aim of this study was to analyze the relationship between resting HRV parameters with MFO and Fat_{max} during exercise in a cohort of young adults (age from 18 to 25) and in a cohort of middle-aged adults (age from 45 to 63).

2. Methods

2.1. Study design and participants

This cross-sectional study was performed using baseline (pre-intervention) data from the ACTIBATE [22] and the FIT-AGEING [23] randomized controlled trials (RCTs). A total of 103 healthy young adults (22.2 ± 2.3 years old, 67% female; from the ACTIBATE cohort) and 67 healthy middle-aged adults (53.1 ± 5.0 years old, 52% female; from the FIT-AGEING cohort) were retrospectively included.

The aims of the study were explained to people interested in participating, which gave their written and oral consent. The common inclusion criteria between both RCTs studies were as follows: (i) not having an oscillatory body weight (a change lower than 3 kg in the last 3 months or lower than 5 kg in the last 5 months); (ii) be sedentary; (iii) not participating in a weight loss program; (iv) not suffering from an acute/chronic disease (e.g., obesity, diabetes, heart failure, among others); (v) having a normal electrocardiogram; (vi) not being a smoker; and, (vii) not being pregnant or lactating. The procedures followed by both RCTs were similar, and extended information about the recruitment and the methodology followed in both RCTs can be found elsewhere [17,18].

2.2. Heart rate variability signal assessment and processing

In both RCTs [22,23], the HRV and HR assessment were carried out between 8 and 9 a.m. following similar procedures. Briefly, the participants were lying motionless on a bed in the supine position (quiet environment and controlled ambient temperature ranging from 22 °C to 24 °C). Participants were instructed to remain silent, not to move excessively and to avoid falling asleep during the HRV assessment. Then, the HRV recording was performed during a 15-min period [24]. HRV signal was recorded using a Polar RS800CX heart rate monitor (Polar Electro, Kempele, Finland) wireless connected to a chest belt with the Polar H3 heart rate sensor (Polar Electro, Kempele, Finland), which is considered to be a valid system [25,26]. Finally, a sampling rate of 1000 Hertz (Hz) was used for the assessment using that system.

Thereafter, from the data obtained during the 15-min period, the first 5-min data were discarded. From the remaining 10-min period, the "best" 5-min period was manually selected using the free version of the Kubios software (v.3.0.0, HRV analysis, University of Eastern Finland) [27] and based on the criteria detailed in Plaza-Florido et al. [28]. In brief, the 5-min period presenting a

Gaussian distribution and no large R-R interval outliers was selected by experienced researchers [28] and used for further processing. The R-R intervals were detrended with the prior smoothness method (setting an alpha at 500 and a cubic interpolation at the predetermined rate of 4 Hz). The medium level of threshold-based artefact correction (using the Kubios software) was used as recently recommended [29]. Briefly, the medium Kubios filter algorithm compares every R-R interval value against a local interval median. The software marks for correction all R-R intervals that are 0.25 s larger or smaller than the local interval median. Then, these artefacts are subsequently interpolated using a cubic spline interpolation (see details in Alcantara et al. [29]).

2.3. Parameters derived from the heart rate signal assessment

The HRV parameters were derived from the R-R signal according to the current guidelines [4]. In time-domain, we computed vagal-related HRV parameters: the standard deviation of normal R-R intervals (SDNN), the square root of the mean of the sum of the squares of the R-R interval differences (RMSSD), and the percentage of R-R intervals that shows a difference higher than 50 ms (pNN50). Regarding the frequency domain, the nonparametric Fast Fourier Transformation algorithm (FFT) was performed using the Welch's periodogram method (using the default values of 256 s for the window width and window overlap of 50%). The power of the high frequency band (HF: 0.15–0.4 Hz; vagal-related) and the power of the low frequency band (LF: 0.4–0.15 Hz; influenced by both sympathetic and vagal activity) were computed after applying the FFT algorithm [27,30]. Then, the LF-to-HF ratio (LF/HF) was computed as an indicator of sympathovagal balance. In addition, we also derived the mean resting heart rate (HR) from the resting R-R signal, as in a previous study [10], the HR was better associated with VO_{2max} than HRV parameters.

