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

3 Guillermo Sanchez-Delgado ^a, Juan M.A. Alcantara ^a, Lourdes Ortiz-Alvarez ^a, Huiwen
4 Xu^a, Borja Martinez-Tellez ^{a,b}, Idoia Labayen ^c, Jonatan R. Ruiz ^a

5

6 ^a PROFITH “PROmoting FITness and Health through physical activity” research group.

7 Department of Physical Education and Sport, Faculty of Sport Sciences, University of
8 Granada, Spain. Ctra de Alfacar s/n C.P. 18071

9 ^b Department of Medicine, Division of Endocrinology, and Einthoven Laboratory for

10 Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The

11 Netherlands. Albinusdreef 2, 2333

12 ^c Department of Health Sciences, Public University of Navarra, Pamplona, Avda.

13 Barañáin s/n. 31008.

14

15 **Corresponding author:**

16 Guillermo Sanchez-Delgado. Department of Physical Education and Sport, Faculty of
17 Sport Sciences. University of Granada, Granada, Spain. Ctra de Alfacar s/n C.P. 18071.

18 E-mail: gsanchezdelgado@ugr.es; tel.: 0034 958242754; Fax: 0034 958244369

19 ABSTRACT

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

28 **Methods:** Seventeen young healthy adults completed two 30-minute indirect
29 calorimetry (IC) measures on two consecutive mornings, using two metabolic carts each
30 day. Two methods for data analysis were used: i) Selection of a predefined time interval
31 (TI) every 5 minutes (*1-5 min, 6-10 min, 11-15 min, 16-20 min, 21-25 min, 26-30 min*);
32 and TI representing the whole measurement period (*0-30 min, 5-30 min, 5-25 min*); and
33 ii) Methods based on the selection of the most stable period (SSt methods) (*3 min SSt, 4*
34 *min SSt, 5 min SSt, 10 min SSt*). Additionally, participants were classified as those
35 achieving SS (CV<10% for VO₂, VCO₂ and VE, and CV<5% for RER) and those who
36 did not.

37 **Results:** RMR and RER measurements were lower when following SSt methods than
38 when following TI methods (all P<0.01). Although no significant differences were
39 found between different lengths of SSt, *5 min SSt* presented the lowest RMR. There
40 were no differences on the inter-day reliability across methods for data analysis (TI and
41 SSt) (all P>0.2), and there was no systematic bias when comparing RMR and RER day
42 1 and day 2 measurements (all P>0.1). Inter-day reliability was similar in individuals

43 who achieved the SS and individuals who did not achieve it. The results were consistent
44 independently of the metabolic cart used.

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

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

50 **ABBREVIATIONS**

51 RMR: Resting metabolic Rate.

52 IC: Indirect calorimetry.

53 VO_2 : Oxygen consumption.

54 VCO_2 : Carbon dioxide production.

55 RER: Respiratory exchange ratio.

56 VE : Minute ventilation.

57 CV: Coefficient of variance.

58 SS: Steady state.

59 SSt: Steady state time.

60 TI: Time interval.

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

62 MGU: Ultima Cardio2 (Medgraphics Corp, Minnesota, USA).

63 ANOVA: Analyses of variance.

64 INTRODUCTION

65 Measuring human resting metabolic rate (RMR) is of key relevance in research and in the
66 clinical setting [1-3]. Among the available methods to measure RMR, indirect calorimetry
67 (IC) through a metabolic cart is the most commonly used in healthy, non-critically ill and
68 ventilated individuals. In IC, energy expenditure is calculated from measured oxygen
69 consumption (VO_2) and carbon dioxide production (VCO_2) by using estimating equations
70 [4, 5]. Additionally, nutrient oxidation rates (i.e. carbohydrate and fat oxidation) can be
71 estimated from IC measurements [6]. Guidelines on how to perform IC evaluations were
72 published more than a decade ago [7] and were recently updated [8]; yet, there are still
73 some issues that need to be clarified [8].

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

84 Methods for data analysis can be grouped in those based on a pre-defined time interval
85 (TI) selection and those based on steady state time (SS_t) approach [10]. Of note is that
86 there is no consensus about time length of data selection in both TI or SS_t methods [8,
87 10, 11], neither about the selection of which gas exchange variables and which pre-
88 defined CV is better for determining SS [8]. High inter-day reliability is a key factor to

89 analyze the magnitude of change in RMR after an intervention [12, 13]. Moreover,
90 although RMR estimation is mainly dependent on VO_2 [4], the ratio between VO_2 and
91 VCO_2 (i.e. RER) is crucial for estimating nutrient oxidation rates [5, 14]. Consequently,
92 achieving a high RER reliability is also key for a method to be able to accurately estimate
93 fuel oxidation. However, to our knowledge there are no studies examining the impact of
94 different methods for data analysis (i.e. TI and SSt) on inter-day RMR and RER
95 reliability.

