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Impact of methods for data selection on the day-to-day reproducibility of resting metabolic rate assessed with four different metabolic carts

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Abstract *Background and aims:* Accomplishing a high day-to-day reproducibility is important to detect changes in resting metabolic rate (RMR) and respiratory exchange ratio (RER) that may be produced after an intervention or for monitoring patients' metabolism over time. We aimed to analyze: (i) the influence of different methods for selecting indirect calorimetry data on RMR and RER assessments; and, (ii) whether these methods influence RMR and RER day-to-day reproducibility.

Methods and results: Twenty-eight young adults accomplished 4 consecutive RMR assessments (30-min each), using the Q-NRG (Cosmed, Rome, Italy), the Vyntus CPX (Jaeger-CareFusion, Höchberg, Germany), the Omnical (Maastricht Instruments, Maastricht, The Netherlands), and the Ultima CardiO2 (Medgraphics Corporation, St. Paul, Minnesota, USA) carts, on 2 consecutive mornings. Three types of methods were used: (i) *short* (periods of 5 consecutive minutes; 6–10, 11–15, 16–20, 21–25, and 26–30 min) and *long time intervals (TI)* methods (6–25 and 6–30 min); (ii) steady state (*SSt* methods); and, (iii) methods filtering the data by thresholding from the mean RMR (*filtering methods*). RMR and RER were similar when using different methods (except RMR for the Vyntus and RER for the Q-NRG). Conversely, using different methods impacted RMR (all $P \leq 0.037$) and/or RER ($P \leq 0.009$) day-to-day reproducibility in all carts. The 6–25 min and the 6–30 min long TI methods yielded more reproducible measurements for all metabolic carts.

Abbreviations: CV, Coefficient of variation; DXA, whole-body dual-energy X-ray absorptiometry; Kcal/day, Kilocalories per day; LoA, Limits of agreement (lower and upper limits); $Mean_{25\text{-min RMR}}$, Mean resting metabolic rate (i.e., mean energy expenditure) used for establishing the cut-off points for the filtering methods; ANOVA, analysis of variance; RER, Respiratory exchange ratio; RMR, Resting metabolic rate; SSt, Steady state method for gas exchange data selection; TI, Time interval method for gas exchange data selection; Ultima, Ultima CardiO2 (Medgraphics Corporation, St. Paul, Minnesota, USA) metabolic cart; VCO_2, Volume of carbon dioxide production; VO_2, Volume of oxygen consumption; Vyntus, Vyntus CPX (Jaeger-CareFusion, Höchberg, Germany) metabolic cart.

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Conclusion: The 6−25 min and 6−30 min should be the preferred methods for selecting data, as they result in the highest day-to-day reproducibility of RMR and RER assessments.

1. Introduction

Assessing resting metabolic rate (RMR) is relevant for clinicians and researchers [1,2], as this component usually accounts for 60−70% of the 24-h energy expenditure. Indirect calorimetry devices, mostly metabolic carts, are the most used systems for assessing RMR [1−7]. Metabolic carts measure oxygen consumption (VO_2) and carbon dioxide production (VCO_2). Later, applying these measures to some equations (e.g., the Weir equation [8]) the RMR and the respiratory exchange ratio (RER) can be estimated [9].

A few years ago, guidelines and recommendations for performing RMR assessments in healthy and spontaneously breathing subjects were published [2]. Nevertheless, certain points remained unclear. One of these unclarified issues is the best method for selecting and discarding gas exchange data to compute an average value. For the RMR assessment, the VO_2 and VCO_2 are continuously measured during a pre-established period of time that usually ranges from 10 to 40 min [2]. Then, it is common to retrospectively discard the first 5-min gas exchange data, thus selecting a shorter period from the remaining recorded data [2,3,10−16]. For that data selection procedure, it has been widely postulated that the steady state time (SSt) method for gas exchange data selection increases the validity of the RMR assessment [10] −assumption that arose from individuals with pathological conditions (e.g., ventilated, suffering from cancer) [10,17]. In brief, the SSt method consists of selecting a period presenting a coefficient of variation (CV) lower than 10% for VO_2 and for VCO_2 [10]. Other authors have also proposed the inclusion of the CV for RER (lower than 5%) and for minute ventilation (lower than 10%) [16,18]. Unfortunately, the accomplishment of the SSt criteria is not always possible [11], and therefore, others methods for gas exchange data selection such as the time interval (TI) and the filtering methods have emerged as potential alternatives [18]. Briefly, when employing the TI methods, a pre-established period of time is selected. When using the filtering methods, all data below or above certain RMR cut-off thresholds are discarded [18]. It should be noted that the different methods (i.e., SSt, TI and filtering) usually provide different RMR and RER estimations [11−13,16,18,19].

Achieving a high day-to-day reproducibility is relevant to detect the changes in RMR that may be produced, for example, after a lifestyle intervention [20,21] −changes that may range from ~100 to 200 kilocalories per day [22]. Similarly, the RER is also fundamental for an accurate estimation of substrate oxidation rates [23] and for an appropriate estimation of metabolic flexibility [24]. Unfortunately, a small number of studies have explored the impact of using different methods for gas exchange data selection on RMR and RER reproducibility (e.g., Refs. [13,16]). We have previously shown that different methods for gas exchange data selection impact the RMR and RER measurements, but not their day-to-day reproducibility at least when using two different metabolic carts [16]. However, because different metabolic carts use different technologies to measure VO_2 and VCO_2, these results cannot be extrapolated to the rest of commercially available devices. Moreover, to our knowledge, there is no study determining RMR and RER reproducibility using filtering methods.

The aims of the present study were: (i) to determine the influence of methods for gas exchange data selection (TIs, SSt, and filtering) on RMR and RER estimations; and, (ii) to study whether these methods influence RMR and RER day-to-day reproducibility in a sample of young, healthy and spontaneously breathing adults using four different commercially available metabolic carts.

