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Heart Rate and Its Variability Are Associated With Resting Metabolic Rate and Substrate Oxidation in Young Women but Not in Men

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ABSTRACT

Background: This study aims to examine the relationship between resting vagal-related heart rate variability (HRV) parameters and heart rate (HR) with resting metabolic rate (RMR) and respiratory exchange ratio (RER) in young adults.

Methods: A total of 74 young adults (22 ± 2 years old, 51 women) were included in this cross-sectional study. HRV was assessed using a HR monitor, whereas RMR and RER were determined by indirect calorimetry.

Results: Linear regression analyses showed a positive association between HR and RER in women (standardized $\beta = 0.384$, $p = 0.008$), while negative associations were observed between vagal-related HRV parameters and RER in women (β ranged from −0.262 to −0.254, all $p \leq 0.042$). No significant association was found between the abovementioned physiological parameters in men.

Conclusion: Here, we show that HR is positively associated with RER in young women but not in men, while vagal-related HRV parameters are inversely related to RMR, therefore suggesting a potential sexual dimorphism between cardiac rhythm and its relationship with markers of cardiometabolic health status.

Trial Registration: ClinicalTrials.gov identifier: NCT02365129.

Francisco J. Amaro-Gahete and Abel Plaza-Florido share last authorship.

1 | Introduction

Heart rate variability (HRV)—the variation in time between consecutive heart beats—is widely employed as a noninvasive indicator of cardiac autonomic nervous system status and cardiovascular health (Force 1996). At the resting state, people with reduced vagal-related HRV parameters present a higher risk of suffering from cardiovascular diseases and mortality (Force 1996; Hillebrand et al. 2013; Lahiri, Kannankeril, and Goldberger 2008), as they are inversely related to cardiometabolic risk factors such as metabolic syndrome markers (Plaza-Florido, Alcantara, Amaro-Gahete, et al. 2020; Plaza-Florido, Sacha, and Alcantara 2022; Stuckey et al. 2014). Likewise, even in apparently healthy people, reduced vagal-related HRV parameters at the resting state can predict the first adverse cardiovascular event (Hillebrand et al. 2013).

The ability of the human body to switch between different endogenous substrates in response to fuel availability is commonly referred as metabolic flexibility (MetF) (Galgani and Fernández-Verdejo 2021). The human body undergoes regular transitions in nutrient utilization between postprandial and fasting states (Frayn 1997). During prolonged fasting periods, the utilization of endogenous fat becomes dominant, increasing fat oxidation (Samra et al. 1996). Conversely, blood endogenous glucose levels rise after food intake, increasing its oxidation (Galgani et al. 2016). A well-known proxy of MetF in resting conditions is the respiratory exchange ratio (RER) (Galgani and Fernández-Verdejo 2021), a parameter that has been also proposed as a noninvasive indicator of cardiometabolic health (Begaye et al. 2020; Kardinaal et al. 2015; Kelley et al. 1999) and that is related to dysfunction in endothelial and cardiac cells (Vallerie and Bornfeldt 2015). In addition, the resting metabolic rate (RMR)—which represents the minimum energy necessary to maintain normal vital signs at the fasting, thermoneutral, and resting state (Alcantara et al. 2022)—is a robust predictor of future body weight gain (Lam and Ravussin 2017). In this sense, higher RMR values have been related to an increased risk of cardiovascular diseases incidence (Li et al. 2023) and has been associated with the presence of cardiometabolic risk factors included in the definition of metabolic syndrome (e.g., high fasting blood circulating glucose or triglycerides) (Soares et al. 2022). Therefore, both MetF and RMR can be considered noninvasive markers of cardiometabolic health status.

The vagus nerve modulates the glucose and lipidic metabolism (Romeo, Lee, and Shoelson 2012), as it releases the acetylcholine, a neurotransmitter which reduces the production of pro-inflammatory cytokines by immune cells (i.e., the cholinergic/anti-inflammatory pathway) (Pavlov et al. 2003). In this regard, at the resting state, higher vagal-related HRV parameters values are associated with better inflammatory status (Williams et al. 2019) and cardiometabolic risk profile (Stuckey et al. 2014). For these reasons, a correct function of the autonomic nervous system is vital to increase the utilization of endogenous fat (Grabner et al. 2021). Thus, it is plausible to hypothesize that, in a healthy population, individuals exhibiting higher vagal-related HRV parameter values at rest may be associated with higher fat oxidation rates (i.e., lower RER values) and lower RMR values. However, it should be noted this relationship may not hold in clinical populations, such as those with atrial fibrillation, where higher vagal-related HRV parameters may have different implications (Hämmerle et al. 2024; Khan, Lip, and Shantsila 2019).

The present study aimed to analyze the association of resting vagal-related HRV parameters with resting RER and RMR in young adults, separately in men and women, as it has been reported a sexual dimorphism regarding the association between vagal-related HRV parameters and cardiometabolic risk factors in adults (Koskinen et al. 2009; Plaza-Florido, Sacha, and Alcantara 2022; Stuckey et al. 2014). Additionally, we explored the association between these resting HRV parameters and substrate utilization. The response to this research question interests both researchers and clinicians because it might suggest a noninvasive and affordable indicator to detect young adults presenting an adverse cardiovascular health profile.

2 | Methods

2.1 | Study Design

The present cross-sectional study used pre-intervention data from the ACTIBATE (Martinez-Tellez et al. 2022; Sanchez-Delgado et al. 2015) randomized controlled trial. We retrospectively included 74 (51 women) young adults from the trial. A flowchart diagram is presented in Figure S1. In brief, the inclusion criteria were: (i) stable body weight (a change ≤ 3 kg in the last 3 months); (ii) sedentary lifestyle; (iii) no participation in a weight loss program; (iv) absence of acute/chronic diseases; (v) normal electrocardiogram; (vi) being nonsmokers; and (vii) not being pregnant or lactating. The trial was approved by the Committee for Research Involving Human Subjects at the University of Granada (Reference no. 924) and Servicio Andaluz de Salud (Centro de Granada, CEI-Granada). In addition, the trial was registered in the ClinicalTrials.gov, and conducted according to the revised version of the Declaration of Helsinki. The study participants were informed about the study's aims, and written informed consent was obtained prior to their enrolment. Extended information concerning the study protocol, aims, and inclusion/exclusion criteria of the ACTIBATE can be found elsewhere (Martinez-Tellez et al. 2022; Sanchez-Delgado et al. 2015).