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

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

110 MATERIAL AND METHODS

111 Participants

112 A total of 20 (n=13 women) Caucasian young healthy adults aged 18-26 years
113 participated in the study. A total of 3 out of 20 participants did not meet the previous
114 conditions for IC measurements on one of the testing days (2 participants performed
115 physical activity in the 24 hours prior to the measurement, and the other one did not met
116 the minimum fasting time requirement). Consequently, they were retrospectively
117 excluded from further statistical analyses. They were non-physically active (<20 minutes
118 <3 days/week), had a stable body weight (body weight changes <3 kg) over the last 3
119 months, were not enrolled in a weight loss program, were non-smokers, did not take any
120 medication, had no acute or chronic illness, and were not pregnant. The study protocol
121 and informed consent were performed in accordance with the Declaration of Helsinki
122 (revision of 2013), and was approved by the Human Research Ethics Committee of both
123 University of Granada (n°924) and Servicio Andaluz de Salud (Centro de Granada, CEI-
124 Granada). Written informed consent was obtained from all the participants before their
125 enrollment.

126 Procedures

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

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

143 IC measurements followed current guidelines [8]. In brief, all measurements were
144 conducted in the same quiet room with dim lighting, with controlled ambient temperature
145 (22-24°C) and humidity (35-45%), and by the same trained staff. Before being evaluated,
146 all participants confirmed that they met previous study conditions and lied on a reclined
147 bed in a supine position and covered by a sheet for the 20 minutes prior to the IC
148 measurement. They were instructed to breathe normally, and not to talk, fidget, or sleep.
149 The same position and instructions were maintained during the two 30-minute
150 measurement periods. Flow calibration was performed by using a 3-L calibration syringe
151 at the beginning of every testing day, and gas analyzers were calibrated using 2 standard
152 gas concentrations following the manufacturer's instruction before every IC
153 measurement.

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

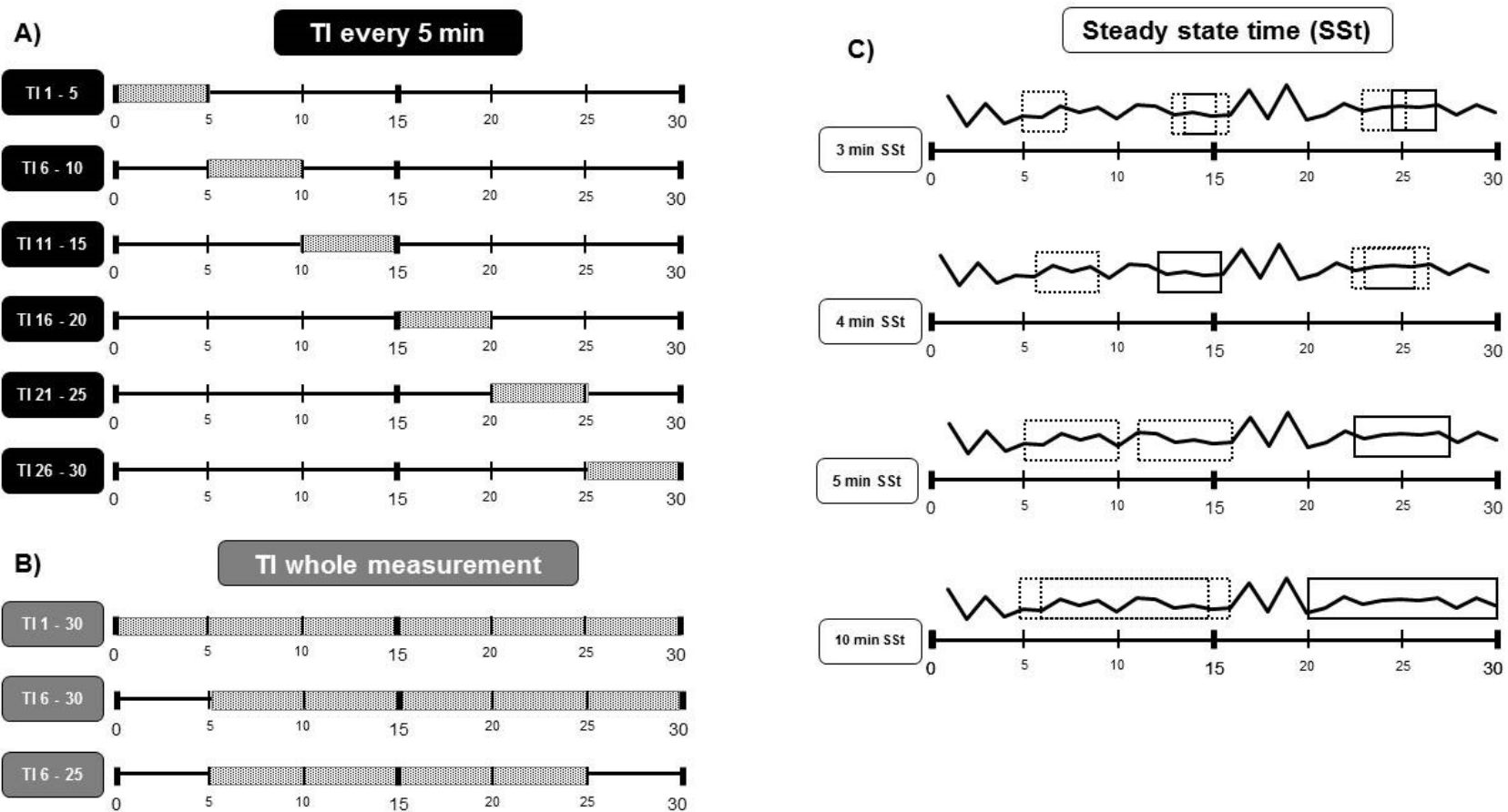
157 **Methods for data analysis and steady state criteria**

