Reliability of resting metabolic rate measurements in young adults: Impact of methods for data analysis

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ABSTRACT

Background & Aims: A high inter-day reliability is a key factor to analyze the magnitude of change in resting metabolic rate (RMR) after an intervention, and the impact of using different methods for data analysis is not known. The aims of this study were: i) to analyze the impact of methods for data analysis on RMR and respiratory exchange ratio (RER) estimation; ii) to analyze the impact of methods for data analysis on inter-day RMR and RER reliability; iii) to compare inter-day RMR and RER reliability across methods for data analysis in participants who achieved steady state (SS) vs. participants who did not achieve SS.

Methods: Seventeen young healthy adults completed two 30-minute indirect calorimetry (IC) measures on two consecutive mornings, using two metabolic carts each day. Two methods for data analysis were used: i) Selection of a predefined time interval (TI) every 5 minutes (*1-5 min; 6-10 min, 11-15 min, 16-20 min, 21-25 min, 26-30 min*); and TI representing the whole measurement period (*0-30 min, 5-30 min, 5-25 min*); and ii) Methods based on the selection of the most stable period (SSt methods) (*3 min SSt, 4 min SSt, 5 min SSt, 10 min SSt*). Additionally, participants were classified as those achieving SS (CV<10% for VO_2, VCO_2 and VE, and CV<5% for RER) and those who did not.

Results: RMR and RER measurements were lower when following SSt methods than when following TI methods (all P<0.01). Although no significant differences were found between different lengths of SSt, *5 min SSt* presented the lowest RMR. There were no differences on the inter-day reliability across methods for data analysis (TI and SSt) (all P>0.2), and there was no systematic bias when comparing RMR and RER day 1 and day 2 measurements (all P>0.1). Inter-day reliability was similar in individuals who achieved the SS and individuals who did not achieve it. The results were consistent independently of the metabolic cart used.

Conclusions: The *5 min SSt* approach should be the method of choice for analyzing IC measures with metabolic carts. However, achieving SS should not be an inclusion criterion in an IC study with young healthy adults.

Keywords: resting energy expenditure; indirect calorimetry; steady state; metabolic cart; CCM Express; Ultima Cardio2.

ABBREVIATIONS

RMR: Resting metabolic Rate.

IC: Indirect calorimetry.

VO_2: Oxygen consumption.

VCO_2: Carbon dioxide production.

RER: Respiratory exchange ratio.

VE: Minute ventilation.

CV: Coefficient of variance.

SS: Steady state.

SSt: Steady state time.

TI: Time interval.

CCM: CCM Express (Medgraphics Corp, Minnesota, USA).

MGU: Ultima CardiO2 (Medgraphics Corp, Minnesota, USA).

ANOVA: Analyses of variance.

INTRODUCTION

Measuring human resting metabolic rate (RMR) is of key relevance in research and in the clinical setting [1-3]. Among the available methods to measure RMR, indirect calorimetry (IC) through a metabolic cart is the most commonly used in healthy, non-critically ill and ventilated individuals. In IC, energy expenditure is calculated from measured oxygen consumption (VO_2) and carbon dioxide production (VCO_2) by using estimating equations [4, 5]. Additionally, nutrient oxidation rates (i.e. carbohydrate and fat oxidation) can be estimated from IC measurements [6]. Guidelines on how to perform IC evaluations were published more than a decade ago [7] and were recently updated [8]; yet, there are still some issues that need to be clarified [8].

When performing IC with metabolic carts, gas exchange is commonly recorded during a relatively short period of time (e.g. 30 minutes), from which a shorter period of recorded data is selected and analyzed (e.g. 5 minutes). It is assumed that the selection of a steady state (SS) period, defined as a period in which gas exchange variables present low variation, increases the validity of the measure [9]. SS is commonly established as a period during which average minute VO_2, VCO_2, respiratory exchange ratio (RER), and/or minute ventilation (VE) coefficient of variance (CV) is lower than a pre-determined percentage (usually 10% for VO_2, VCO_2, and VE, and 5% for RER) [9]. However, as SS is not always feasible to achieve, other methods for data analysis have been proposed [10].

Methods for data analysis can be grouped in those based on a pre-defined time interval (TI) selection and those based on steady state time (SSt) approach [10]. Of note is that there is no consensus about time length of data selection in both TI or SSt methods [8, 10, 11], neither about the selection of which gas exchange variables and which pre-defined CV is better for determining SS [8]. High inter-day reliability is a key factor to analyze the magnitude of change in RMR after an intervention [12, 13]. Moreover, although RMR estimation is mainly dependent on VO_2 [4], the ratio between VO_2 and VCO_2 (i.e. RER) is crucial for estimating nutrient oxidation rates [5, 14]. Consequently, achieving a high RER reliability is also key for a method to be able to accurately estimate fuel oxidation. However, to our knowledge there are no studies examining the impact of different methods for data analysis (i.e. TI and SSt) on inter-day RMR and RER reliability.

The assumption that SS provides more valid RMR and RER measurements comes mainly from studies performed with ventilated patients [9, 15]. However, it is unknown whether this also applies to healthy non-ventilated people [15, 16]. On the other hand, it has been shown that RMR is consistently lower when following SSt than when following TI methods in healthy individuals achieving SS [10]. This suggests that achieving SS could provide a more valid RMR measure, given that RMR is considered the lowest energy expenditure in an awake person [10]. However, whether the inter-day RMR or RER reliability is higher in individuals achieving the SS compared to those that do not achieve the SS needs to be studied.