2.4. MFO and Fat_{max} assessment: conditions before performing the submaximal exercise test

In both RCTs [22,23] the MFO and Fat_{max} assessments were carried following similar procedures. In brief, participants arrived at the research center by public transportation or by a motorized vehicle, avoiding any physical activity prior the test. Furthermore, participants came to the lab in a fasted state of 5–6 h or 10–12 h for the ACTIBATE and the FIT-AGEING studies, respectively. In addition, they were also instructed to avoid moderate and/or intense physical activity for the 24 and 48 h prior testing, respectively. Compliance with these criteria was verified before the test.

2.5. MFO and Fat_{max} assessment: determination of the maximum comfortable walking speed before performing the submaximal exercise test

To estimate MFO and Fat_{max}, the participants performed a submaximal graded exercise test consisting on walking on a treadmill (H/P/cosmos pulsar, H/P/cosmos sports & medical GmbH, Nussdorf-Traunstein, Germany) following a validated exercise protocol and procedures [31–33]. Briefly, before starting the test, the *"maximum comfortable walking speed"* was determined. Participants were instructed to walk for 30 s at 4 km/h, 45 s at 5.5 km/h, and then, the speed was increased by 1 km/h in 45 s intervals until the abovementioned maximum comfortable walking speed was achieved [31–33].

2.6. MFO and Fat_{max} assessment: submaximal exercise test and MFO and Fat_{max} estimation

The participants took a 3-min rest after determining the comfortable walking speed. Immediately after, the submaximal walking test started at a speed of 3.5 km/h (0% slope) with an increment of 1 km/h every 3-min until the maximum comfortable walking speed was reached. Afterwards, the slope was increased by 2% every 3-min, trying to maintain the individualized speed until the respiratory exchange ratio (RER) was 1.0. Also, on a different day (3–14 days before/after the submaximal test), VO_{2max} was assessed as detailed below.

During the entire submaximal test, the gas exchange was measured using indirect calorimetry (CPX Ultima CardiO2 metabolic cart [Medgraphics Corp., MN, US]), and daily flow calibration (using a standard 3 L calibration syringe) and the calibration of the gas analyzers were performed following the manufacturer's recommendations. Thus, from the measured oxygen consumption and carbon dioxide production, the fat oxidation in grams per minute (considering a value of 0 for urinary nitrogen excretion) was estimated using the equation proposed by Frayn [34]. To estimate fat oxidation, the last 1-min data of each 3-min exercise stage were used. Then, these values were plotted according to exercise intensity (expressed as $\%VO_{2max}$; see below) building a third-degree polynomial curve and aiming to calculate MFO and Fat_{max} [32,35].

2.7. VO_{2max} assessment

The VO_{2max} was determined using a maximal graded exercise test (modified Balke protocol [36]) in the same treadmill mentioned above. In brief, the participant walked 1-min at 5.3 km/h and then, every 1-min, the treadmill slope was increased 1% until the participant reported to be exhausted. During the entire graded exercise, the gas exchange was recorded using indirect calorimetry (the CPX Ultima CardiO2; i.e., the same metabolic cart used to assess the MFO and Fat_{max}). Both, flow and gases analyzers were calibrated prior to each test following the recommendations by the manufacturer. On the other hand, participants were instructed not to consume any stimulant substance (24 h) nor eat before the test (3–5 h). Furthermore, they were also instructed to avoid moderate and/or intense physical activity for the 24 and 48 h prior testing, respectively.

For VO_{2max} determination, data obtained in the above-described exercise test were downloaded from the

metabolic cart every 5 s. Then, the highest VO_2 value (5 s data) and the VO_2 values immediately before and after that maximum value (i.e., 5 s data prior and 5 s data after the highest VO_2 value) were averaged and used for further analyses. An extended explanation about the VO_{2max} test and procedures can be found elsewhere [22,23].