2.2 | Study Visit

Participants were instructed to refrain from engaging in both, moderate physical activity (24 h) and vigorous physical activity (48 h) before the study visit. The evening before the visit, they were advised to consume a standardized meal (ad libitum). The meal consisted of an egg omelet, boiled rice, and tomato sauce. Participants were asked to avoid physical activity after waking up and to commute to the research center by motorized transportation. Additionally, they arrived in the fasting state (ensuring a 12 h fast) as recommended by current RMR guidelines (Fullmer et al. 2015). After checking they accomplished all the previously mentioned study conditions, we assessed participants' body weight and height using an electronic column scale (Seca model 799, Seca, Hamburg, Germany), with participants wearing light clothing and being barefoot. Body mass index (BMI; in kg/m^2) was also calculated. Moreover, the participants'

body composition was determined by dual-energy x-ray absorptiometry (Discovery Wi scanner, Hologic Inc., Bedford, MA, USA). Subsequently, participants laid on a bed, in the supine position, and we assessed resting HRV and gas exchange as detailed below. Assessments took place early in the morning, in the same quiet room with controlled ambient conditions, and dim lighting. During the test, the participants were covered with a bed sheet and instructed to maintain silence, remain awake, refrain from fidgeting, and breathe normally.

2.3 | HR Signal Acquisition, Processing, and Derivation of HRV Parameters

We measured heart rhythm using a Polar RS800CX HR monitor wireless synchronized to a Polar H3 HR sensor (Polar Electro Oy Inc., Kempele, Finland), with a sampling frequency of 1000 Hz, during an uninterrupted period of 15 min. After the assessment, we processed the heart rhythm data using the free version of the Kubios software (v.3.0.0, HRV analysis, University of Eastern Finland) excluding the first 5-min period from the entire recording to avoid signal interferences/artifacts (Plaza-Florido, Sacha, and Alcantara 2021). Then, we manually selected the 5-min period that presented a Gaussian distribution, and no large R–R interval outlier values (Plaza-Florido, Alcantara, Migueles, et al. 2020). In the Kubios software, the medium level of threshold-based artifact correction was used as recommended for young adults (Alcantara, Plaza-Florido, et al. 2020), and the R–R intervals were detrended (prior smoothness method) setting alpha at 500 and a cubic interpolation at 4 Hz. We derived the following vagal-related HRV parameters: in time-domain (Force 1996), (i) the square root of the mean of the sum of the squares of the R–R interval differences (RMSSD); (ii) the standard deviation (SD) of normal R–R intervals (SDNN); and, (iii) the percentage of R–R intervals that shows a difference higher than 50 ms (pNN50). In the frequency-domain (Force 1996), we derived the power of the high frequency band (HF; 0.15–0.4 Hz). Finally, we calculated the mean resting HR from the abovementioned 5-min period.

2.4 | Resting Gas Exchange Measurement

Resting volumes of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured using either a CCM Express or a CPX Ultima CardiO2 metabolic cart (Medical Graphics Corp., St. Paul, Minnesota, USA). Both systems determine gas exchange in a breath-by-breath mode, require the use of a Directconnect low flow sensor attached to a face mask (Medical Graphics Corp., St. Paul, Minnesota, USA), measure VO_2 using the same galvanic fuel cell, and assess VCO_2 using the same non-dispersive infrared analyzer (Alcantara et al. 2018). Before the beginning of each test, metabolic carts' flow rate (using a 3-L syringe) and gas analyzers (using standard gasses bottles) were calibrated strictly following the manufacturers' instructions.

The VO_2 and VCO_2 gas exchange was measured for 30 min, data that were subsequently processed using the metabolic carts' software (MGCDiagnostic Breeze Suite 8.1.0.54 SP7 software; Medical Graphics Corp., St. Paul, MN, USA) to retrieve data for every minute. Then, we used an Excel spreadsheet and the first 5-min period of data were retrospectively discarded following current recommendations (Fullmer et al. 2015). From the remaining 25 min data, we selected the most stable 5-min period determined as the coefficient of variation (CV) for VO_2, VCO_2, minute ventilation $\leq 10\%$, and for RER $\leq 5\%$, as extensively detailed elsewhere (Alcantara, Delgado, et al. 2020; Sanchez-Delgado et al. 2018). We calculated the RER as the VCO_2-to-VO_2 ratio, estimated the RMR (in kilocalories per day [kcal/day]) using the equation proposed by Weir (1949), and estimated carbohydrate and fat oxidation rates (CHOOx and FATOx, respectively) using the equations proposed by Frayn (1983). We assumed no urinary nitrogen excretion for both RMR and substrate oxidation.

2.5 | Statistical Analysis

The normal distribution of the variables was checked using the Kolmogorov–Smirnov test and the visual inspection of histograms. The descriptive data were presented as mean and SD for the variables that exhibited a normal distribution, while we computed the median and interquartile (IQ) range for those that presented a skewed distribution. We used *t*-tests and Mann–Whitney *U* tests to compare between-group characteristics. We used analysis of covariance (ANCOVA) to compare whether RMR values differ between men and women after accounting for lean mass, as it is the major determinant of RMR (Johnstone et al. 2005). For analytical purposes, the variables that showed a skewed distribution were transformed with the natural logarithm (Ln).

We performed simple and multiple linear regressions to examine the association of resting vagal-related HRV parameters with RER, RMR, CHOOx, and FATOx. The same regression analyses were conducted using resting HR instead of HRV parameters. Multiple linear regression models were selected for their ability to control for potential confounding variables. Specifically, we included age, fat mass, lean mass, and duration of fasting (in hours) as covariates in the models. This approach allowed us to statistically isolate the influence of resting vagal-related HRV parameters and HR on the dependent variables while accounting for these relevant factors. Concretely, age and fat and lean masses are intimately related to resting metabolism. Theoretically, in healthy individuals, energy expenditure increases in parallel with lean mass and decreases with aging (Pontzer et al. 2021). Meanwhile, the duration of fasting is directly related to resting substrate oxidation. In theory, in healthy individuals, the longer is the fasting duration, the higher is the FATOx (thus, the lower the RER values) (Galgani and Fernández-Verdejo 2021). To identify potential sex-specific differences in the associations, we performed the analyses separately in men and women. To ensure the validity of the multiple linear regression models, we first depicted a matrix scatter plot for each regression model and added a fit line to visually check whether the regression model could be expressed in a linear manner. Next, we explored whether the expected mean error of the regression model was zero, checking if the mean of the unstandardized residual values was zero, and examined if the residual values followed a normal distribution using the Kolmogorov–Smirnov test. Additionally, we assessed the homoscedasticity by using the Breusch–Pagan test to check whether the variance of the error was constant.