2. Methods

2.1. Subjects

A total of 28 young, healthy and spontaneously breathing, adults were included in this study. This is part of a more comprehensive trial aiming to determine the validity of the metabolic carts [25]. We used a repeated-measures design over 2 consecutive mornings (see below; Fig. 1A). Participants had their RMR and RER assessed during 30 min with each of the 4 metabolic carts, with a 20-minute period between measurements. The order of the metabolic carts was randomized and counterbalanced on the first visit, and repeated on the second visit. Moreover, the time of measurement for each participant was also replicated on the second visit. The inclusion criteria of the study were: (i) being older than 18 years old; (ii) having a body mass index (BMI) between 18.5 and 40 kg/m^2 (inclusive); (iii) having a stable body weight over the last 3 months (changes $\leq$3 kg) and not being enrolled in a weight loss program; (iv) non-smokers; (v) under no medication that could directly affect RMR; (vi) not suffering from chronic or acute illness; and (vii) not being pregnant or lactating. The study protocol and written informed consent were approved by the Human Research Ethics Committee of the University of Granada (No. 836), and followed the Declaration of Helsinki (revised version

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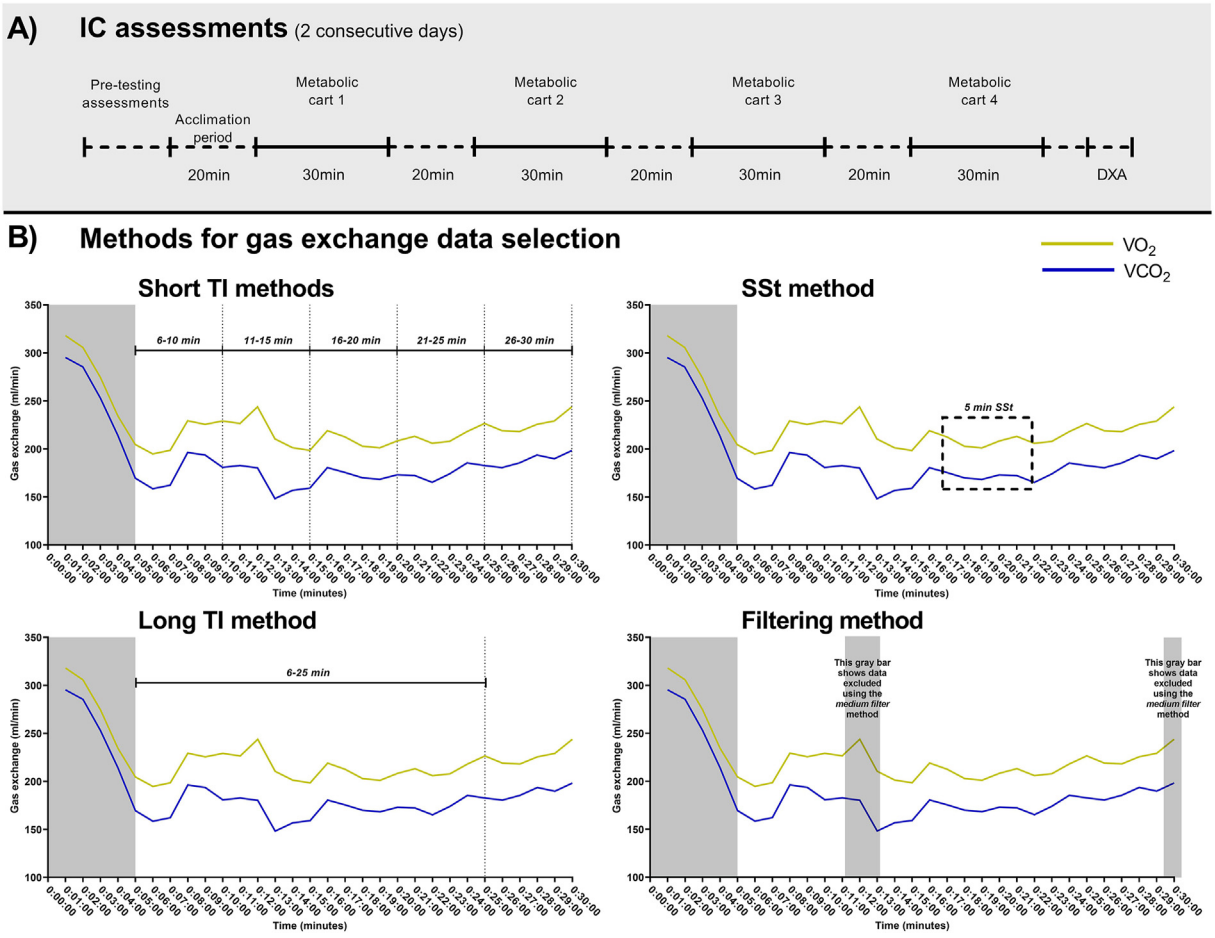

Figure 1 Study procedures (replicated on both visits, 24 h apart). In Panel A, the pre-testing assessments included the measurements of weight and height. IC: indirect calorimetry. Metabolic cart 1–4 denotes the indirect calorimetry assessments (30-min each) using the different metabolic carts (i.e., the Q-NRG, the Vyntus, the Omnical and the Ultima) in a random order. DXA: dual-energy X-ray absorptiometry assessment. Weight, height and DXA assessments were performed only on visit 1. Panel B depicts some examples of the methods for gas exchange selection. The upper (short) and lower (long) left panels represent examples of the Time Interval (TI) methods. The upper right panel represents an example the 5 min Steady State time (SSt) method. The lower right panel represents an example of the medium filter method (the two gray bars represent data excluded by the medium filter method). The yellow line represents the volume of oxygen consumption (VO_2), while the blue line represents the volume of carbon dioxide production (VCO_2). The first 5-min of measurements were discarded as suggested by current recommendations [2].