2.8. Dietary intake assessment

Three non-consecutive 24 h dietary recalls (one included a weekend day) were used to assess food consumption. All recalls were performed by a qualified dietitian team and subsequently analyzed using the EvalFINUT® software (https://www.finut.org/evalfinut/). The software includes the U.S. Department of Agriculture and the *Base de Datos Española de Composición de Alimentos* databases, which were used to estimate energy and macronutrient (e.g., fat) intake from food consumption. Extended information about the methodology for the assessment of the participant's dietary intake can be found elsewhere [22,23].

2.9. Physical activity assessment

Levels of physical activity were determined during 7 consecutive days using wrist-worn accelerometer (ActiGraph GT3X+, Pensacola, FL, US), being the participant allowed to remove it during water activities. A sampling frequency of 100 Hz was used to record the raw accelerations [37]. Then, the version 6.13.3 of the ActiLife software (ActiGraph, Pensacola, FL, US) was used for converting raw accelerations data into ".csv" format files. In brief, ".csv" format files were processed in the R software (version 3.1.2; https://www.cran.r-project.org) and using the GGIR software (version 1.6–0; https://cran.r-project.org/web/packages/GGIR). Then, the time spent in different intensities (e.g., moderate-to-vigorous physical activity [MVPA]) was estimated using the Euclidean Norm Minus One (ENMO) and age-specific thresholds [10,38,39]. Extended information about the data processing and physical activity intensities determination can be found elsewhere [10,40].

2.10. Statistical analysis

The descriptive characteristics of the participants are reported as mean and standard deviation (SD) for the variables that exhibited a normal distribution. The median and interquartile (IQ) range are presented for non-normally distributed variables. The normal distribution of the variables was checked using the Kolmogorov-Smirnov test and the visualization of histograms. Then, variables that did not follow a normal distribution were transformed using a natural logarithm (ln). All the analyses were performed separately for each cohort due to an interaction effect (*Age* × *Study population*) was observed in a previous study [10].

Simple linear regression models were used to study the associations of resting HRV parameters computed in time- and frequency-domains (i.e., SDNN, RMSSD, and pNN50, and HF, LF, LF/HF ratio, respectively) and HR with MFO (expressed as grams per minute) and Fat_{max} (expressed as $\%VO_{2max}$). Then, we used multiple linear regressions models to study the associations of resting HRV parameters and HR with MFO and Fat_{max} adjusting for potential confounders as sex, age, MVPA, $VO_{2max,}$ and fat intake [10,16].

The results are presented as standardized β coefficients and *p* values, unless stated otherwise. P values ≤ 0.05 were considered as significant. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) statistical software (v.25, IBM Corporation, NY, US). Figures were created using the GraphPad Prism software (v.8.0.2, CA, US).

3. Results

Table 1 shows the characteristics of the participants from the ACTIBATE (hereinafter, *young adults*) and the FIT-AGEING (hereinafter, *middle-aged adults*) cohorts. Fig. 1 presents the unadjusted associations of resting HRV parameters and HR with MFO (Fig. 1A–G) and Fat_{max} (Fig. 1H–N) in the cohort of young adults. We did not observe significant associations (standardized β coefficients ranged from −0.063 to 0.094, and all $P \geq 0.347$; Fig. 1). Regarding the cohort of middle-aged adults, Fig. 2 shows the unadjusted associations of resting HRV parameters and HR with MFO (Fig. 2A–G) and Fat_{max} (Fig. 2H–N). We did not observe significant associations (standardized β coefficients ranged from −0.234 to 0.090, and all $P \geq 0.056$; Fig. 2).

Table 2 shows multiple linear regression models between resting HRV parameters and HR with MFO and Fat_{max} adjusting for potential confounders (i.e., including sex, age, MVPA, VO_{2max}, and fat intake as covariates). In the cohort of young adults, no associations of resting HRV parameters and HR with MFO were observed (standardized β coefficients ranged from −0.161 to 0.046; and all $P \geq 0.103$; Table 2). Similar results were observed for the abovementioned associations with Fat_{max} (standardized β coefficients ranged from −0.102 to 0.072; and all $P \geq 0.360$; Table 2). Concerning the cohort of middle-aged adults, only a weak and negative association was identified between the pNN50 and MFO (standardized β coefficient = −0.279, and P = 0.033; Table 2). Finally, no association was observed between resting HRV parameters and HR with Fat_{max} (standardized β coefficients ranged from −0.120 to 0.042, and all $P \geq 0.371$; Table 2).