The aims of this study were: i) to analyze the impact of methods for data analysis (TI and SSt) on RMR and RER measurements in young adults; ii) to analyze the impact of methods for data analysis (TI and SSt) on inter-day RMR and RER reliability; iii) to compare inter-day RMR and RER reliability across methods for data analysis (TI and SSt) in participants who achieved SS vs. participants who did not achieve SS.

MATERIAL AND METHODS

Participants

A total of 20 (n=13 women) Caucasian young healthy adults aged 18-26 years participated in the study. A total of 3 out of 20 participants did not meet the previous conditions for IC measurements on one of the testing days (2 participants performed physical activity in the 24 hours prior to the measurement, and the other one did not met the minimum fasting time requirement). Consequently, they were retrospectively excluded from further statistical analyses. They were non-physically active (<20 minutes <3 days/week), had a stable body weight (body weight changes <3 kg) over the last 3 months, were not enrolled in a weight loss program, were non-smokers, did not take any medication, had no acute or chronic illness, and were not pregnant. The study protocol and informed consent were performed in accordance with the Declaration of Helsinki (revision of 2013), and was approved by the Human Research Ethics Committee of both University of Granada (nº924) and Servicio Andaluz de Salud (Centro de Granada, CEI-Granada). Written informed consent was obtained from all the participants before their enrollment.

Procedures

The study was conducted between February and April 2016. IC was measured via a repeated-measures design over 2 consecutive days. Measurements were conducted between 7.30 AM and 11 AM, and each participant was given an appointment at the same time on both days. Participants arrived to the laboratory by car or by bus (avoiding any physical activity after waking up) in a fasted state (at least 8 hours). They were instructed to refrain from moderate or vigorous physical activity 24 and 48 hours before the testing day, respectively. On each testing day, before performing the measurements, participants had to confirm that they met the aforementioned study conditions.

On both testing days, IC measurements were performed during two consecutive 30-minute periods with two different metabolic carts: CCM Express (CCM) and Ultima CardiO2 (MGU) (Medgraphics Corp, Minnesota, USA), using neoprene face-mask without external ventilation. The device order was replicated on both testing days, and it was counterbalanced between participants. Both devices measure VO_2 and VCO_2 using a breath-by-breath technique for determining the gas exchange. VCO_2 measurement is performed using a non-dispersive infrared analyzer, and VO_2 is measured using a galvanic fuel cell [17, 18].

IC measurements followed current guidelines [8]. In brief, all measurements were conducted in the same quiet room with dim lighting, with controlled ambient temperature (22-24ºC) and humidity (35-45%), and by the same trained staff. Before being evaluated, all participants confirmed that they met previous study conditions and lied on a reclined bed in a supine position and covered by a sheet for the 20 minutes prior to the IC measurement. They were instructed to breathe normally, and not to talk, fidget, or sleep. The same position and instructions were maintained during the two 30-minute measurement periods. Flow calibration was performed by using a 3-L calibration syringe at the beginning of every testing day, and gas analyzers were calibrated using 2 standard gas concentrations following the manufacturer's instruction before every IC measurement.

On day 1, we measured participants' weight and height using a Seca scale and stadiometer (model 799, Electronic Column Scale, Hamburg, Germany). Participants wore light clothing and no shoes during the measurements.

Methods for data analysis and steady state criteria

We used two types of methods for data analysis based on TI and SSt periods. (Figure 1): (i) TI every 5 minutes, and TI representing the whole measurement period; and (ii) SSt methods. TI every 5 minutes: mean values of every consecutive 5-minute period (i.e. from the 1st to the 5th minute, from the 6th to the 10th, etc.), hereinafter referred as *1-5 min*, *6-10 min*, *11-15 min*, *16-20 min*, *21-25 min*, and *26-30 min* (Figure 1A). TI representing the whole measurement period: mean values for the whole measurement period (i.e. *1-30 min*), and mean values for the whole measurement period except for the first 5 minutes (i.e. *6-30 min*) [8] or the first and the last 5 minutes (i.e. *6-25 min*) [19] (Figure 1B). For the SSt methods, we calculated the CV of VO_2, VCO_2, VE, and RER for every period of 3, 4, 5, and 10 minutes [7, 11], excluding the first 5 minutes of data collection (i.e. for *3 min SSt*, CVs were calculated from 6th to 8th minute, from 7th to 9th, etc.) (Figure 1C). Thereafter, we selected the periods of 3, 4, 5, or 10 minutes that met most of the following criteria: i) CV<10% for VO_2, ii) CV<10% for VCO_2, iii) CV<10% for VE, and iv) CV<5% for RER. Finally, among the periods that met most of those criteria we selected the 3, 4, 5, and 10-minute periods with the lowest average between CVs of VO_2, VCO_2, VE, and RER, for being used as *3 min SSt*, *4 min SSt*, *5 min SSt* and *10 min SSt*, respectively (Figure 1C). Finally, mean VO_2 and VCO_2 obtained by each method for data analysis were entered into Weir´s abbreviated equation [4] (see below) to estimate energy expenditure, and RER was calculated as VCO_2/VO_2:

$$RMR\ (\text{Kcal/min}) = 3.941\ \times\ VO_2(\text{l/min}) + 1.106\ \times\ VCO_2(\text{l/min})$$