We also tested whether the errors were independent, ensuring no autocorrelation was present. Finally, we assessed potential multicollinearity among predictor variables by determining the variance inflation factor (VIF) and tolerance for each regression model (O'Brien 2007). For all regression analyses, the VIF and tolerance were ≤ 2.4 and < 1.0 respectively, suggesting no multicollinearity was present. Finally, we used *t*-tests to compare within-group resting substrate oxidation rates.

The Statistical Package for the Social Sciences (SPSS) statistical software (v.25, IBM Corporation, Armonk, NY, USA) was used to perform all the analyses, while figures were designed with the GraphPad Prism software (v.8.0.2, Boston, MA, USA). Statistical significance was set at $p < 0.05$, and results concerning regression analyses are presented as standardized regression β coefficients and *p* values.

3 | Results

Table 1 shows the characteristics of young adults included in the study. In brief, men were heavier, taller, and had higher BMI values and more lean mass compared to women (all $p \leq 0.001$). Women had lower RMR values compared to men ($p < 0.001$), although these differences disappeared after adjusting for lean mass ($p = 0.086$). Resting FATOx was higher in men compared to women (Table 1). No differences were observed on resting HR and vagal-related HRV parameters (all $p \geq 0.111$; Table 1). Regarding within-group substrate oxidation comparisons, we noticed that CHOOx was higher than FATOx in women ($p < 0.001$), while no differences were observed in men ($p = 0.141$).

Figure 1 provides the associations of resting vagal-related HRV parameters and HR with RER and RMR in young men and women, separately. No associations were observed between vagal-related HRV parameters and RER (Figure 1A–D), while a positive association between HR and RER was noted in women ($\beta = 0.309$, $p = 0.027$; Figure 1E). Vagal-related HRV parameters (RMSSD, SDNN, and HF) were inversely associated with RMR in women (β ranged from -0.300 to -0.315, all $p \leq 0.033$; Figure 1F,G,I). Concerning substrate oxidation, a positive association was observed between HR and CHOOx in women ($\beta = 0.331$, $p = 0.018$; Table S1), while no significant association was observed for FATOx (all $p \geq 0.137$; Table S1). We did not note any significant

TABLE 1 | Descriptive characteristics of the participants.

	All ($n = 74$)		Men ($n = 23$)		Women ($n = 51$)		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	***p***
Age (years)	22.18	2.37	22.70	2.27	21.94	2.40	0.206
Weight (kg)	69.56	16.44	84.13	16.48	62.99	11.55	**< 0.001**
Height (cm)	168.15	8.76	176.03	7.17	164.59	6.91	**< 0.001**
BMI (kg/m^2)	24.49	4.71	27.17	5.18	23.27	3.98	**0.001**
Body composition							
Fat mass (kg)	24.53	9.11	26.56	11.41	23.62	7.82	0.201
Lean mass (kg)	41.21	9.55	53.02	6.07	35.89	4.89	**< 0.001**
Energy metabolism							
Resting RER	0.83	0.07	0.81	0.09	0.83	0.05	0.119
RMR (kcal/day)	1425.88	279.66	1615.83	325.54	1340.22	208.50	**< 0.001**
Resting CHOOx (g/min)	0.11	0.07	0.11	0.10	0.11	0.05	0.936
Resting FATOx (g/min)	0.06	0.03	0.07	0.03	0.05	0.02	**0.002**
HR (bpm)	68.00	9.62	65.34	11.89	69.20	8.27	0.111
Vagal-related HRV parameters	**Median**	**IQ**	**Median**	**IQ**	**Median**	**IQ**	
RMSSD (ms)	56.00	[41.90]	61.74	[46.14]	55.15	[38.61]	0.939
SDNN (ms)	51.08	[34.65]	51.96	[30.70]	50.63	[40.64]	0.893
pNN50 (%)	33.62	[33.71]	33.44	[44.67]	33.81	[32.53]	0.930
HF (ms^2)	1125.18	[1896.64]	1126.73	[2213.01]	1123.62	[1657.33]	0.958

Note: Data are presented either as mean and standard deviation (SD) or as median and interquartile range (IQ). *p* values from between-group comparisons were obtained using either *t*-tests or Mann–Whitney *U* tests. Bold numbers represent a *p* value < 0.05.
Abbreviations: BMI, body mass index; CHOOx, carbohydrate oxidation in grams per minute (g/min); FATOx, fat oxidation in grams per minute (g/min); HF, power of the high frequency band (0.15–0.4 Hz) in millisecond squared; HR, mean heart rate in beats per minute (bpm); pNN50, percentage number of pairs of adjacent normal R–R intervals differing by more than 50 ms; RER, respiratory exchange ratio; RMR, resting metabolic rate in kilocalories per day (kcal/day); RMSSD, the squared root of the mean of the sum of the squares of successive normal R–R interval differences in milliseconds (ms); SDNN, standard deviation of all normal R–R intervals in milliseconds (ms).