4. Discussion

Overall, this study shows no significant association of resting HRV parameters in time- and frequency-domains with MFO and Fat_{max} estimated through a graded exercise test in two different cohorts of healthy sedentary young and middle-aged adults, respectively. Nevertheless, only a weak association was observed between one HRV parameter in time-domain (the pNN50) and MFO in the cohort of middle-aged adults. In line with HRV, similar results were

Table 1 Descriptive characteristics of the participants.

	Young adults						Middle-aged adults					
	All (n = 103)		Men (n = 34)		Women (n = 69)		All (n = 67)		Men (n = 32)		Women (n = 35)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	22.2	2.3	22.5	2.3	22.0	2.3	53.1	4.9	53.8	5.1	52.5	4.8
Weight (kg)	71.6	17.7	87.1	18.1	64.0	11.4	76.2	15.2	88.4	10.2	64.9	9.2
Height (cm)	168.5	8.7	176.3	6.9	164.7	6.8	168.1	9.9	176.4	6.2	160.5	5.8
BMI (kg/m^2)	25.1	4.9	28.0	5.6	23.6	3.9	26.8	3.8	28.5	3.6	25.2	3.4
FM (kg)	25.5	9.2	28.1	11.5	24.2	7.6	30.0	8.4	31.2	9.6	29.0	7.2
FM (%)	35.9	7.2	31.9	7.5	37.9	6.3	39.7	8.9	34.7	7.8	44.3	7.3
Lean mass index (Kg/m^2)	14.7	2.6	17.4	2.2	13.3	1.4	15.3	2.9	17.6	2.1	13.2	1.8
MVPA (min/day)	88.2	32.3	77.3	31.8	93.6	31.2	95.3	36.7	96.3	34.7	94.2	39.4
MFO (g/min)	0.35	0.11	0.30	0.09	0.37	0.11	0.31	0.09	0.29	0.10	0.34	0.08
Fat_{max} (% VO_2max)	43.8	14.6	41.2	13.7	45.1	15.0	44.6	9.5	45.4	7.3	43.8	11.2
Fat intake (g/day)	85.8	28.4	89.5	31.5	83.9	26.8	36.9	8.3	37.8	5.9	35.9	10.1
VO_{2max} relative to BW (ml/[kg/BW]/min)	41.5	7.7	43.5	10.2	40.6	5.9	30.5	5.7	33.3	4.6	27.9	5.4
HR (bpm)	69.0	9.9	66.0	11.4	70.0	8.9	63.0	8.5	62.0	9.5	64.0	7.5
HRV parameters	Median	IQ	Median	IQ	Median	IQ	Median	IQ	Median	IQ	Median	IQ
RMSSD (ms)	48.0	[41.4]	50.4	[45.1]	48.0	[41.5]	27.1	[22.7]	29.1	[30.0]	25.6	[21.3]
pNN50 (%)	30.5	[40.0]	28.4	[41.1]	30.9	[38.7]	5.8	[20.9]	7.2	[23.6]	5.7	[19.2]
SDNN (ms)	45.8	[32.0]	50.0	[32.7]	44.9	[32.6]	28.3	[18.1]	28.4	[21.9]	28.3	[16.0]
HF (ms^2)	870.0	[1816.7]	943.4	[2178.0]	845.2	[1756.2]	240.0	[563.0]	265.8	[571.5]	218.1	[526.5]
LF (ms^2)	663.3	[1210.6]	716.4	[1164.3]	605.0	[1129.3]	339.9	[510.6]	357.5	[382.0]	295.6	[524.2]
LF/HF ratio	0.8	[0.9]	0.8	[1.2]	0.8	[0.8]	1.2	[2.3]	1.7	[2.4]	0.8	[1.7]

Data are presented either as mean and SD or as median and interquartile range (IQ). Abbreviations: Bpm, beats per minute; BMI, body mass index; BW, body weight; VO_{2max} relative to BW, the highest peak oxygen consumption relative to body weight; Fat_{max}, intensity relative to the highest peak oxygen uptake value (i.e., VO_{2max}) at which MFO is produced; FM, fat mass; HF, high frequency; HR, heart rate; LF, low frequency; MFO, maximal fat oxidation; ms, milliseconds; MVPA, moderate-vigorous physical activity; pNN50, percentage number of pairs of adjacent normal R–R intervals differing by more than 50 ms; RMSSD, the squared root of the mean of the sum of the squares of successive normal R–R interval differences; SDNN, standard deviation of all normal R–R intervals.

observed between the association of resting HR with MFO and Fat_{max}.