2013). All participants gave their written and oral consent prior to their enrollment.

2.2. Metabolic carts used for the resting metabolic rate assessments

Participants' gas exchange was measured using 4 metabolic carts: the Q-NRG (Cosmed, Rome, Italy), the Vyntus CPX (Jaeger-CareFusion, Höchberg, Germany; thereinafter called *Vyntus*), the Omnical (Maastricht Instruments, Maastricht, The Netherlands), and the Ultima CardiO2 (Medgraphics Corporation, St. Paul, Minnesota, USA; thereinafter called *Ultima*). The Q-NRG, the Vyntus and the Omnical metabolic carts were equipped with a canopy-hood system for the gas exchange collection, while the Ultima was equipped with a face-tent system. Detailed information, characteristics and other technical issues can be found elsewhere [25]. All the metabolic carts were calibrated (i.e., flow and gas analyzers calibrations) before each measurement by the same researchers, strictly following the manufacturers' recommendations and instructions.

2.3. Resting metabolic rate assessment procedures

The participants came to the research center (avoiding any physical activity since they woke up) by public transportation or motorized vehicle and confirmed having consumed a standardized *ad-libitum* diet, including a dinner 12 h before the start of the first RMR assessment (9 am) on both study visits [25]. The standardized ad-libitum diet was the same before both visits, as shown elsewhere [25]. In addition, participants were instructed to avoid alcohol and caffeine consumption for a minimum of 12 h before attending the research center [2]. All tests were performed according to current methodological guidelines

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[2]. Briefly, participants stayed motionless (in the supine position) on a bed, and were covered by a bed sheet for at least 20 min before the first measurement (acclimation period prior to the RMR assessment; see Fig. 1A). Additionally, they confirmed not having performed any moderate and/or vigorous intensity (previous 24 and 48 h, respectively) physical activity. Participants were asked to lay on the bed (in the supine position) during the last 15 min of every period between measurements (see Fig. 1A) and were also instructed to breathe normally, and not to talk, fidget or sleep during all assessments. We strictly followed the same procedures on both study visits. On both testing days, room temperature and humidity were monitored. In average, room temperature and humidity were maintained at 25° and 38% respectively, with no significant within- (all $P \geq 0.289$) or between-day (all $P \geq 0.105$) differences.

The 30-min measured VO_2 and VCO_2 data were downloaded from all metabolic carts and averaged every 1-minute using a Microsoft Excel® spreadsheet (Microsoft Corp, Redmond, Washington, USA). Later, those 1-min data points yielding non-physiological resting RER values (above 1.0 or below 0.7), and the first 5 min period data were retrospectively discarded following current methodological recommendations [2].

2.4. Methods for gas exchange data selection

Using the minute-to-minute data, we utilized different TI, SSt, and filtering methods (see Fig. 1B) to select the VO_2 and VCO_2 data, which in turn were used for calculating the RMR and RER measurement provided by each method for each metabolic cart and visit (see Fig. 1B). Of note, we followed used methods for gas exchange data selection proposed in previous literature [10–19].

2.4.1. Time interval methods

We used both, short TIs (periods of 5 min) and long TIs (periods of 20 and 25 min) to select the gas exchange data. We established pre-defined short TIs of 6–10 min, 11–15 min, 16–20 min, 21–25 min, and 26–30 min (see Fig. 1B upper left panel); and, long TIs of 6–25 min and 6–30 min (see Fig. 1B lower left panel). For each TI method, the average VO_2 and VCO_2 were computed and later used to estimate the RMR and RER.

2.4.2. Steady state time methods

For every period of 3, 4, 5, and 10 consecutive minutes, we computed the CV for VO_2, VCO_2, and RER. Subsequently, these CVs for VO_2, VCO_2, and RER were averaged obtaining a single *mean CV* for each period of 3, 4, 5, and 10 min. The SSt periods selected for final analyses were those achieving the SSt criteria ($CV \leq 10\%$ for VO_2 and for VCO_2, and $CV \leq 5\%$ for RER) and presenting the lowest *mean CV* (see Fig. 1B upper right panel). For each of the selected periods –i.e., one for each duration: 3, 4, 5, and 10 min– accomplishing the abovementioned criteria, the average VO_2 and VCO_2 were calculated and later used to estimate the RMR and RER [16].

2.4.3. Filtering methods

The mean VO_2 and VCO_2 for the available 25 min data were used to calculate the $mean_{25\text{-}min}$ RMR. Then, 3 filtering methods were applied, so we excluded any 1-min data points that were: ±15% of the $mean_{25\text{-}min}$ RMR when using the low filter, ±10% of the $mean_{25\text{-}min}$ RMR when using the medium filter, and ±5% of the $mean_{25\text{-}min}$ RMR when using the strong filter. Finally, for the 1-min data points that passed each filter, the average VO_2 and VCO_2 were calculated and later used to estimate the RMR and RER (see Fig. 1B lower right panel). Of note, since the filtering methods might include a variable number of valid data points, we computed the number of included data points for each participant and the filtering method.

2.5. Resting metabolic rate and respiratory exchange ratio determination

For each method and participant the RMR was estimated using the Weir's abbreviated equation [8] (expressed in kilocalories per day [kcal/day]). The RER was computed as VCO_2/VO_2.

2.6. Urine collection and sample analysis

Participants collected 12-h urine samples before arriving at the research center. Participants were asked to collect their urine after the standardized dinner (9 pm) and to continue the collection during the entire fasting period (until the start of the RMR gas exchange measurement at 9 am) [25]. We measured total urine volume and urea concentration (by an enzymatic method; Spinreact, UREA-37_R1, Girona, Spain). Then, we estimated urinary nitrogen concentration using a regression equation as detailed elsewhere [26] and subsequently included that value in the Weir's equation.