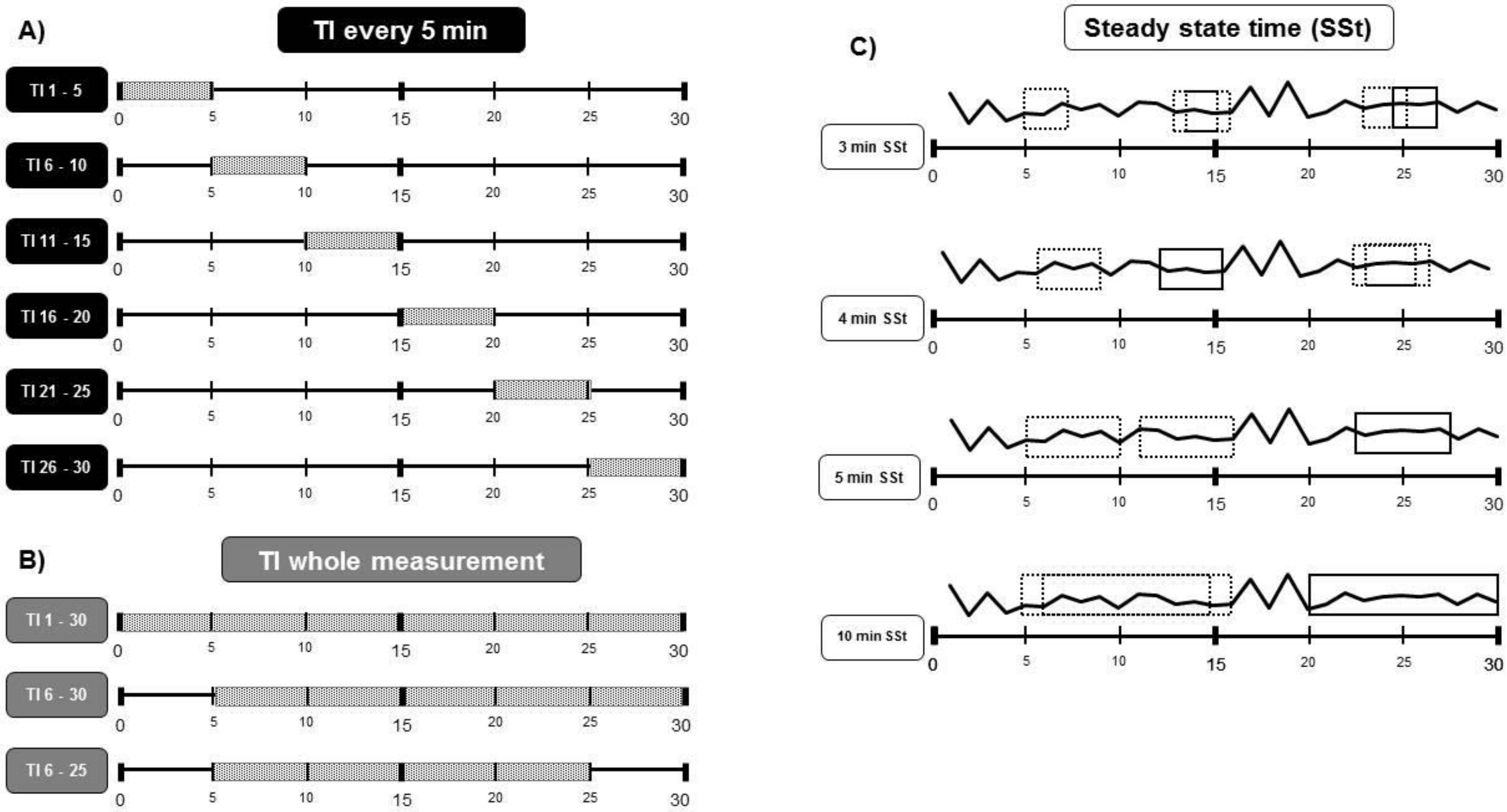

Figure 1. Methods for data analysis. A=Time interval (TI) methods every 5 minutes; B= TI for the whole measurement. Pointed blocks represent selected data for each method for data analysis in A and B panels; C= Steady state time (SSt) methods. Y axe represents resting metabolic rate (simulated data). Blocks represent the 3, 4, 5, or 10-minute periods that met the most of the following criteria: CV<10% for VO_2, VCO_2, and VE, and CV<5% for RER, among the 30-minute record, after having discarded the first 5 minutes recorded. The solid lined blocks represent the period with the lowest average between CVs of VO_2, VCO_2, VE, and RER, and thus, the period of time selected in each method for data analysis. Dashed lined blocks represent periods with the same number of CVs criteria achieved as the selected period but with a higher average between CVs of VO_2, VCO_2, VE, and RER.

To compare inter-day RMR and RER reliability across methods for data analysis in participants who achieved SS vs. participants who did not achieve SS, we classified participants as those achieving CV<10% for VO_2, VCO_2 and VE and CV<5% for RER (SS criteria) and those who failed to comply the SS criteria on any of the two testing days. This classification was performed for every method for data analysis. Therefore, a total of 13 methods for data analysis were tested, and were further grouped in those achieving SS vs. not achieving SS.

The selected CV cut-off points are probably the most used ones in literature [8]. In addition, a CV cut-off point of 10% for VO_2 and VCO_2 has been proved to accurately predict total energy expenditure in ventilated patients [9]. However, there is no consensus on how to define SS, neither on CV cut-off points, nor in the combination of gas exchange variables. Therefore, we selected the most used CV cut-off points [8], and we decided to classify participants taking into account the four gas exchange variables. This is the most strict combination criteria, which would allow to test whether achieving SS would result in better inter-day reliability. Nevertheless, we performed additional analyses classifying participants just based on VO_2 and VCO_2 CV criteria.

Statistical analysis

Gas exchange parameters including VO_2, VCO_2, VE, RMR, and RER were averaged each minute with the Breeze Suite (8.1.0.54 SP7, MGCDiagnostic®) software and downloaded to an Excel spreadsheet where the CVs and outputs of the different methods for data analysis were calculated. Results are presented as means ± standard deviation, unless otherwise stated. The analyses were conducted using the Statistical Package for Social Sciences (SPSS, v. 21.0, IBM SPSS Statistics, IBM Corporation), and the level of significance was set to <0.05.