To the best of our knowledge, this is the first study addressing the associations of HRV parameters during resting conditions with MFO and Fat_{max} (during exercise) in healthy sedentary young and middle-aged adults. Low vagal activity during resting conditions (represented by low HRV values) has been related to a higher cardiometabolic risk (e.g., high fasting glucose, triglycerides) [5,8]. In this regard, our findings will be discussed in the context of existing literature reporting the relationship between other CVD risk factors (metabolic syndrome markers) assessed during resting conditions with MFO and Fat_{max} determined while exercising. Our findings concur with another cross-sectional study that reported unclear associations of CVD risk factors (different metabolites and proteins circulating in plasma and measured during resting conditions) and MFO and Fat_{max} during a treadmill exercise test using data from the same two cohorts [16]. In the Amaro-Gahete et al. study, an inverse and weak association was identified between the fatty liver index and Fat_{max} in healthy sedentary young adults [16]. However, in a different cross-sectional study (carried-out in healthy sedentary young adults), Montes-de-Oca-García et al. observed that some CVD risk factors such as plasma triglycerides or waist circumference were inversely associated with MFO and Fat_{max} during an exercise test [15].

These two previous studies mentioned above [15,16] suggested that the VO_{2max} was better associated (i.e., showed higher β and p values) with CVD risk factors than MFO and Fat_{max} during exercise in sedentary healthy young adults [15,16] and in middle-aged adults [16]. Similarly, in a previous study [10], we observed significant associations between resting HRV parameters with VO_{2max} in the same two cohorts of healthy young and middle-aged adults included in the present work. However, the associations disappeared after including physical activity as a covariate [10]. In the present study, as previously stated, we did not detect any significant relationship between resting HRV parameters with MFO and Fat_{max} during exercise. Therefore, and considering all together, these studies suggested that CVD risk factors are better associated with VO_{2max} than with MFO and Fat_{max} during exercise in sedentary healthy young and middle-aged adults.

It is also necessary to remark some limitations of this study. First, this is a cross-sectional study; hence, it is not possible to assume a causal relationship. Second, the participants in both cohorts were healthy; therefore, these results could not be extrapolated to other persons with diseases (e.g., obesity, type 2 diabetes). Third, regarding the MFO exercise test and HRV parameters, it has been reported a low day-to-day reproducibility, which may influence to an unknown extent the results across studies [35,41]. Therefore, other studies with a repeated measures design are needed to test if our results