2.7. Anthropometric and body composition assessment

On the first visit (see Fig. 1A), participant's body weight and height were measured (Seca model 799, Electronic Column Scale, Hamburg, Germany), while participants were barefoot and wearing light clothes. We computed BMI as body weight (in kg)/height (in m^2). Finally, body composition (fat and lean masses) was determined by whole-body dual-energy X-ray absorptiometry (DXA; Discovery Wi, Hologic, Inc., Bedford, Massachusetts, USA).

2.8. Statistical analysis

Results are presented as mean ± standard deviation (SD), and adjusted R^2 for linear regression analyses. Analyses were conducted using the Statistical Package for Social Sciences (SPSS; v. 22.0, IBM SPSS Statistics, IBM Corporation, Chicago, Illinois, USA) and the level of significance was set at $P \leq 0.05$. Figures were created using Graph Pad Prism (GraphPad Software, v. 8.4.1, California, USA). All the analyses were performed separately for each metabolic cart and no between devices comparisons were made.

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2.8.1. Impact of methods for gas exchange data selection on resting metabolic rate assessments

A repeated-measures analysis of variance (ANOVA) was used to test differences in mean RMR, RER, VO_2, and VCO_2 among methods on both visits. Then, the Bonferroni correction was used to perform *post-hoc* comparisons.

2.8.2. Variance in resting metabolic rate explained by its classical predictors

We studied the associations of RMR (an average of both visits) with its classical predictors [27] including body weight and composition (lean mass and fat mass [both in kg]), and sex as an approach for indirectly determining the validity of the RMR estimation yielded by each method for gas exchange data selection as previously proposed [18,19]. To that end, we performed simple linear regression analyses to study the association between RMR estimations among methods and body weight (Model 1), and multiple linear regressions to analyze the associations between RMR estimations among methods and body composition (lean and fat mass) and sex (Model 2).

2.8.3. Impact of methods for gas exchange data selection on the RMR and RER day-to-day reproducibility

For every participant and metabolic cart, and for every method for gas exchange data selection, we computed the absolute day-to-day differences (e.g., $|$RMR Visit 1 − RMR Visit 2$|$), and the day-to-day CV (e.g., [SD RMR/mean RMR] × 100). Then, repeated-measures ANOVA, with *post-hoc* Bonferroni comparisons, were used to test differences among methods in both, the absolute day-to-day differences and the CVs for RMR, RER, VO_2, and VCO_2.

In addition, as proposed by Bland and Altman [28], to study the day-to-day reproducibility we calculated the mean bias (also known as mean difference; computed as Visit 1 − Visit 2) and the 95% lower and upper limits of agreement (LoA). Finally, to analyze the day-to-day differences in the gas exchange parameters among methods, we conducted paired *t*-test analyses.

3. Results

The participants (17 men, 11 women) were 25.3 ± 4.6 years old, had a body weight of 69.9 ± 12.3 kg, and a height of 171.0 ± 7.6 cm (BMI: 23.8 ± 2.9 kg/m^2). Fat mass, fat mass percentage, and lean mass were 17.3 ± 6.0 kg, 25.5 ± 8.6%, and 48.7 ± 11.0 kg, respectively.

3.1. Impact of methods for gas exchange data selection on resting metabolic rate assessments

We observed that, while all participants achieved the SSt criteria (regardless the time length and the metabolic cart used), 5 and 8 participants did not have valid data points for the strong filter method criteria on visit 1 and on visit 2 (Table S1). In addition, we observed a wide range of "valid" data included among filtering methods. The number of valid data points included for each of the filtering methods is presented in Table S2. Thus, we excluded the strong filter method from the main analyses.

Fig. 2 shows mean for RMR (Panels A–D) and RER (Panels E–H) measures in visit 1 for all methods for data selection. Repeated-measures ANOVA showed significant differences across methods in RMR for the Vyntus (Fig. 2B) and in RER for the Q-NRG (Fig. 2E). In all other cases, no differences were detected on RMR and RER across methods (Fig. 2). We observed similar results for RMR and RER measures obtained during visit 2 (Figure S1). Measured VO_2 and VCO_2 values across different methods for data selection are presented in Table S3. Repeated-measures ANOVA showed differences in VO_2 on both visits (both $P < 0.003$), and in VCO_2 on visit 2 ($P = 0.011$) for the Vyntus. On both visits, no differences were observed in VO_2 or VCO_2 across methods for the Q-NRG, the Omnical, and the Ultima metabolic carts (all $P \geq 0.199$; Table S3).

3.2. Variance in resting metabolic rate explained by its classical predictors

We observed that, regardless the regression model used, the RMR values provided by the 6–25 min and the 6–30 min methods yielded higher explained variance (adjusted R^2 ranged from 0.59 to 0.95; Table S4). These results were similar for all metabolic carts (Table S4).

3.3. Impact of methods for gas exchange data selection on RMR and RER day-to-day reproducibility

Fig. 3 shows mean absolute values of day-to-day RMR (Panels A–D) and RER (Panels E–H) differences among methods for gas exchange data selection. Repeated-measures ANOVA showed significant differences in day-to-day RMR differences for the Q-NRG, the Omnical, and the Ultima (all $P \leq 0.037$; Fig. 3A, C, and D), as well as in day-to-day RER differences (all $P \leq 0.009$; Fig. 3E, G, and H). For all metabolic carts, we observed lower absolute day-to-day RMR and RER differences (i.e., higher reproducibility) when using the 6–25 min and the 6–30 min methods (Fig. 3; light grey columns). These results remained unaltered when using the day-to-day CVs instead of the absolute values of day-to-day differences (repeated-measures ANOVA analyses: all $P \leq 0.047$; data not shown).