Impact of methods for data analysis on RMR and RER measurements

A repeated-measures analysis of variance (ANOVA) was used to test differences in RMR and RER measurements across methods for data analysis for both CCM and MGU metabolic carts on both testing days. LSD Tuckey and Bonferroni corrections were used to perform post hoc comparisons.

Impact of methods for data analysis on inter-day reliability

We compared the absolute value of inter-day differences in RMR and RER values (e.g. |RMR Day1 – RMR Day2|) with every method for data analysis using repeated-measures ANOVA for both CCM and MGU metabolic carts. Inter-day RMR and RER reliability for every method of data analysis was also assessed using the Bland-Altman method [20]. Day 1 measurements were subtracted from day 2 measurements, so a positive difference indicates that day 2 measurements were higher than day 1. Bias was measured by using a 2-sided *t*-test to determine if there was a significant difference between RMR and RER measures on day 2 vs. day 1.

Inter-day reliability across methods for data analysis in participants achieving SS vs. not achieving SS

The absolute value of inter-day differences in RMR and RER (e.g. |RMR Day1 – RMR Day2|) in each method for data analysis and for both CCM and MGU metabolic carts were compared between those participants who achieved the SS criteria and those who did not, using independent sample *t*-tests.

RESULTS

The included participants (n=17, 11 women) were 23.2±1.9 years old. Mean weight and height were 63.3±11.5 Kg and 168±9 cm respectively (body mass index: 18.6 to 26.2 kg/m^2). All participants had valid data for the MGU, and all except one had valid data for the CCM (n=16).

Impact of methods for data analysis on RMR and RER measurements

Figure 2 shows mean values of day 1 measurements for RMR and RER across different methods for data analysis. Repeated-measures ANOVA indicated significant differences in mean RMR and RER for both CCM and MGU metabolic carts (all $P<0.01$). The lowest RMR value was obtained when following the *5 min SSt* method for both CCM and MGU. The lowest RER value was also obtained following the *5 min SSt* for the CCM, but not for the MGU, where *1-5 min* method resulted in lower RER value. LSD Tuckey post hoc comparisons revealed significant differences between SSt and TI methods. SSt obtained lower values, but we found no significant differences when comparing between different lengths of SSt methods. Nevertheless, significant differences disappeared after Bonferroni corrections. Results were similar in the measurements preformed on day 2 (data not shown).