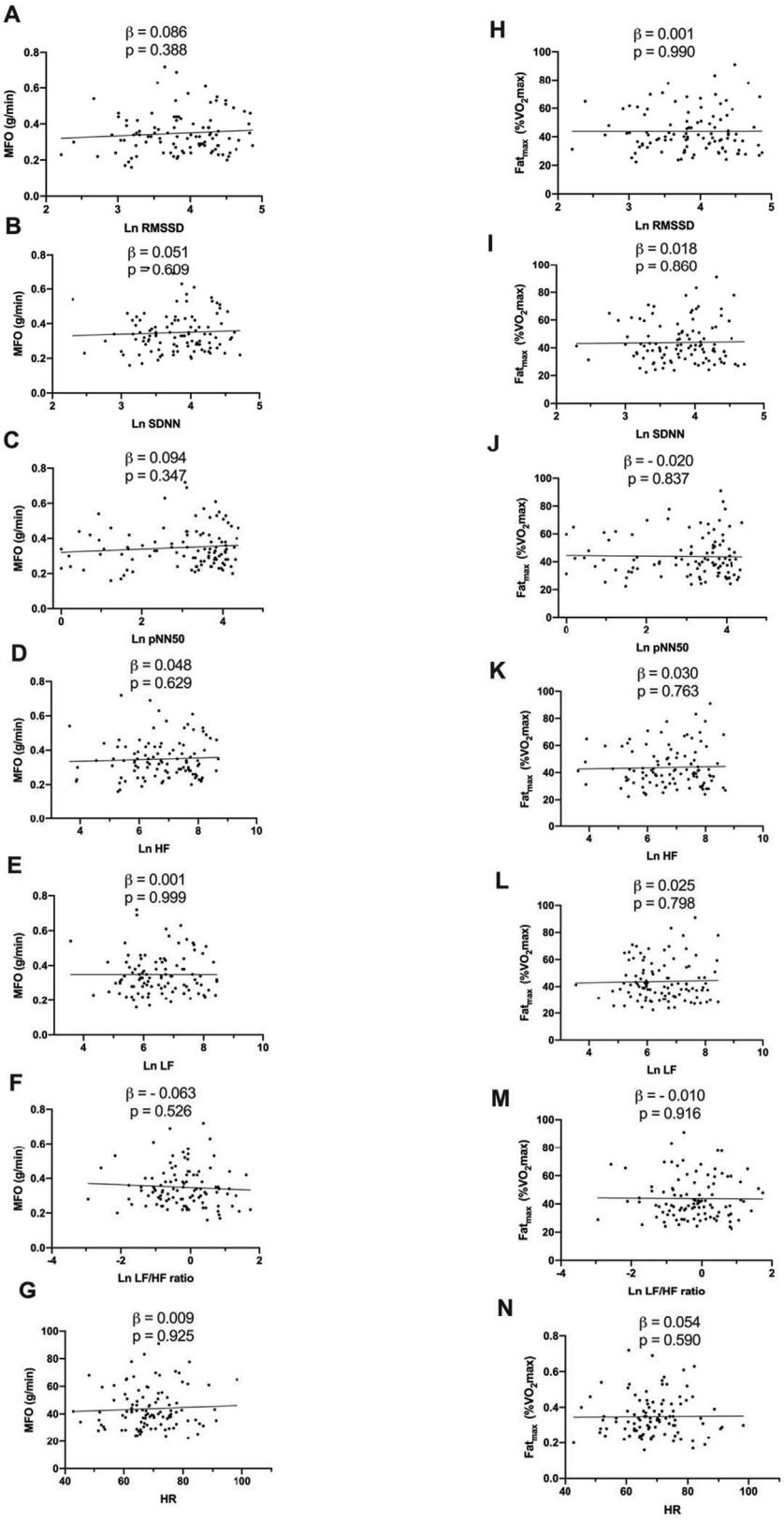

Figure 1 Association of resting HRV parameters and HR with MFO and the intensity at which MFO is elicited (Fat_{max}) in sedentary healthy young adults. Ln: napierian logarithm. β (standardized regression coefficient) and p values for simple linear regression analyses. Panels A–G present the associations of HRV parameters and HR with MFO, while panels H–N present the associations of HRV parameters and HR with Fat_{max}. HF, high frequency; HR, heart rate (i.e., heart beats per minute); LF, low frequency; pNN50, percentage number of pairs of adjacent normal R–R intervals differing by more than 50 ms; RMSSD, the squared root of the mean of the sum of the squares of successive normal R–R interval differences; SDNN, standard deviation of all normal R–R intervals.