Table 1 shows the day-to-day mean bias, the 95% upper and lower LoA, and the day-to-day CV for every method for gas exchange data selection and metabolic cart. Paired *t*-test showed no significant RMR and RER day-to-day bias in any of the methods for data selection for the Vyntus, the Omnical, and the Ultima metabolic carts (Table 1). In contrast, significant RMR day-to-day differences were observed for the Q-NRG with the 21–25 min, the 10 min SSt, the low filter, and the medium filters methods (all $P < 0.030$; Table 1), as well as for the 26–30 min method in the RER estimations ($P = 0.047$; Table 1). Interestingly, although RMR and RER day-to-day bias and LoA were similar among methods, the 6–25 min and the 6–30 min

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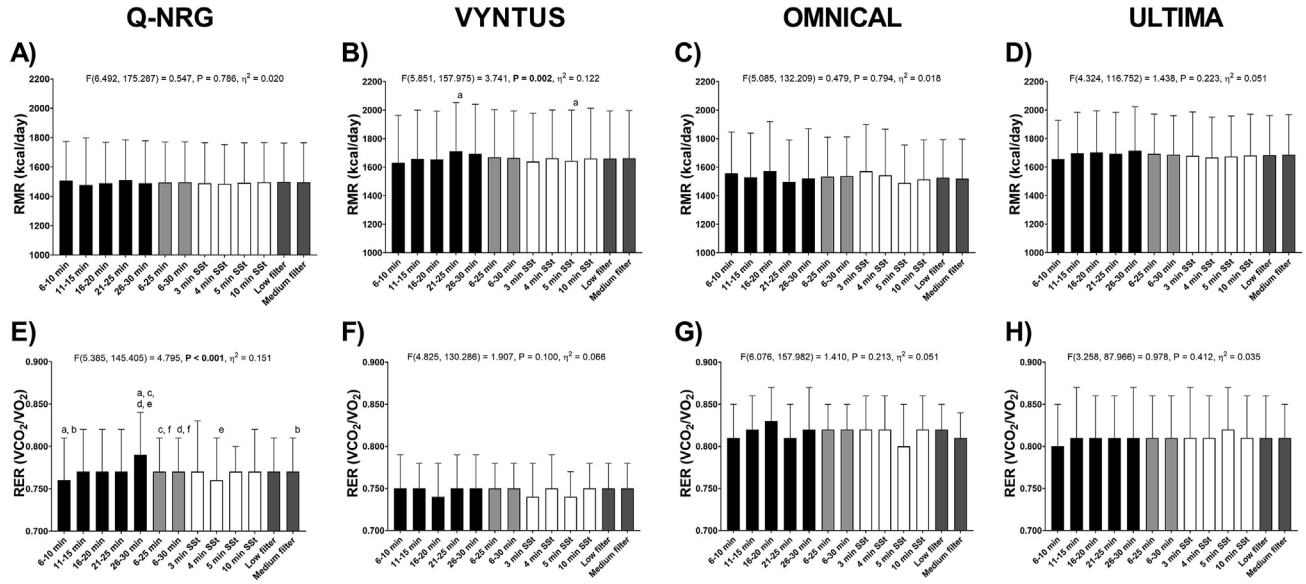

Figure 2 Differences among methods for gas exchange data selection and metabolic carts on resting metabolic rate (RMR; Panels A–D) and respiratory exchange ratio (RER; Panels E–H) during visit 1. Black columns represent the short time interval (TI) methods. Light grey columns represent the long TI methods. White columns represent the steady state time (SSt) periods. Dark grey columns represent the filtering methods. P-values are obtained from repeated-measures analysis of variance (ANOVA; n = 28). Significant P values are presented in bold numbers. Identical letters indicate statistical differences as determined by post-hoc Bonferroni analysis. Data are presented as mean and standard deviation (SD). Vyntus: Vyntus CPX metabolic cart. Ultima: Ultima CardiO2 metabolic cart. Min: minutes. VCO_2: volume of carbon dioxide production. VO_2: volume of oxygen consumption.

methods showed the narrowest LoA and lower day-to-day CVs (Table 1). In brief, the VO_2 and VCO_2 results were similar to the abovementioned for RMR and RER and are presented in Table S5.

4. Discussion

The main findings of the present study show that despite RMR and RER values are similar independently of the