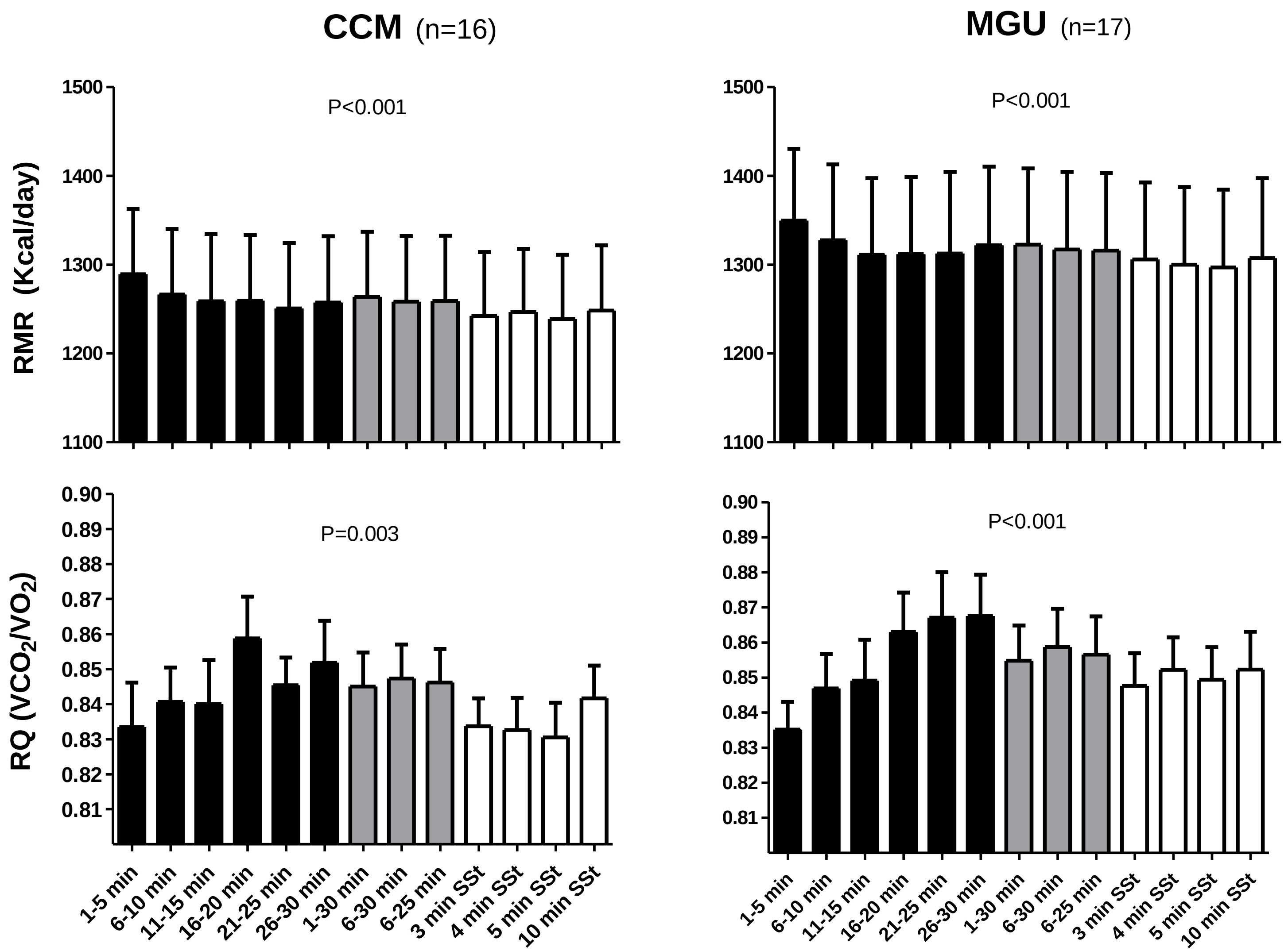

Figure 2. Day 1 resting metabolic rate (RMR) and respiratory exchange ratio (RER) measurements across methods for data analysis (steady state time and time interval) in the CCM and MGU metabolic carts. Black columns represent time intervals of 5 minutes; Grey columns represent time intervals for longer time periods; and White columns represent steady state time (SSt) periods. SSt is defined as the period (3, 4, 5, or 10 minutes) with the lowest coefficient of variance for VO2, VCO2, RER, and VE (i.e. the most stable period of *n* minutes). P from analysis of variance.

Impact of methods for data analysis on inter-day reliability

Repeated-measures ANOVA indicated no significant effect of method for data analysis in absolute value of inter-day RMR and RER differences on both CCM and MGU metabolic carts (Figure 3, all $P>0.2$). Results remained unaltered when using inter-day percentages instead of absolute values (all $P>0.2$, data not shown).

Table 1 shows inter-day mean bias (Day2 - Day1) and the 95% limit of agreement (mean difference±1.96 standard deviation of the difference) for every method for data analysis with the CCM and MGU metabolic carts. Paired *t*-test showed no significant RMR or RER inter-day mean differences in any of the methods for data analysis (all $P>0.1$). The limits of agreement were quite similar across methods, and no method presented the narrowest limits of agreement for all analysis. We observed that *10 min SSt*, *5 min SSt*, *6-25 min*, and *10 min SSt* presented the narrowest limits of agreement for RMR-CCM, RMR-MGU, RER -CCM, and RER -MGU, respectively.