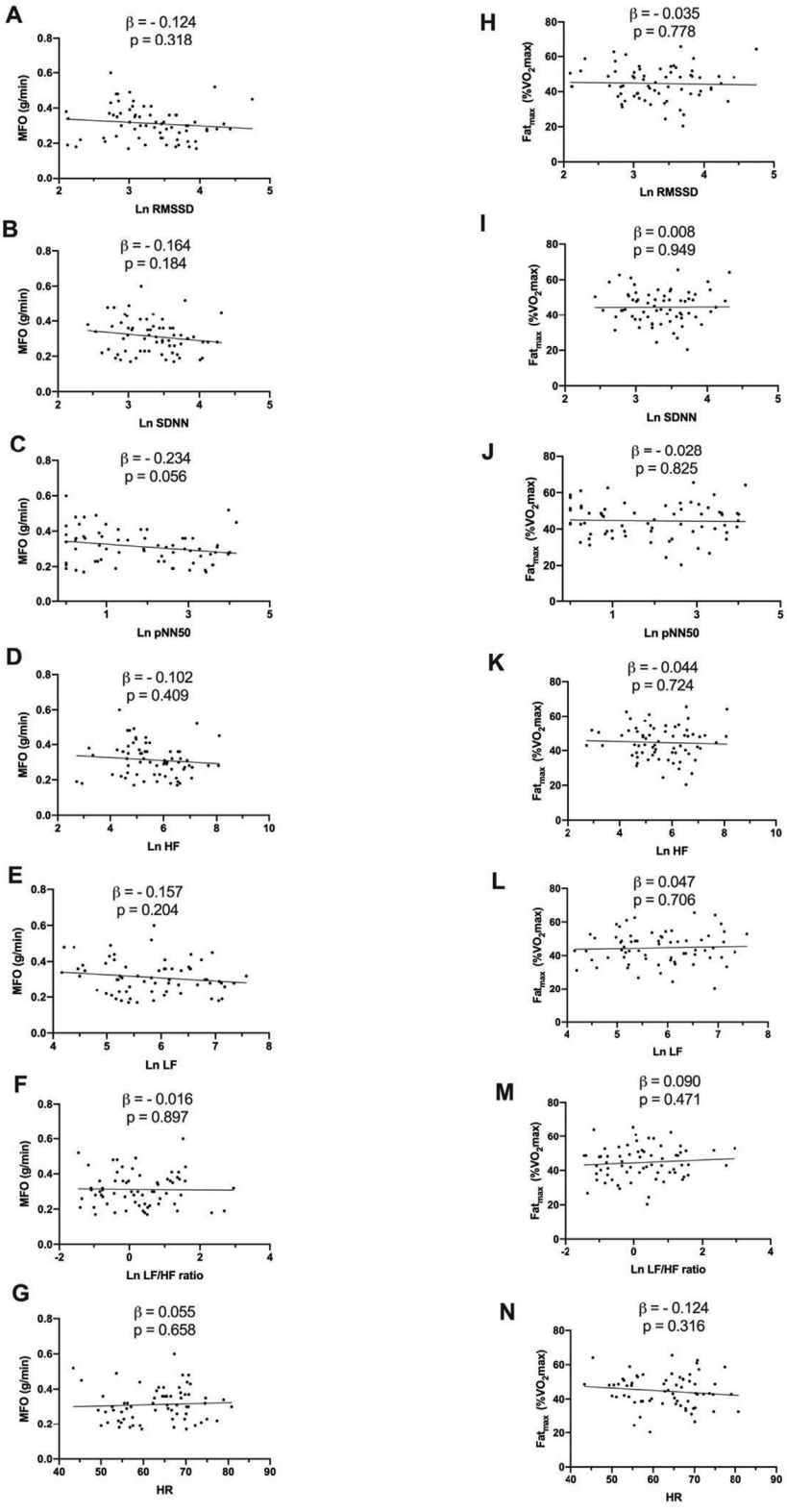

Figure 2 Association of resting heart rate variability (HRV parameters and HR with MFO and the intensity at which MFO is elicited (Fat_{max}) in sedentary healthy middle-aged adults. Ln: napierian logarithm. β (standardized regression coefficient) and p values for simple linear regression analyses. Panels A–G present the associations of HRV parameters and HR with MFO, while panels H–N present the associations of HRV parameters and HR with Fat_{max}. HF, high frequency; HR, heart rate (i.e., heart beats per minute); LF, low frequency; pNN50, percentage number of pairs of adjacent normal R–R intervals differing by more than 50 ms; RMSSD, the squared root of the mean of the sum of the squares of successive normal R–R interval differences; SDNN, standard deviation of all normal R–R intervals.

Table 2 Association of resting HRV parameters and HR with MFO and the intensity at which the MFO is elicited (Fat_{max}) in healthy young and middle-aged adults.