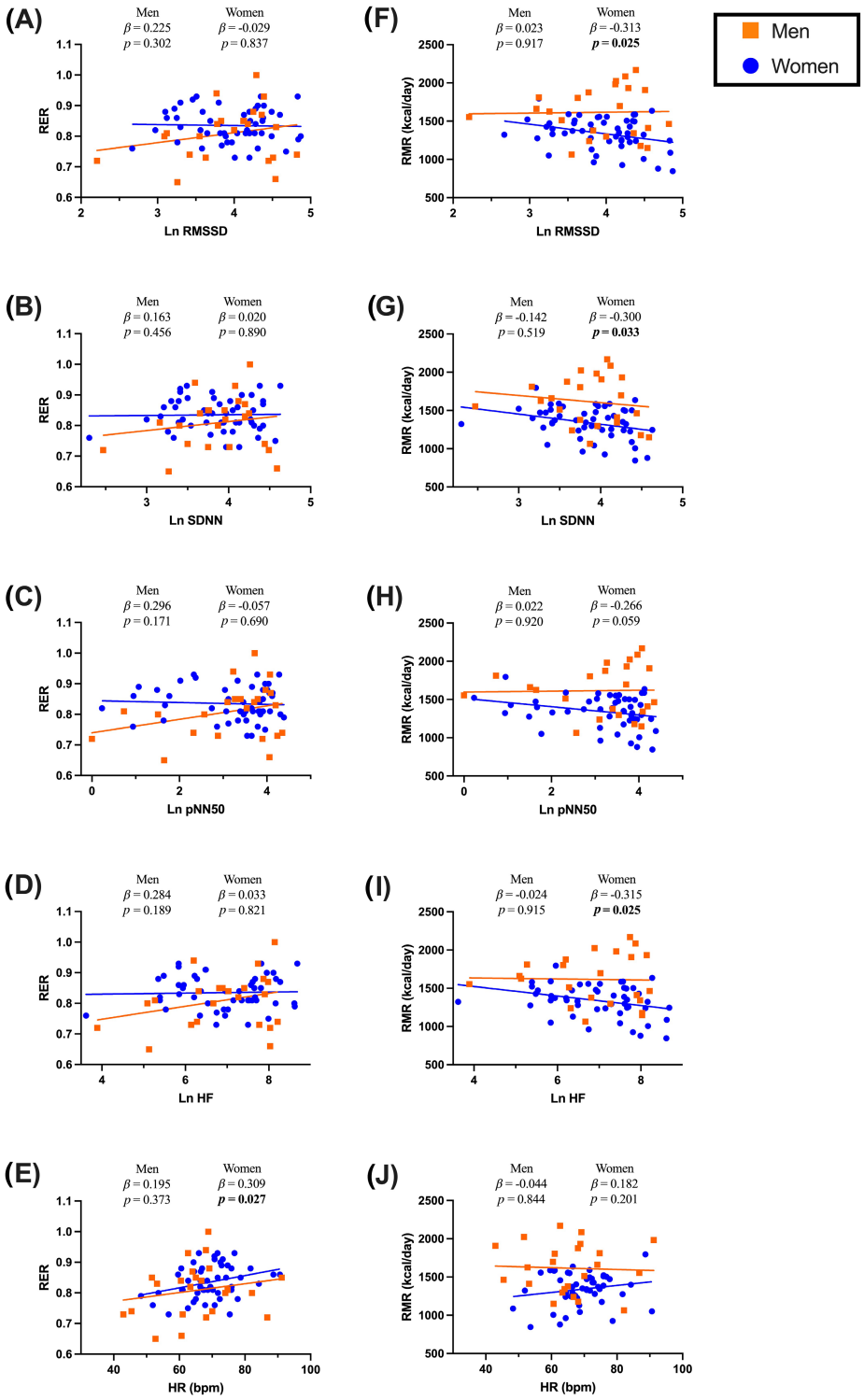

FIGURE 1 | Association of resting vagal-related heart rate variability (HRV) parameters and resting heart rate (HR) with respiratory exchange ratio (RER, panels A–E) and resting metabolic rate (RMR, panels F–J) in young adults. Results are presented as β (standardized regression coefficient) and p values for simple linear regression analyses. Bold numbers represent a $p < 0.05$. HF, high frequency; HR, heart beats per minute; Ln, napierian logarithm; 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.

relationship between vagal-related HRV parameters and HR with substrate oxidation in men ($p \geq 0.074$; Table S1).

Table 2 shows the association of resting vagal-related HRV parameters and HR with RER and RMR after considering age, fat mass, lean mass, and duration of fasting as potential confounders, while Table S2 shows the same associations analyses but including substrate oxidation (CHOOx and FATOx) instead of RER and RMR in the statistical models. In women, the positive association of HR with RER and CHOOx ($\beta = 0.384$ and 0.414,

TABLE 2 | Association between resting vagal-related heart rate variability parameters and mean heart rate with respiratory exchange ratio and resting metabolic rate in young adults.

	Men ($n=23$)		Women ($n=51$)	
	β	p	β	p
RER				
RMSSD (Ln)	0.173	0.487	−0.063	0.674
SDNN (Ln)	0.161	0.512	0.011	0.939
pNN50 (Ln)	0.235	0.335	−0.119	0.431
HF (Ln)	0.256	0.298	0.002	0.992
HR (bpm)	0.102	0.728	**0.384**	**0.008**
RMR (kcal/day)				
RMSSD (Ln)	0.074	0.713	**−0.254**	**0.042**
SDNN (Ln)	0.028	0.889	−0.233	0.060
pNN50 (Ln)	0.105	0.598	−0.203	0.112
HF (Ln)	0.125	0.536	**−0.262**	**0.037**
HR (bpm)	0.212	0.365	0.240	0.057

Note: Results are presented as standardized regression β coefficient and p values for multiple linear regression analyses. Bold numbers represent a p value <0.05. Regression analyses are adjusted by age, body composition, and fasting hours. The napierian logarithm (Ln) transformation was calculated for resting vagal-related heart rate variability parameters.
Abbreviations: HF, power of the high frequency band (0.15–0.4 Hz); HR, mean heart rate in beats per minute (bpm); pNN50, percentage number of pairs of adjacent normal R–R intervals differing by more than 50 ms; RER, respiratory exchange ratio; RMR, resting metabolic rate in kilocalories per day (kcal/day); 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.

both $p \leq 0.008$; Tables 2 and S2), and the inverse association of vagal-related HRV parameters (RMSSD and HF) with RMR ($\beta = -0.254$ and -0.262, both $p \leq 0.042$; Table 2) remained significant after including covariates. There were no statistically significant associations between resting vagal-related HRV parameters and HR with RER, RMR, and substrate oxidation in men (all $p \geq 0.190$; Tables 2 and S2).

4 | Discussion

The findings of this study reveal an inverse association between vagal-related HRV parameters and RMR, and a positive association of HR with RER and CHOOx, after adjusting for potential confounder factors, in women but not in men. Our results support the idea that resting vagal-related HRV parameters could be noninvasive cardiometabolic health indicators, and that a sexual dimorphism might be present when associating these HRV parameters and HR with gas exchange related parameters during resting conditions.