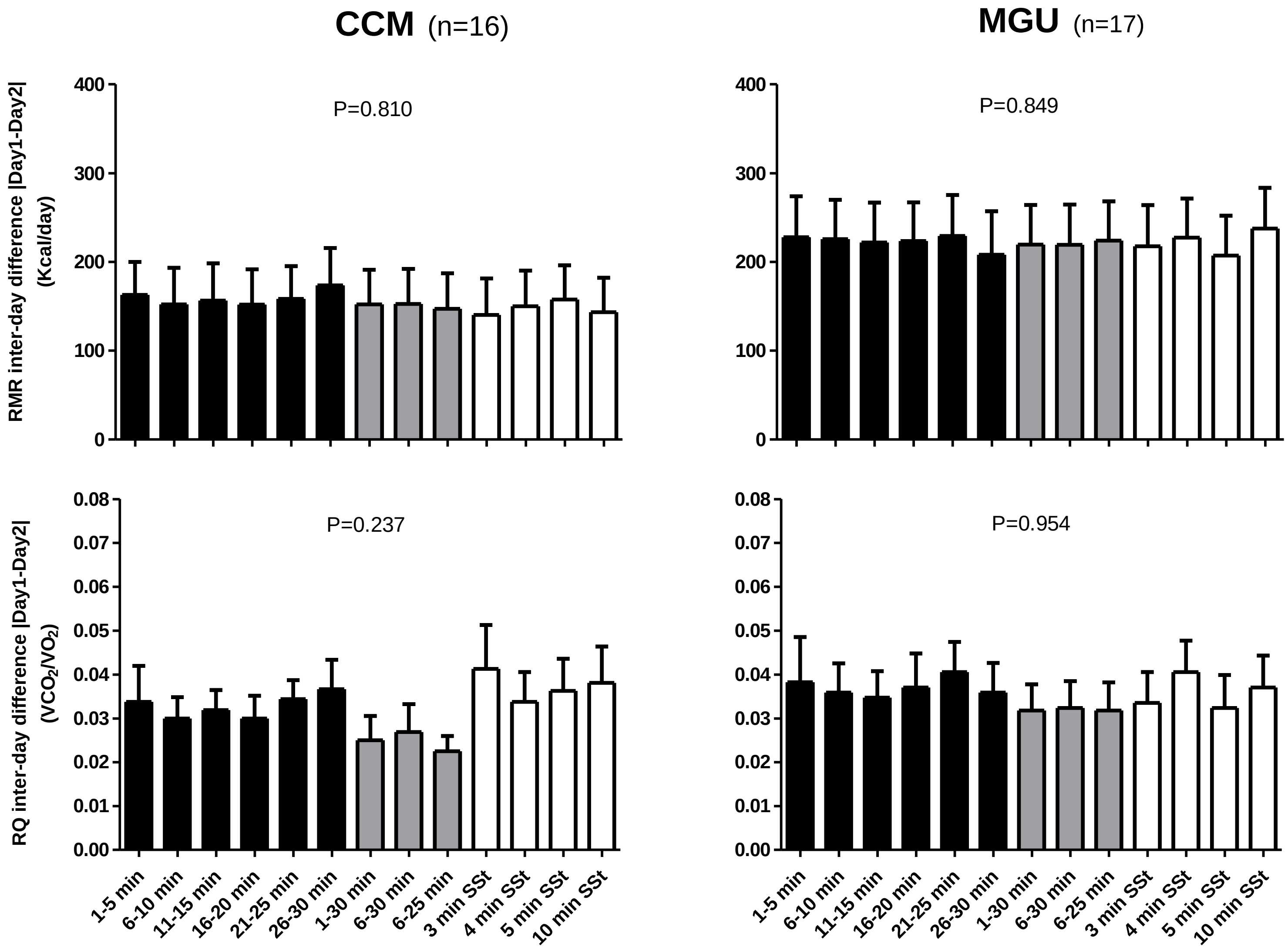

Figure 3. Inter-day reliability of resting metabolic rate (RMR) and respiratory exchange ratio (RER) across methods for data analysis (steady state and time interval) in the CCM and MGU metabolic carts. Y axis represents absolute values of the inter-day differences (e.g. |RMR Day1 – RMR Day2|). Black columns represent time intervals of 5 minutes; Grey columns represent time intervals for longer time periods; and White columns represent steady state time (SSt) periods. SSt is defined as the period (3, 4, 5, or 10 minutes) with the lowest coefficient of variance for VO2, VCO2, RER, and VE (i.e. the most stable period of *n* minutes). P from analysis of variance.

Table 1. Resting metabolic rate (RMR) and respiratory exchange ratio (RER) inter-day reliability across methods for data analysis by metabolic cart (CCM and MGU).

	CMM (n=16)			MGU (n=17)		
	Bias	(Lower limit ; Higher limit)	P	Bias	(Lower limit ; Higher limit)	P
RMR (Kcal/day)						
1-5 min	51	(-385 ; 488)	0.364	87	(-490 ; 665)	0.231
6-10 min	48	(-396 ; 493)	0.396	45	(-539 ; 629)	0.533
11-15 min	53	(-398 ; 505)	0.361	69	(-502 ; 640)	0.334
16-20 min	58	(-373 ; 488)	0.3	59	(-513 ; 631)	0.408
21-25 min	61	(-361 ; 483)	0.267	77	(-508 ; 662)	0.295
26-30 min	46	(-424 ; 517)	0.443	67	(-507 ; 641)	0.352
1-30 min	53	(-376 ; 482)	0.339	67	(-499 ; 634)	0.342
6-30 min	53	(-380 ; 486)	0.34	63	(-508 ; 635)	0.374
6-25 min	55	(-371 ; 481)	0.318	62	(-512 ; 637)	0.383
3 min SSt*	50	(-375 ; 475)	0.359	77	(-489 ; 644)	0.277
4 min SSt*	48	(-388 ; 484)	0.39	83	(-485 ; 651)	0.244
5 min SSt*	53	(-382 ; 487)	0.347	75	(-468 ; 619)	0.27
10 min SSt*	55	(-358 ; 468)	0.301	69	(-534 ; 672)	0.361
RER						
1-5 min	0.01	(-0.09 ; 0.10)	0.549	0	(-0.11 ; 0.12)	0.786
6-10 min	0	(-0.07 ; 0.07)	0.934	0.01	(-0.08 ; 0.09)	0.578
11-15 min	0	(-0.07 ; 0.08)	0.821	0.01	(-0.08 ; 0.10)	0.362
16-20 min	0	(-0.07 ; 0.07)	0.989	0	(-0.10 ; 0.10)	0.88
21-25 min	0.01	(-0.06 ; 0.09)	0.226	0	(-0.10 ; 0.10)	0.946
26-30 min	0.02	(-0.21 ; 0.25)	0.478	0	(-0.09 ; 0.09)	0.828
1-30 min	0.01	(-0.06 ; 0.07)	0.417	0	(-0.08 ; 0.08)	0.746
6-30 min	0.01	(-0.07 ; 0.08)	0.469	0	(-0.08 ; 0.09)	0.761
6-25 min	0	(-0.05 ; 0.06)	0.627	0	(-0.08 ; 0.09)	0.748
3 min SSt*	0.01	(-0.11 ; 0.12)	0.658	0.01	(-0.09 ; 0.10)	0.641
4 min SSt*	0.02	(-0.07 ; 0.1)	0.134	0	(-0.10 ; 0.10)	0.945
5 min SSt*	0.01	(-0.07 ; 0.1)	0.206	0	(-0.10 ; 0.09)	0.693
10 min SSt*	0.01	(-0.05 ; 0.06)	0.451	0.01	(-0.07 ; 0.09)	0.459

Data are mean bias (Day2 - Day1) and the 95% limits of agreement (mean difference ±1.96 standard deviation of the difference). P from paired T-test for Day1 vs. Day2. *Steady state time (SSt) period is defined as the period (3, 4, 5, or 10 minutes) with the lowest coefficient of variance for VO2, VCO2, RER, and VE (i.e. the most stable period of *n* minutes).

Inter-day reliability across methods for data analysis in participants achieving SS vs. participants not achieving SS

Table 2 shows the comparisons between participants who achieved SS and those who did not on inter-day differences in RMR and RER in each method for data analysis and for both CCM and MGU metabolic carts. All participants, except one, achieved the SS criteria when following the *3*, *4*, and *5 min SSt* method for data analysis. There were no significant mean differences between participants who achieved the SS criteria and those who did not, except in RMR-MGU following the *6-10 min* method (98±108 vs. 296±180 Kcal/day, respectively, P=0.027). Results were similar when the SS criteria were based on just VO_2 and VCO_2 CV (data not shown).

Table 2. Resting metabolic rate (RMR) and respiratory exchange ratio (RER) inter-day reliability across methods for data analysis between participants who achieved steady state (Steady State) and participants who did not (non-Steady state), and by metabolic cart (CCM and MGU).