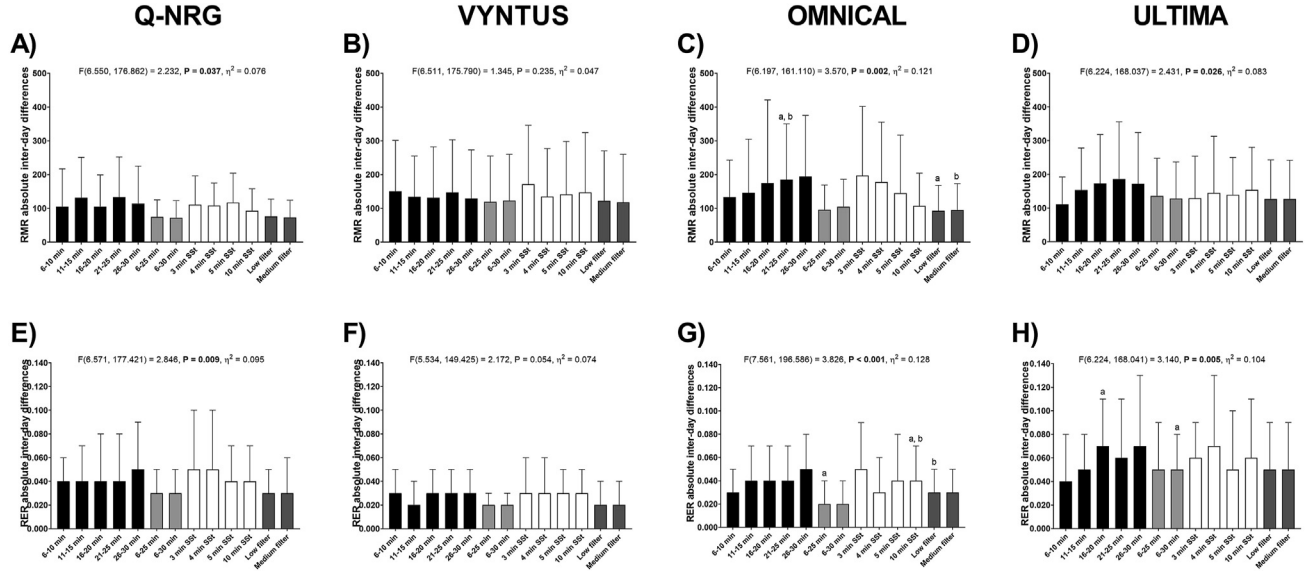

Figure 3 Day-to-day reproducibility (i.e., absolute difference between day 1 and 2) of resting metabolic rate (RMR; Panels A–D) and respiratory exchange ratio (RER; Panels E–H) estimates yield by different methods for gas exchange data selection and metabolic carts. Y axis represent the absolute day-to-day differences (e.g., |RMR Visit 1 − RMR Visit 2|). Black columns represent the short time interval (TI) methods. Light grey columns represent the long TI methods. White columns represent the steady state time (SSt) periods. Dark grey columns represent the filtering methods. P-values come from repeated-measures analysis of variance (ANOVA; n = 28). Significant P values are presented in bold numbers. Identical indicatory letters show statistical differences as determined by post-hoc Bonferroni analysis. Data are presented as mean and standard deviation (SD). Vyntus: Vyntus CPX metabolic cart. Ultima: Ultima CardiO2 metabolic cart. Min: minutes. Absolute day-to-day differences for RMR are presented as kilo-calories per day. VCO_2: volume of carbon dioxide production. VO_2: volume of oxygen consumption. RER was calculated as VCO_2/VO_2.

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Table 1 Day-to-day reproducibility of resting metabolic rate (RMR) and respiratory exchange ratio (RER) yielded by each method for gas exchange data selection and metabolic cart.