To our knowledge, this is the first study addressing the associations of resting vagal-related HRV parameters and HR with RER (i.e., a surrogate of MetF) and/or RMR in young adults. Reduced vagal-related HRV parameters during resting are linked to cardiometabolic risk factors used in the definition of metabolic syndrome (Plaza-Florido, Alcantara, Migueles, et al. 2020; Plaza-Florido, Sacha, and Alcantara 2022; Stuckey et al. 2014). Indeed, studies conducted in young adults showed the associations between vagal-related HRV parameters and cardiometabolic risk factors (Koskinen et al. 2009; Meyer et al. 2016; Soares-Miranda et al. 2012), while lower fasting RER (Galgani and Fernández-Verdejo 2021) and RMR values (Soares et al. 2022) seem to be predictors of a healthier cardiometabolic profile. Others which included some participants from the present one, have reported weak associations between the abovementioned metabolic syndrome parameters and vagal-related HRV parameters (Plaza-Florido, Alcantara, Migueles, et al. 2020), and also suggested that HR (rather than vagal-related HRV parameters) was better related to cardiorespiratory fitness (Plaza-Florido et al. 2022), which is an indicator of cardiometabolic health (Imboden et al. 2020). In the present study, we observed that HR was positively associated with both, RER and CHOOx, while vagal-related HRV parameters such as RMSSD and HF were negatively linked with RMR in women. Theoretically and attending to the MetF definition, during prolonged fasting periods endogenous fat supply increases (Galgani and Fernández-Verdejo 2021); thus, in a metabolically healthy individual we should observe a higher FATOx compared to the CHOOx, or in other words, we should found lower fasting RER values. Our within-group multiple linear regression analyses demonstrate that women presenting higher HR yielded higher RER values during resting conditions ($\beta = 0.384$, $p = 0.008$; Table 2) as well as higher CHOOx rates ($\beta = 0.414$, $p = 0.004$; Table S2). Therefore, in the women group, we observed higher resting CHOOx rates compared to resting FATOx rates ($p < 0.001$), suggesting an impaired MetF. In addition, after conducting the same within-group multiple linear regression analyses, we found that women exhibiting lower resting vagal-related HRV parameters yielded higher RMR values (both $\beta < -0.253$, both $p < 0.05$; Table 2). In this regard, it should be highlighted that higher RMR values are related to increased cardiometabolic risk factors included in the definition of metabolic syndrome (Soares et al. 2022), which are involved in the pathogenesis of cardiovascular diseases. In this line, a recent study performed Mendelian randomization analysis and reported that higher RMR causally predicted the development of several cardiovascular diseases (Li et al. 2023).

The associations observed in the present study reinforce that resting vagal-related HRV parameters could be used as noninvasive cardiometabolic health indicators. Furthermore, our study showed that both, resting vagal-related HRV parameters and HR are related to resting RER and RMR in women but not in men, an issue that may be of relevance for future studies addressing the MetF in the resting and fasting state. In a previous review, Ventura-Clapier et al. (2020) reported sex and gender differences in term of cardiovascular diseases incidence, a point that would be explained by metabolic issues. In fact, the interaction between mitochondrial function, sexual hormones, gene expression profiles, and epigenetic marks may contribute to these differences observed between men and women (Ventura-Clapier et al. 2020). Although this is an observational cross-sectional study, we can hypothesize the possible physiological mechanisms that underlie the sex-specific associations found in our study. Based on the cholinergic/anti-inflammatory pathway (Pavlov et al. 2003; Williams et al. 2019), we could infer that immune cells from those individuals presenting lower values on vagal-related HRV parameters will have an increased

pro-inflammatory activity and oxidative stress, which in the ultimate instance, is energetically expensive (Straub 2017). Interestingly, the presence of significant associations only in women could suggest a sexual dimorphism. It has been reported that, in general terms, women present a lower basal inflammation than men (Martínez de Toda et al. 2023). Some preclinical studies (Takase et al. 2007, 2009) showed that female rats released more acetylcholine and had more cholinergic neurons in the brain in cortical areas that regulates cardiac control via the autonomic nervous system (Sentis et al. 2024) than male rats. Moreover, in general terms, females shown a preponderance of vagal over sympathetic control of cardiac function compared with males (Dart, Du, and Kingwell 2002). The synthesis and clearance of catecholamines and acetylcholine (both affect cardiac autonomic function) are regulated by sex hormones such as testosterone, estrogen, and progesterone (Dart, Du, and Kingwell 2002). For example, estrogen (levels of this hormone are significantly higher in women than in men) enhances the synthesis and release of acetylcholine, thus influencing parasympathetic activity for more details, see (Dart, Du, and Kingwell 2002). These observations could partially explain, based on the cholinergic/anti-inflammatory pathway, the inverse association we observed in the present study between vagal-related HRV parameters and RMR in women. In a metabolically healthy individual, prolonged fasting periods should lead to a higher FATOx compared to CHOOx rates (Galgani and Fernández-Verdejo 2021). However, in our study, we found the opposite in women, where a positive relationship between HR and CHOOx rates was found. This observation suggests a potential impairment in MeF during the resting state, characterized by elevated RER values during fasting. Such an impairment in MeF could result in intramyocellular lipid accumulation due to reduced fatty acid oxidation (Kelley and Mandarino 2000). This ectopic fat accumulation is concerning as it could contribute to chronic, low-grade systemic inflammation, a key factor in the pathogenesis of obesity and cardiometabolic diseases (Valenzuela et al. 2023). Obesity enhances the secretion of pro-inflammatory adipocytokines, which are associated with the pathological expansion of adipose tissue (Valenzuela et al. 2023). This pro-inflammatory state not only suppresses the release of anti-inflammatory adipocytokines but also exacerbates the risk of developing obesity-related diseases, thereby negatively impacting overall health (Valenzuela et al. 2023).

It should be highlighted however all the relationship we found in our study may not hold in clinical populations, where higher vagal-related HRV parameters may have different implications (Hämmerle et al. 2024; Khan, Lip, and Shantsila 2019). On the other hand, we do not know whether our results could be partially influenced by differences in sample size between groups (23 men vs. 51 women). Also, the current work design cannot elucidate the potential mechanisms underlying the inverse association between vagal-related HRV parameters and resting gas exchange parameters. Nevertheless, the findings of the present study suggest that resting vagal-related HRV parameters and HR could serve as noninvasive markers for cardiometabolic health, particularly in women. The observed sexual dimorphism implies that women may exhibit different autonomic and metabolic responses during fasting states, potentially increasing their risk for metabolic syndrome and related cardiovascular conditions. Clinically, these insights emphasize the importance of considering sex-specific factors when assessing metabolic health and designing interventions. Future research should focus on mechanistic studies exploring the role of inflammation and autonomic regulation in these associations. Clinical trials performing longitudinal analyses with larger, balanced sample sizes across sexes are also needed to confirm these findings and understand their implications in both healthy and clinical populations.

Certain limitations are present in our study. We did not perform a priori sample size calculations as this is a retrospective study using baseline data from a randomized controlled trial. Thus, the imbalance in the number of participants per group (23 men vs. 51 women) should be acknowledged. This discrepancy arose because the participants were drawn from the ACTIBATE study, which was not originally designed to ensure equal representation of men and women for cross-sectional analyses (Martinez-Tellez et al. 2022; Sanchez-Delgado et al. 2015). As a result, our findings primarily focus on within-group analyses to mitigate potential biases, with between-group analyses included cautiously and primarily for exploratory purposes. Regarding the study design, this is a cross-sectional study; thus, we cannot establish a causal relationship. We did not control and measure the breathing pattern in order to avoid potential disturbances that may influence the resting status of the participants during the gas exchange and heart rhythm recordings. However, while some studies have found that vagal-related HRV parameters are affected by breathing (e.g., HF), the influence of breathing on other HRV parameters is not clear (Sidorenko, Kraemer, and Wessel 2016; Wessel, Riedl, and Kurths 2009). In addition, we determined the heart rhythm using a HR monitor (the Polar RS800CX), instead of an electrocardiograph; however, this system has been validated to assess HRV and HR at resting (Tsitoglou et al. 2018; Williams et al. 2017), and the heart rhythm was determined under well-controlled laboratory conditions.