	Young adults (n = 103)		Middle-aged adults (n = 67)	
	β	P value	β	P value
MFO (g/min)				
RMSSD (Ln)	- 0.036	0.729	- 0.161	0.213
SDNN (Ln)	- 0.103	0.318	- 0.156	0.228
pNN50 (Ln)	- 0.017	0.872	**- 0.279**	**0.033**
HF (Ln)	- 0.086	0.408	- 0.150	0.253
LF (Ln)	- 0.161	0.103	- 0.143	0.258
LF/HF ratio (Ln)	- 0.081	0.429	0.040	0.761
HR (bpm)	0.046	0.656	0.065	0.609
Fat_{max} (%VO_{2max})				
RMSSD (Ln)	- 0.069	0.533	0.033	0.809
SDNN (Ln)	- 0.072	0.513	0.042	0.760
pNN50 (Ln)	- 0.102	0.360	0.008	0.956
HF (Ln)	- 0.038	0.730	0.030	0.829
LF (Ln)	- 0.085	0.425	- 0.004	0.976
LF/HF ratio (Ln)	- 0.051	0.640	- 0.037	0.788
HR (bpm)	0.072	0.513	- 0.120	0.371

β (standardized regression coefficient) and p values for linear regression analyses. These analyses are adjusted by age, sex, cardiorespiratory fitness, fat intake, and moderate-vigorous physical activity. For analytical purposes, the napierian logarithm (Ln) was calculated for HRV parameters. Abbreviations: Bpm, beats per minute; BMI, body mass index; BW, body weight; $Fat_{max,}$ intensity relative to the highest peak oxygen uptake value (i.e., VO_{2max}) at which MFO is produced; FM, fat mass; HF, high frequency; HR, heart rate; LF, low frequency; MFO, maximal fat oxidation; pNN50, percentage number of pairs of adjacent normal R–R intervals differing by more than 50 ms; RMSSD, the squared root of the mean of the sum of the squares of successive normal R–R interval differences; SDNN, standard deviation of all normal R–R intervals.

are replicated. Furthermore, we have used a 3-min stage MFO exercise protocol, issue that may underestimate carbohydrate oxidation and overestimate lipid oxidation [42]. Finally, the Polar RS800CX heart rate monitor was used to quantify the R-R or HRV signal during resting instead of using an electrocardiograph. Nevertheless, the Polar RS800CX heart rate monitor is a valid instrument to measure the HRV signal during resting conditions in healthy adults [25,26].

Several strengths can be identified in this work. First, some potential confounders such as the VO_{2max} and MVPA were obtained using the gold standard techniques (indirect calorimetry and accelerometry). In addition, we considered fat intake as a potential confounder in statistical analyses because the inter-individual variation in MFO during exercise can be influenced by fat intake [43]. Second, in both RCTs studies, the data were obtained following similar standard operating protocols [22,23]. Furthermore, resting HRV parameters and HR values were obtained using short-term recordings (10–15 min), which are widely used in research and clinical settings due to its feasibility and because it is possible to better control confounders parameters compared to long-duration recordings (e.g., ≈24 h recordings) [24]. Finally, the HRV signal was processed with the Kubios software, which is widely used for its clinical validity, and we used the *medium filter* based on the recommendations made by a previous study [29].

5. Conclusions

The results of this study suggest no significant associations of resting HRV parameters with MFO and Fat_{max} during a treadmill exercise test in sedentary healthy young and middle-aged adults. Nevertheless, future studies are needed to corroborate our findings in populations with different clinical conditions (e.g., CVD, type 2 diabetes, etc.), using different exercise protocols (e.g., cycloergometer, rowing machine, etc.) and designs (e.g., repeated measures design).

Author contributions

Conceptualization, A.P.-F., J.M.A.A.; Data curation, A.P.-F., J.M.A.A., F.J.A.-G; Formal analysis, A.G.A., A.P.-F., and J.M.A.A.; Methodology, A.P.-F., J.M.A.A.; Writing original draft, A.G.A., A.P.-F., and J.M.A.A; Writing – review & editing, A.G.-A., A.P.-F., F.J.A.-G., J.S., J.R.R., J.M.A.A.

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Declaration of competing interest

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

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