	Q-NRG			Vyntus			Omnical			Ultima		
	Mean difference (SD)	95% LoA (lower; upper)	CV (SD)	Mean difference (SD)	95% LoA (lower; upper)	CV (SD)	Mean difference (SD)	95% LoA (lower; upper)	CV (SD)	Mean difference (SD)	95% LoA (lower; upper)	CV (SD)
RMR (kcal/day)												
6–10 min	46 (147)	(−243; 334)	5.6 (7.1)	−19 (214)	(−438; 401)	6.5 (6.6)	2 (174)	(−339; 344)	5.9 (4.4)	−24 (137)	(−293; 246)	4.7 (3.4)
11–15 min	−16 (179)	(−366; 334)	6.8 (6.9)	−11 (182)	(−368; 345)	5.9 (5.5)	47 (213)	(−370; 463)	6.8 (6.9)	12 (199)	(−379; 403)	6.3 (5.1)
16–20 min	29 (139)	(−243; 302)	5.0 (4.3)	−41 (197)	(−427; 344)	5.4 (6.0)	65 (297)	(−517; 646)	7.7 (9.0)	21 (227)	(−424; 466)	7.2 (5.7)
21–25 min	72 (165) *	(−251; 395)	6.5 (6.2)	1 (216)	(−424; 424)	6.0 (6.2)	42 (247)	(−442; 525)	8.7 (6.9)	17 (254)	(−481; 515)	7.8 (6.7)
26–30 min	34 (157)	(−274; 341)	5.6 (6.1)	−37 (192)	(−413; 339)	5.5 (5.9)	−12 (268)	(−537; 514)	8.8 (8.0)	19 (231)	(−434; 471)	7.0 (6.2)
6–25 min	33 (85)	(−134; 199)	3.6 (2.5)	−22 (181)	(−376; 333)	5.1 (5.7)	29 (119)	(−205; 262)	4.4 (3.0)	10 (178)	(−338; 358)	5.7 (4.6)
6–30 min	32 (84)	(−132; 196)	3.6 (2.8)	−18 (184)	(−379; 344)	5.2 (5.8)	39 (128)	(−212; 290)	4.9 (3.5)	7 (170)	(−326; 340)	5.5 (4.6)
3 min SSt	44 (134)	(−219; 307)	5.4 (3.8)	−57 (240)	(−529; 414)	7.2 (7.1)	39 (284)	(−517; 596)	8.6 (8.2)	−12 (181)	(−367; 343)	5.3 (4.9)
4 min SSt	43 (121)	(-194; 280)	5.2 (3.0)	−17 (197)	(−404; 369)	5.5 (5.5)	2 (253)	(−493; 498)	7.8 (7.2)	−39 (220)	(−471; 392)	5.8 (5.6)
5 min SSt	25 (145)	(−259; 309)	5.5 (3.5)	−23 (211)	(−437; 390)	6.0 (6.8)	−5 (227)	(−450; 439)	6.6 (7.5)	13 (179)	(−338; 363)	5.8 (4.4)
10 min SSt	50 (103) *	(−152; 252)	4.6 (3.3)	−26 (230)	(−478; 426)	6.1 (7.3)	14 (145)	(−271; 299)	4.9 (4.1)	7 (201)	(−387; 401)	6.4 (5.0)
Low filter	40 (84) *	(−124; 204)	3.7 (2.5)	−25 (191)	(−400; 349)	5.2 (6.3)	25 (118)	(−207; 257)	4.3 (3.2)	12 (173)	(−327; 352)	5.4 (4.9)
Medium filter	37 (82) *	(−124; 198)	3.6 (2.4)	−22 (185)	(−384; 340)	5.1 (6.1)	19 (123)	(−221; 259)	4.3 (3.2)	13 (172)	(−325; 351)	5.4 (4.8)
RER												
6–10 min	−0.005 (0.047)	(−0.098; 0.088)	3.7 (2.2)	−0.001 (0.034)	(−0.067; 0.065)	2.6 (1.9)	0.009 (0.040)	(−0.07; 0.088)	3.0 (1.9)	0.006 (0.062)	(−0.115; 0.127)	4.0 (4.0)
11–15 min	0.003 (0.047)	(−0.089; 0.094)	3.4 (2.6)	−0.008 (0.028)	(−0.063; 0.048)	2.0 (1.9)	0.013 (0.049)	(−0.084; 0.109)	3.4 (2.7)	0.004 (0.061)	(−0.116; 0.124)	4.5 (3.1)
16–20 min	−0.002 (0.054)	(−0.107; 0.103)	3.6 (3.1)	−0.007 (0.034)	(−0.074; 0.059)	2.5 (2.1)	0.012 (0.052)	(−0.09; 0.114)	3.6 (2.8)	−0.012 (0.079)	(−0.167; 0.142)	5.9 (3.4)
21–25 min	0.001 (0.054)	(−0.107; 0.107)	3.6 (3.2)	0.004 (0.038)	(−0.071; 0.079)	3.1 (1.7)	0.004 (0.052)	(−0.097; 0.105)	3.5 (2.6)	−0.021 (0.078)	(−0.174; 0.132)	5.4 (4.3)
26–30 min	0.022 (0.056)*	(−0.088; 0.133)	4.3 (3.4)	−0.005 (0.037)	(−0.078; 0.068)	3.0 (1.9)	−0.002 (0.058)	(−0.115; 0.111)	3.9 (2.9)	−0.029 (0.093)	(−0.212; 0.154)	6.4 (5.4)
6–25 min	0.004 (0.036)	(−0.066; 0.074)	2.5 (2.0)	−0.004 (0.026)	(−0.054; 0.047)	2.1 (1.3)	0.008 (0.029)	(−0.05; 0.065)	1.9 (1.7)	−0.010 (0.066)	(−0.139; 0.119)	4.7 (3.5)
6–30 min	0.001 (0.038)	(−0.075; 0.074)	2.9 (1.9)	−0.003 (0.026)	(−0.055; 0.049)	2.1 (1.4)	0.010 (0.031)	(−0.05; 0.07)	2.2 (1.7)	−0.006 (0.063)	(−0.129; 0.117)	4.6 (3.2)
3 min SSt	0.002 (0.071)	(−0.136; 0.141)	4.7 (4.2)	−0.014 (0.040)	(−0.093; 0.064)	3.0 (2.7)	−0.001 (0.062)	(−0.122; 0.12)	4.3 (3.1)	0.001 (0.069)	(−0.134; 0.135)	5.2 (3.2)
4 min SSt	−0.011 (0.066)	(-0.141; 0.119)	4.2 (4.1)	−0.007 (0.041)	(−0.087; 0.074)	2.9 (2.5)	0.006 (0.042)	(−0.077; 0.088)	2.8 (2.3)	−0.006 (0.089)	(−0.181; 0.168)	5.8 (4.9)
5 min SSt	0.011 (0.055)	(−0.097; 0.119)	4.1 (3.2)	−0.006 (0.032)	(−0.068; 0.057)	2.5 (1.8)	−0.004 (0.057)	(−0.115; 0.108)	3.6 (3.3)	0.002 (0.070)	(−0.135; 0.14)	4.6 (4.1)
10 min SSt	0.007 (0.049)	(−0.089; 0.102)	3.2 (3.0)	−0.007 (0.033)	(−0.072; 0.058)	2.5 (1.9)	0.009 (0.049)	(−0.088; 0.105)	3.4 (2.6)	−0.009 (0.079)	(−0.163; 0.145)	5.4 (4.2)
Low filter	0.006 (0.037)	(−0.067; 0.079)	2.6 (2.2)	−0.004 (0.027)	(−0.057; 0.049)	2.1 (1.5)	0.008 (0.033)	(−0.057; 0.072)	2.2 (1.9)	−0.008 (0.065)	(−0.135; 0.12)	4.6 (3.5)
Medium filter	0.007 (0.040)	(−0.072; 0.085)	2.8 (2.4)	−0.003 (0.027)	(−0.056; 0.049)	2.0 (1.5)	0.005 (0.033)	(−0.061; 0.07)	2.3 (1.8)	−0.009 (0.066)	(−0.138; 0.12)	4.5 (3.7)

Results are presented as mean difference (visit 1 − visit 2) and standard deviation (SD), 95% lower and upper limits of agreement (LoA), and day-to-day coefficient of variation (CV) expressed as percentage and (SD). Vyntus: Vyntus CPX metabolic cart. Ultima: Ultima CardiO2 metabolic cart. SSt: Steady state time method. * denotes statistically significant P values (i.e., $P < 0.05$) from paired *t*-test (visit 1 vs. visit 2). RMR is expressed as kilocalories per day (kcal/day). N = 28.

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method for gas exchange data selection used, the day-to-day reproducibility of these parameters is indeed influenced by the chosen method for gas exchange data selection. The most reproducible RMR and RER values were obtained with the 6–25 min and the 6–30 min (long TI) methods for all the investigated metabolic carts. The convenience of using the long TI method is further reinforced by the fact that the RMR values it yielded were better associated with RMR classical predictors (body weight, lean and fat masses, and sex), thus suggesting these methods provide the most valid RMR estimations. Therefore, all results support the preferred use of the long TI method for gas exchange data selection, at least when using the 4 calorimeters studied here, which adds relevant information to current guidelines for assessing RMR in healthy and non-ventilated patients.