5 | Conclusion

The present study shows an inverse relationship between vagal-related HRV parameters and RMR, and a positive association of HR with RER and CHOOx in young women but not in men. These findings suggest a possible sexual dimorphism when relating noninvasive cardiac autonomic indicators with gas exchange variables during resting conditions. Future studies using larger sample sizes and longitudinal designs are guaranteed to contrast or confirm our findings.

Author Contributions

Conceptualization: J.M.A.A. and A.P.-F. Data curation: J.M.A.A., F.J.A.-G., and A.P.-F. Formal analysis: J.M.A.A., A.G.-A., and A.P.-F. Methodology: J.M.A.A. and A.P.-F. Writing – original draft: J.M.A.A. and A.P.-F. Writing – review and editing: J.M.A.A., A.G.-A., F.J.A.-G., and A.P.-F. All authors read and approved the final version.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Alcantara, J. M. A., G. Sanchez-Delgado, B. Martinez-Tellez, E. Merchan-Ramirez, I. Labayen, and J. R. Ruiz. 2018. "Congruent Validity and Inter-Day Reliability of Two Breath by Breath Metabolic Carts to Measure Resting Metabolic Rate in Young Adults." *Nutrition, Metabolism, and Cardiovascular Diseases* 28, no. 9: 929–936. https://doi.org/10.1016/j.numecd.2018.03.010.

Alcantara, J. M. A., G. S. Delgado, F. J. A. Gahete, J. E. Galgani, and J. R. Ruiz. 2020. "Impact of the Method Used to Select Gas Exchange Data for Estimating the Resting Metabolic Rate, as Supplied by Breath-by-Breath Metabolic Carts." *Nutrients* 12, no. 487: 1–14. https://doi.org/10.3390/nu12020487.

Alcantara, J. M. A., J. E. Galgani, L. Jurado-Fasoli, et al. 2022. "Validity of Four Commercially Available Metabolic Carts for Assessing Resting Metabolic Rate and Respiratory Exchange Ratio in Non-Ventilated Humans." *Clinical Nutrition* 41: 746–754. https://doi.org/10.1016/J.CLNU.2022.01.031.

Alcantara, J. M. A., A. Plaza-Florido, F. J. Amaro-gahete, et al. 2020. "Impact of Using Different Levels of Threshold-Based Artefact Correction on the Quantification of Heart Rate Variability in Three Independent Human Cohorts." *Journal of Clinical Medicine* 9, no. 2: 1–11. https://doi.org/10.3390/JCM9020325.

Begaye, B., K. Vinales, T. Hollstein, et al. 2020. "Impaired Metabolic Flexibility to High-Fat Overfeeding Predicts Future Weight Gain in Healthy Adults." *Diabetes* 69, no. 2: 181–192. https://doi.org/10.2337/DB19-0719.

Dart, A. M., X. J. Du, and B. A. Kingwell. 2002. "Gender, Sex Hormones and Autonomic Nervous Control of the Cardiovascular System." *Cardiovascular Research* 53, no. 3: 678–687. https://doi.org/10.1016/S0008-6363(01)00508-9.

Force, T. 1996. "Heart Rate Variability. Standards of Measurement, Physiological Interpretation, and Clinical Use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology." *European Heart Journal* 17: 354–381.

Frayn, K. N. 1983. "Calculation of Substrate Oxidation Rates In Vivo From Gaseous Exchange." *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology* 55, no. 2: 628–634. https://doi.org/10.1152/jappl.1983.55.2.628.

Frayn, K. N. 1997. "Integration of Substrate Flow In Vivo: Some Insights Into Metabolic Control." *Clinical Nutrition (Edinburgh, Scotland)* 16, no. 6: 277–282. https://doi.org/10.1016/S0261-5614(97)80012-X.

Fullmer, S., S. Benson-Davies, C. P. Earthman, et al. 2015. "Evidence Analysis Library Review of Best Practices for Performing Indirect Calorimetry in Healthy and Non-Critically Ill Individuals." *Journal of the Academy of Nutrition and Dietetics* 115, no. 9: 1417–1446.e2. https://doi.org/10.1016/j.jand.2015.04.003.

Galgani, J. E., and R. Fernández-Verdejo. 2021. "Pathophysiological Role of Metabolic Flexibility on Metabolic Health." *Obesity Reviews* 22, no. 2: 1–14. https://doi.org/10.1111/obr.13131.

Galgani, J. E., C. Gómez, M. L. Mizgier, et al. 2016. "Assessment of the Role of Metabolic Determinants on the Relationship Between Insulin Sensitivity and Secretion." *PLoS One* 11, no. 12: 1–16. https://doi.org/10.1371/journal.pone.0168352.

Grabner, G. F., H. Xie, M. Schweiger, and R. Zechner. 2021. "Lipolysis: Cellular Mechanisms for Lipid Mobilization From Fat Stores." *Nature Metabolism* 3: 1445–1465. https://doi.org/10.1038/s42255-021-00493-6.

Hämmerle, P., S. Aeschbacher, V. Schlageter, et al. 2024. "Heart Rate Variability and Stroke or Systemic Embolism in Patients With Atrial Fibrillation." *Heart Rhythm* 1–8: 1509–1516. https://doi.org/10.1016/j.hrthm.2024.05.028.

Hillebrand, S., K. B. Gast, R. De Mutsert, et al. 2013. "Heart Rate Variability and First Cardiovascular Event in Populations Without Known Cardiovascular Disease: Meta-Analysis and Dose-Response Meta-Regression." *Europace* 15, no. 5: 742–749. https://doi.org/10.1093/europace/eus341.

Imboden, M. T., L. A. Kaminsky, J. E. Peterman, et al. 2020. "Cardiorespiratory Fitness Normalized to Fat-Free Mass and Mortality Risk." *Medicine and Science in Sports and Exercise* 52, no. 7: 1532–1537. https://doi.org/10.1249/MSS.0000000000002289.