	CMM (n=16)					MGU (n=17)				
	n	*Steady state	n	non-Steady state	P	n	*Steady state	n	non-Steady state	P
RMR (Kca/day)										
1-5 min	3	270 (258)	13	138 (114)	0.473	3	147 (153)	14	245 (198)	0.44
6-10 min	2	374 (347)	14	121 (115)	0.488	6	98 (108)	11	296 (180)	**0.027**
11-15 min	5	80 (54)	11	191 (190)	0.226	10	186 (191)	7	273 (178)	0.361
16-20 min	4	203 (297)	12	135 (95)	0.68	7	214 (210)	10	230 (168)	0.863
21-25 min	5	208 (244)	11	136 (84)	0.552	6	247 (266)	11	210 (159)	0.824
26-30 min	8	219 (213)	8	131 (61)	0.293	6	196 (263)	11	215 (176)	0.858
1-30 min	5	207 (241)	11	128 (105)	0.366	8	170 (215)	9	264 (151)	0.306
6-30 min	6	190 (226)	10	131 (108)	0.483	9	196 (224)	8	245 (144)	0.607
6-25 min	6	182 (229)	10	126 (110)	0.514	9	203 (221)	8	247 (138)	0.633
3 min SSt*	16	140 (164)	0		NC	16	226 (193)	1	80	NC
4 min SSt*	16	150 (161)	0		NC	16	233 (187)	1	144	NC
5 min SSt*	15	163 (158)	1	83	NC	16	213 (190)	1	120	NC
10 min SSt*	11	157 (179)	5	115 (91)	0.634	14	254 (206)	3	159 (29)	0.129
RER										
1-5 min	3	0 (0)	13	0.04 (0.03)	0.077	3	0.02 (0.02)	14	0.04 (0.04)	0.458
6-10 min	2	0.02 (0.02)	14	0.03 (0.02)	0.396	6	0.04 (0.02)	11	0.04 (0.03)	0.958
11-15 min	5	0.02 (0.02)	11	0.03 (0.02)	0.334	10	0.03 (0.03)	7	0.04 (0.02)	0.441
16-20 min	4	0.02 (0.02)	12	0.03 (0.02)	0.283	7	0.03 (0.03)	10	0.04 (0.04)	0.284
21-25 min	5	0.03 (0.02)	11	0.04 (0.02)	0.821	6	0.03 (0.02)	11	0.04 (0.03)	0.068
26-30 min	8	0.05 (0.02)	8	0.07 (0.14)	0.61	6	0.03 (0.02)	11	0.04 (0.03)	0.544
1-30 min	5	0.01 (0.02)	11	0.03 (0.02)	0.197	8	0.03 (0.02)	9	0.04 (0.02)	0.348
6-30 min	6	0.02 (0.02)	10	0.03 (0.03)	0.414	9	0.03 (0.02)	8	0.04 (0.03)	0.39
6-25 min	6	0.02 (0.02)	10	0.02 (0.01)	0.734	9	0.03 (0.02)	8	0.04 (0.03)	0.417
3 min SSt*	16	0.04 (0.04)	0		NC	16	0.03 (0.03)	1	0.03	NC
4 min SSt*	16	0.03 (0.03)	0		NC	16	0.04 (0.03)	1	0.07	NC
5 min SSt*	15	0.03 (0.03)	1	0.07	NC	16	0.03 (0.03)	1	0.02	NC
10 min SSt*	11	0.03 (0.01)	5	0.02 (0.02)	0.522	14	0.04 (0.03)	3	0.05 (0.02)	0.799

Data are presented as absolute mean differences between day 1 and day 2 (e.g. |RMR Day1 – RMR Day2|) and (standard deviation). P from paired T-Test comparing participants who achieved steady state (SS) and participants who did not. "NC": Not computable. *Steady state (SS) is defined as the time period where VO2, VCO2, VE vary by <10% and RER varies by <5%. When these criteria are not met, measurement is defined as non-SS. **Steady state time (SSt) period is defined as the period (3, 4, 5, or 10 minutes) with the lowest coefficient of variance for VO2, VCO2, RER and VE (i.e. the most stable period of *n* minutes).

DISCUSSION

The main findings of this study suggest that: i) RMR and RER measurements are lower when following SSt methods than when following TI methods in young healthy adults using the CCM or MGU metabolic carts. Although no significant differences were found between different lengths of SSt, *5 min SSt* seems to present the lowest RMR and RER values; ii) there are no differences on the inter-day reliability across methods for data analysis (TI and SSt), and there is no systematic bias when comparing RMR and RER day 1 and day 2 measurements; iii) inter-day reliability seems to be comparable between participants who achieved the SS and participants who did not. Of note is that the results were consistent independently of the metabolic cart used. Taken together, these findings suggest that *5 min SSt* should be the method of choice, and that not achieving SS should not be an inclusion criterion in an IC study with young adults using either the CCM or MGU metabolic cart.

We observed that *5 min SSt* was the method which obtained the lowest RMR value in both metabolic carts, and the lowest RER in one of the metabolic carts (CCM). These results concur with those reported by Irving et al. [10]. They reported that RMR values obtained by *5 min SSt* method was lower than values obtained with TI methods of several lengths. However, there are some differences between our study and the one by Irving et al. [10]: (i) they excluded participants who did not achieve the SS, (ii) they did not include other SSt periods in their analysis, and (iii) they did not compare RER values between methods for data analysis. Nevertheless, as RMR is considered to be the lowest energy expenditure on an awake person, our results also suggest that *5 min SSt* may provide the most valid RMR measurement. Nonetheless, it should be noted that when comparing mean RMR measurements between different SSt methods, maximum differences were of 20 Kcal/day, which may not be of clinical relevance, and no statistical differences were found. Reeves et al. [11] also showed similar RMR differences when comparing *3 min SSt*, *4 min SSt*, and *5 min SSt*, which also concur with our results.