The RMR is commonly defined as the necessary energy for maintaining normal body functions and homeostasis in an awake person while resting in conditions of ambient thermoneutrality [2]. Thus, taking into account the RMR definition, one may hypothesize that the method providing the lowest RMR estimations may be the most valid method –although an underestimation of the RMR value should not be neglected. Nevertheless, we did not observe differences on RMR among methods for gas exchange data selection (except for the Vyntus metabolic cart; Fig. 2B and Figure S1B). These results partially disagree with previous studies that showed that both, TI and SSt methods provided lower estimations for RMR and RER [11,13,16]. Furthermore, in a previous study using two different Medgraphics Corporation metabolic carts (an older version of the Ultima CardiO2 and the CCM Express), we suggested that the strong filter method could be an alternative for those subjects that are not able to achieve the SSt criteria during the RMR assessment, as no significant differences were observed among them [18]. Here, using 4 different metabolic carts, we did not observe differences between the SSt and the Filtering methods, thus suggesting that their estimations are mostly similar (Fig. 2 and Figure S1). It should be noted, however, that the participants included in the present study all accomplished the SSt criteria. On the other hand, we should mention that not all participants achieved the strong filter criteria on both visits (see Table S1). This issue should not be ignored when considering the application of the strong filter method, as it may result in missed data (even when the SSt criteria was accomplished by all participants as mentioned previously). In this study, our primary objective was to investigate the impact of different methods for gas exchange data selection on RMR and RER, rather than conducting a comparison of RMR and RER measurements obtained across different metabolic carts. See Ref. [25] for comprehensive information regarding the comparison of metabolic carts, encompassing their validity, precision, and comparability.

The variance in RMR explained by its classical predictors [27] may be considered an indirect approach for determining the accuracy of the RMR estimation. Thus, one may hypothesize that the method providing the highest explained variance may be the method providing the most valid RMR estimations. Here, we observed that the variance in RMR explained by its classical predictors is in agreement with previous studies. Previous literature reported that the variance in RMR explained by body weight ranged from 36% to 56% [18,19,29–32], while in our study ranged from 36% to 73% across methods and metabolic carts (Model 1, Table S3). Including body composition minimally increases the explained variance by the different methods (Model 2, Table S3). This explained variance including body composition in the model also concurs with previous studies [18,19,29,31,33].

Accomplishing a high RMR and RER day-to-day reproducibility (i.e., a low day-to-day difference on RMR and RER values) is of great relevance to detect changes produced by any intervention [20,21] or for monitoring patients' metabolism over time. Therefore, if the procedure to select the VO_2 and VCO_2 data impacts reproducibility, it is a very relevant aspect of the good practice to assess RMR and RER. It should be noted that, while RMR is mostly dependent of VO_2, the RER depends on both VO_2 and VCO_2, thus the methods could impact the RMR and RER day-to-day reproducibility in a different manner. In the present study, we observed that regardless of the metabolic cart used the 6–25 min or the 6–30 min methods resulted in lower RMR and RER day-to-day differences than when applying the short TI, the SSt, and the filtering methods (Fig. 3). Despite significance was not reached in most of the *post-hoc* comparisons, the RMR day-to-day absolute differences were ≈100 kcal/day, a result that should be considered as could be clinically significant, which might suggest that this study was underpowered to detect such differences. Of note, the CVs for the RMR and RER day-to-day differences were <6% and <5% for the 6–25 and 6–30 min methods for gas exchange data selection. It should be noted that this RMR day-to-day reproducibility is within the range that has been suggested for the human biological reproducibility determined using metabolic carts [34]. In addition, we observed lower mean bias and narrower LoA (see Table 1) than those observed in our previous study [16]. Regarding RER, we observed day-to-day absolute differences ≈0.020 for the Q-NRG, the Vyntus, and the Omnical, and ≈0.040 for the Ultima (Fig. 3E–H) metabolic carts. Importantly, these RER day-to-day differences could negatively influence the nutrient oxidation estimations and bias the results in a clinically significant manner (e.g., to determine metabolic flexibility before and after an intervention). However, more studies are needed to determine if these results also apply to other metabolic cart systems or brands, or even to different populations (e.g., younger and/or older subjects, ill and/or ventilated hospitalized patients).

Our results should be considered with caution, as certain limitations have to be acknowledged. All participants were young, healthy, and spontaneously breathing, adults thus we cannot assure that our results can be extrapolated to older people or mechanically ventilated patients. Although our results are similar when using 4 different systems (the Q-NRG, the Vyntus CPX, the

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Omnical, and the Ultima CardiO2 metabolic carts), we cannot assure whether our findings would apply to other metabolic carts or to other gases collection systems (e.g., face-mask) which have been proved to result in different RMR and/or RER estimation compared to a canopy collection system [35,36]. Finally, we did not control the menstrual cycle [37–39], thus its possible influence on the results cannot be ascertained. Nevertheless, that possible influence could be –at least partially– mitigated by the fact that we performed the assessments within 24 h and a within-subject study design.

5. Conclusion

Our findings support the use of long time interval methods for gas exchange data selection as the preferred method for determining the RMR and the RER when using metabolic carts. The results indicate that both, the 6–25 min and the 6–30 min methods resulted in the best RMR and RER day-to-day reproducibility and were better associated with RMR classical predictors. These findings are robust as results are similar when using 4 different indirect calorimetry systems.

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Declaration of competing interest

The authors report no conflicts of interest.

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The authors' responsibilities were as follows – JMAA, JRR and GS-D: designed the research; JMAA, LJ-F, MDM and EM-R: conducted the experiments; JMAA: analyzed the data; JMAA: wrote the original draft; JMAA, LJ-F, MDM, EM-R, FJA-G, IL, JRR and GS-D: critically revised the manuscript and discussed the results; JMAA, JRR and GS-D: were primarily responsible for the final content; and all authors: read and approved the final version. The authors report no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2023.07.017.

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