Johnstone, A. M., S. D. Murison, J. S. Duncan, K. A. Rance, and J. R. Speakman. 2005. "Factors Influencing Variation in Basal Metabolic Rate Include Fat-Free Mass, Fat Mass, Age, and Circulating Thyroxine but Not Sex, Circulating Leptin, or Triiodothyronine." *American Journal of Clinical Nutrition* 82, no. 5: 941–948. https://doi.org/10.1093/ajcn/82.5.941.

Kardinaal, A. F. M., M. J. Van Erk, A. E. Dutman, et al. 2015. "Quantifying Phenotypic Flexibility as the Response to a High-Fat Challenge Test in Different States of Metabolic Health." *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology* 29, no. 11: 4600–4613. https://doi.org/10.1096/FJ.14-269852.

Kelley, D. E., B. Goodpaster, R. R. Wing, and J. A. Simoneau. 1999. "Skeletal Muscle Fatty Acid Metabolism in Association With Insulin Resistance, Obesity, and Weight Loss." *American Journal of Physiology* 277, no. 6: E1130–E1141. https://doi.org/10.1152/AJPENDO.1999.277.6.E1130.

Kelley, D. E., and L. J. Mandarino. 2000. "Fuel Selection in Human Skeletal Muscle in Insulin Resistance: A Reexamination." *Diabetes* 49, no. 5: 677–683. https://doi.org/10.2337/DIABETES.49.5.677.

Khan, A. A., G. Y. H. Lip, and A. Shantsila. 2019. "Heart Rate Variability in Atrial Fibrillation: The Balance Between Sympathetic and Parasympathetic Nervous System." *European Journal of Clinical Investigation* 49, no. 11: 1–8. https://doi.org/10.1111/eci.13174.

Koskinen, T., M. Kähönen, A. Jula, et al. 2009. "Metabolic Syndrome and Short-Term Heart Rate Variability in Young Adults: The Cardiovascular Risk in Young Finns Study." *Diabetic Medicine* 26, no. 4: 354–361. https://doi.org/10.1111/j.1464-5491.2009.02686.x.

Lahiri, M. K., P. J. Kannankeril, and J. J. Goldberger. 2008. "Assessment of Autonomic Function in Cardiovascular Disease. Physiological Basis and Prognostic Implications." *Journal of the American College of Cardiology* 51: 1725–1733. https://doi.org/10.1016/j.jacc.2008.01.038.

Lam, Y. Y., and E. Ravussin. 2017. "Indirect Calorimetry: An Indispensable Tool to Understand and Predict Obesity." *European Journal of Clinical Nutrition* 71, no. 3: 318–322. https://doi.org/10.1038/ejcn.2016.220.

Li, Y., H. Zhai, L. Kang, Q. Chu, X. Zhao, and R. Li. 2023. "Causal Association Between Basal Metabolic Rate and Risk of Cardiovascular Diseases: A Univariable and Multivariable Mendelian Randomization Study." *Scientific Reports* 13, no. 1: 12487. https://doi.org/10.1038/s41598-023-39551-2.

Martínez de Toda, I., M. González-Sánchez, E. Díaz-Del Cerro, G. Valera, J. Carracedo, and N. Guerra-Pérez. 2023. "Sex Differences in Markers of Oxidation and Inflammation. Implications for Ageing." *Mechanisms of Ageing and Development* 211: 111797. https://doi.org/10.1016/J.MAD.2023.111797.

Martinez-Tellez, B., G. Sanchez-Delgado, F. M. Acosta, et al. 2022. "No Evidence of Brown Adipose Tissue Activation After 24 Weeks of Supervised Exercise Training in Young Sedentary Adults in the ACTIBATE Randomized Controlled Trial." *Nature Communications* 13, no. 1: 5259. https://doi.org/10.1038/S41467-022-32502-X.

Meyer, M. L., N. M. Gotman, E. Z. Soliman, et al. 2016. "Association of Glucose Homeostasis Measures With Heart Rate Variability Among Hispanic/Latino Adults Without Diabetes: The Hispanic Community Health Study/Study of Latinos (HCHS/SOL)." *Cardiovascular Diabetology* 15, no. 1: 45. https://doi.org/10.1186/s12933-016-0364-y.

O'Brien, R. M. 2007. "A Caution Regarding Rules of Thumb for Variance Inflation Factors." *Quality and Quantity* 41, no. 5: 673–690. https://doi.org/10.1007/s11135-006-9018-6.

Pavlov, V. A., H. Wang, C. J. Czura, S. G. Friedman, and K. J. Tracey. 2003. "The Cholinergic Anti-Inflammatory Pathway: A Missing Link in Neuroimmunomodulation." *Molecular Medicine* 9: 125–134. https://doi.org/10.1007/bf03402177.

Plaza-Florido, A., J. M. A. Alcantara, J. H. Migueles, et al. 2020. "Inter- and Intra-Researcher Reproducibility of Heart Rate Variability Parameters in Three Human Cohorts." *Scientific Reports* 10, no. 1: 11399. https://doi.org/10.1038/S41598-020-68197-7.

Plaza-Florido, A., J. M. A. Alcantara, F. J. Amaro-Gahete, J. Sacha, and F. B. Ortega. 2020. "Cardiovascular Risk Factors and Heart Rate Variability: Impact of the Level of the Threshold-Based Artefact Correction Used to Process the Heart Rate Variability Signal." *Journal of Medical Systems* 45, no. 1: 2. https://doi.org/10.1007/S10916-020-01673-9.

Plaza-Florido, A., F. J. Amaro-Gahete, F. M. Acosta, J. Sacha, and J. M. A. Alcantara. 2022. "Heart Rate Rather Than Heart Rate Variability Is Better Associated With Cardiorespiratory Fitness in Adults." *European Journal of Sport Science* 22, no. 6: 836–845. https://doi.org/10.1080/17461391.2021.1892198.

Plaza-Florido, A., J. Sacha, and J. M. A. Alcantara. 2021. "Short-Term Heart Rate Variability in Resting Conditions: Methodological Considerations." *Kardiologia Polska* 79, no. 7–8: 745–755. https://doi.org/10.33963/KP.A2021.0054.

Plaza-Florido, A., J. Sacha, and J. M. A. Alcantara. 2022. "Associations Between Different Heart Rate Variability Ratios and Cardiometabolic Risk Factors in Young Adults." *American Journal of Human Biology: The Official Journal of the Human Biology Council* 34: e23797. https://doi.org/10.1002/AJHB.23797.

Pontzer, H., Y. Yamada, H. Sagayama, et al. 2021. "Daily Energy Expenditure Through the Human Life Course." *Science* 373: 808–812. https://doi.org/10.1126/SCIENCE.ABE5017.