Interestingly, Reeves et al. [11] showed that only 54% of the participants were able to achieve SS on *5 min SSt*, while Irving et al. [10] reported that 84% of participants achieved SS on *5 min SSt*. Horner et al. [16] observed that 93% of participants achieved SS on *5 min SSt* (considering a CV<10% for VO_2, RER, and VE) and that 47% of participants achieved the SS on *10 SSt* in 30 min of measuring. These results are in agreement with our study. We observed that 93.7% and 94.1% (CCM and MGU, respectively) of participants achieved the SS on *5 min SSt* on both testing days, and only 68.7% and 82.3% (CCM and MGU, respectively) achieved the SS on *10 min SSt* on both testing days. Surprisingly, we found a higher percentage of participants that achieved SS than most of previous studies, except for Horner et al. [16]. It is to note that Reeves et al. [11] included patients with cancer, and that the participants in Irving et al. [10] study were considerably older than those taking part in our study. Thus, it is plausible that both health status [9], sex [11], and age influence the ability to achieve SS. Further studies are needed to confirm this hypothesis.

A high RMR reliability is important in order to be able to detect changes resulting from an intervention or for between-individual comparisons on a cross-sectional study [12, 13]. Haugen et al. [21] reported 79±11 Kcal/day absolute inter-day RMR differences when measuring healthy individuals with a canopy system (model 2900 Metabolic Cart; SensorMedics). Cooper et al. [19] showed a 10.9% mean inter-day variance of RMR measured with an older MGU model. In our study, the inter-day variability following the *5 min SSt* method was 158±154 Kcal/day (13.5±15.3%) for the CCM, and 219±185 Kcal/day (18.3±17.2%) for the MGU (Figure 3). Of note is that the reliability was similar when using different methods for data analysis (TI and SSt). Future studies are needed to confirm if these results also apply to other metabolic carts such as those used by Haugen et al. [21] or Cooper et al. [19].

Although factors influencing RMR inter-day reliability have been explored in several studies [19, 21], not all of them have also studied RER inter-day reliability [21]. RER equally depends on VCO_2 and VO_2, whereas RMR depends mainly on VO_2. Consequently, using different methods for data analysis could have a different impact on RMR and RER inter-day reliability. In a study comparing six metabolic carts, Cooper et al. [19] showed that RER inter-day reliability was considerably better than RMR inter-day reliability. Indeed, there were no differences between the RER inter-day reliability obtained with the gold-standard metabolic cart (Deltatrac metabolic monitor), whereas important differences between metabolic carts were found for RMR inter-day reliability [19]. Taken together, these findings [19] suggest than RER has a better inter-day reliability than RMR. In line with this, we found that RER inter-day reliability was not influenced by the selected method for data analysis. Nonetheless, it should be noted that limits of agreement of inter-day RER differences (Table 1) might not be considered clinically acceptable. It is to note that we did not control the composition of previous meals, which could affect RER inter-day reliability. In addition, RER inter-day reliability has been shown to be slightly worse in IC performed with a MGU metabolic cart (an older model than the one used in our study) than with several other metabolic carts [19], which could also explain why we found these high inter-day RER differences.

Achieving SS is generally considered necessary to obtain a valid measure of RMR and RER [9-11, 15]. However, we found that participants who achieved SS did not consistently present higher inter-day RMR or RER reliability than participants who did not achieve SS in any of the methods used for data analysis. Although an unequal distribution of participants in both groups (SS vs. non-SS) hampers deeper analysis, our results suggest that achieving SS does not improve inter-day reliability. These findings concur with those by Horner et al. [16]. They showed no higher repeatability on participants achieving SS than in those not achieving SS when analyzing data following the TI methods. If confirmed, these results should be considered in future IC studies, as it might be that excluding participants who do not achieve SS, and consequently loosing statistical power, has no advantage in terms of inter-day reliability.

The results of this study should be considered with caution as there are some limitations. Participants were healthy young adults, and we do not know if these findings can be extended to older or unhealthy people. We strictly controlled the fasting time (8 hours) prior to IC measurements, which is considered a mandatory condition to measure RER [22]. However, the composition of previous meals was not standardized, which affect the RER measurements [23]. Whereas our results are similar when using the CCM and MGU metabolic carts, we do not know if our findings apply to other metabolic carts, or even to other gases collection systems such as canopy which may affect the RMR estimation (e.g. canopy) [24, 25]. Due to the relatively low sample size, we were not able to analyze the data in men and women separately. Further studies with larger sample sizes are needed to confirm the impact of achieving SS on inter-day RMR and RER reliability.

In summary, our findings suggest that inter-day RMR and RER reliability is not influenced by the use of different methods for data analysis (TI and SSt) and that it is not better in participants who achieved SS. This finding implies that participants who do not achieve SS should not be excluded from data analysis. Moreover, our data confirm the use of the *5 min SSt* as the optimal method for analyzing RMR and RER from IC. The *5 min SSt* presented the lowest RMR value, and the proportion of participants able to achieve SS following this method was higher than with other methods for data analysis.

These findings are further reinforced by the fact that the results are similar when using two different metabolic carts.

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AUTHOR´S CONTRIBUTION

GSD, JMA, IL, and JRR conceived the study; GSD, JMA, BMT, and JRR designed the study; JMA, LOA, and HX did the data collection; GSD performed the statistical analyses and drafted the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST SOURCES

The authors confirm that there are no conflicts of interest.

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