Romeo, G. R., J. Lee, and S. E. Shoelson. 2012. "Metabolic Syndrome, Insulin Resistance, and Roles of Inflammation – Mechanisms and Therapeutic Targets." *Arteriosclerosis, Thrombosis, and Vascular Biology* 32: 1771–1776. https://doi.org/10.1161/ATVBAHA.111.241869.

Samra, J. S., M. L. Clark, S. M. Humphreys, I. A. Macdonald, and K. N. Frayn. 1996. "Regulation of Lipid Metabolism in Adipose Tissue During Early Starvation." *American Journal of Physiology* 271, no. 3 Pt 1: E541–E546. https://doi.org/10.1152/AJPENDO.1996.271.3.E541.

Sanchez-Delgado, G., J. Alcantara, L. Ortiz-Alvarez, et al. 2018. "Reliability of Resting Metabolic Rate Measurements in Young Adults: Impact of Methods for Data Analysis." *Clinical Nutrition* 37, no. 5: 1618–1624. https://doi.org/10.1016/j.clnu.2017.07.026.

Sanchez-Delgado, G., B. Martinez-Tellez, J. Olza, et al. 2015. "Activating Brown Adipose Tissue Through Exercise (ACTIBATE) in Young Adults: Rationale, Design and Methodology." *Contemporary Clinical Trials* 45: 416–425. https://doi.org/10.1016/j.cct.2015.11.004.

Sentis, A. I., J. Rasero, P. J. Gianaros, and T. D. Verstynen. 2024. "Cortical and Subcortical Brain Networks Predict Prevailing Heart Rate." *Psychophysiology*: e14641. https://doi.org/10.1111/PSYP.14641.

Sidorenko, L., J. F. Kraemer, and N. Wessel. 2016. "Standard Heart Rate Variability Spectral Analysis: Does It Purely Assess Cardiac Autonomic Function?" *Europace: European Pacing, Arrhythmias, and Cardiac Electrophysiology: Journal of the Working Groups on Cardiac Pacing, Arrhythmias, and Cardiac Cellular Electrophysiology of the European Society of Cardiology* 18, no. 7: 1085. https://doi.org/10.1093/EUROPACE/EUW078.

Soares, M., Y. Zhao, E. Calton, et al. 2022. "The Impact of the Metabolic Syndrome and Its Components on Resting Energy Expenditure." *Metabolites* 12, no. 8: 1–8. https://doi.org/10.3390/metabo12080722.

Soares-Miranda, L., G. Sandercock, S. Vale, et al. 2012. "Metabolic Syndrome, Physical Activity and Cardiac Autonomic Function." *Diabetes/Metabolism Research and Reviews* 28, no. 4: 363–369. https://doi.org/10.1002/dmrr.2281.

Straub, R. H. 2017. "The Brain and Immune System Prompt Energy Shortage in Chronic Inflammation and Ageing." *Nature Reviews Rheumatology* 13, no. 12: 743–751. https://doi.org/10.1038/nrrheum.2017.172.

Stuckey, M. I., M. P. Tulppo, A. M. Kiviniemi, and R. J. Petrella. 2014. "Heart Rate Variability and the Metabolic Syndrome: A Systematic Review of the Literature." *Diabetes/Metabolism Research and Reviews* 30, no. 8: 784–793. https://doi.org/10.1002/dmrr.2555.

Takase, K., F. Kimura, T. Yagami, and D. Mitsushima. 2009. "Sex-Specific 24-h Acetylcholine Release Profile in the Medial Prefrontal Cortex: Simultaneous Measurement of Spontaneous Locomotor Activity in Behaving Rats." *Neuroscience* 159, no. 1: 7–15. https://doi.org/10.1016/J.NEUROSCIENCE.2008.12.039.

Takase, K., D. Mitsushima, T. Funabashi, and F. Kimura. 2007. "Sex Difference in the 24-h Acetylcholine Release Profile in the Premotor/Supplementary Motor Area of Behaving Rats." *Brain Research* 1154, no. 1: 105–115. https://doi.org/10.1016/J.BRAINRES.2007.04.001.

Tsitoglou, K. I., Y. Koutedakis, P. C. Dinas, et al. 2018. "Validation of the Polar RS800CX for Assessing Heart Rate Variability During Rest, Moderate Cycling and Post-Exercise Recovery." *F1000Research* 7: 1501. https://doi.org/10.12688/f1000research.16130.1.

Valenzuela, P. L., P. Carrera-Bastos, A. Castillo-García, D. E. Lieberman, A. Santos-Lozano, and A. Lucia. 2023. "Obesity and the Risk of Cardiometabolic Diseases." *Nature Reviews. Cardiology* 20, no. 7: 475–494. https://doi.org/10.1038/S41569-023-00847-5.

Vallerie, S. N., and K. E. Bornfeldt. 2015. "Metabolic Flexibility and Dysfunction in Cardiovascular Cells." *Arteriosclerosis, Thrombosis, and Vascular Biology* 35, no. 9: e37–e42. https://doi.org/10.1161/ATVBAHA.115.306226.

Ventura-Clapier, R., J. Piquereau, A. Garnier, M. Mericskay, C. Lemaire, and B. Crozatier. 2020. "Gender Issues in Cardiovascular Diseases. Focus on energy metabolism." *Biochimica et Biophysica Acta – Molecular Basis of Disease* 1866, no. 6: 165722. https://doi.org/10.1016/j.bbadis.2020.165722.

Weir, J. B. d. V. 1949. "New Methods for Calculating Metabolic Rate With Special Reference to Protein Metabolism." *Journal of Physiology* 109, no. 1–2: 1–9. https://doi.org/10.1113/jphysiol.1949.sp004363.

Wessel, N., M. Riedl, and J. Kurths. 2009. "Is the Normal Heart Rate "Chaotic" due to Respiration?" *Chaos (Woodbury, N.Y.)* 19, no. 2: 28508. https://doi.org/10.1063/1.3133128.

Williams, D. W. P., M. N. Jarczok, R. J. Ellis, T. K. Hillecke, J. F. Thayer, and J. Koenig. 2017. "Two-Week Test-Retest Reliability of the Polar® RS800CX™ to Record Heart Rate Variability." *Clinical Physiology and Functional Imaging* 37, no. 6: 776–781. https://doi.org/10.1111/CPF.12321.

Williams, D. W. P., J. Koenig, L. Carnevali, et al. 2019. "Heart Rate Variability and Inflammation: A Meta-Analysis of Human Studies." *Brain, Behavior, and Immunity* 80: 219–226. https://doi.org/10.1016/j.bbi.2019.03.009.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.