158 We used two types of methods for data analysis based on TI and SSt periods. (Figure 1):

159 (i) TI every 5 minutes, and TI representing the whole measurement period; and (ii) SSt

160 methods. TI every 5 minutes: mean values of every consecutive 5-minute period (i.e. from
 161 the 1st to the 5th minute, from the 6th to the 10th, etc.), hereinafter referred as *1-5 min*, *6-*
 162 *10 min*, *11-15 min*, *16-20 min*, *21-25 min*, and *26-30 min* (Figure 1A). TI representing the
 163 whole measurement period: mean values for the whole measurement period (i.e. *1-30*
 164 *min*), and mean values for the whole measurement period except for the first 5 minutes
 165 (i.e. *6-30 min*) [8] or the first and the last 5 minutes (i.e. *6-25 min*) [19] (Figure 1B). For
 166 the SSt methods, we calculated the CV of VO₂, VCO₂, VE, and RER for every period of
 167 3, 4, 5, and 10 minutes [7, 11], excluding the first 5 minutes of data collection (i.e. for 3
 168 *min SSt*, CVs were calculated from 6th to 8th minute, from 7th to 9th, etc.) (Figure 1C).
 169 Thereafter, we selected the periods of 3, 4, 5, or 10 minutes that met most of the following
 170 criteria: i) CV<10% for VO₂, ii) CV<10% for VCO₂, iii) CV<10% for VE, and iv) CV<5%
 171 for RER. Finally, among the periods that met most of those criteria we selected the 3, 4,
 172 5, and 10-minute periods with the lowest average between CVs of VO₂, VCO₂, VE, and
 173 RER, for being used as *3 min SSt*, *4 min SSt*, *5 min SSt* and *10 min SSt*, respectively
 174 (Figure 1C). Finally, mean VO₂ and VCO₂ obtained by each method for data analysis
 175 were entered into Weir's abbreviated equation [4] (see below) to estimate energy
 176 expenditure, and RER was calculated as VCO₂/VO₂:

$$177 \quad RMR \text{ (Kcal/min)} = 3.941 \times VO_2 \text{ (l/min)} + 1.106 \times VCO_2 \text{ (l/min)}$$



178

179 **Figure 1.** Methods for data analysis. A=Time interval (TI) methods every 5 minutes; B= TI for the whole measurement. Pointed blocks represent
 180 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
 181 (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,
 182 and $CV < 5\%$ for RER, among the 30-minute record, after having discarded the first 5 minutes recorded. The solid lined blocks represent the
 183 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.
 184 Dashed lined blocks represent periods with the same number of CVs criteria achieved as the selected period but with a higher average between
 185 CVs of VO_2 , VCO_2 , VE, and RER.

186 To compare inter-day RMR and RER reliability across methods for data analysis in
187 participants who achieved SS vs. participants who did not achieve SS, we classified
188 participants as those achieving CV<10% for VO₂, VCO₂ and VE and CV<5% for RER
189 (SS criteria) and those who failed to comply the SS criteria on any of the two testing days.
190 This classification was performed for every method for data analysis. Therefore, a total
191 of 13 methods for data analysis were tested, and were further grouped in those achieving
192 SS vs. not achieving SS.

193 The selected CV cut-off points are probably the most used ones in literature [8]. In
194 addition, a CV cut-off point of 10% for VO₂ and VCO₂ has been proved to accurately
195 predict total energy expenditure in ventilated patients [9]. However, there is no consensus
196 on how to define SS, neither on CV cut-off points, nor in the combination of gas exchange
197 variables. Therefore, we selected the most used CV cut-off points [8], and we decided to
198 classify participants taking into account the four gas exchange variables. This is the most
199 strict combination criteria, which would allow to test whether achieving SS would result
200 in better inter-day reliability. Nevertheless, we performed additional analyses classifying
201 participants just based on VO₂ and VCO₂ CV criteria.

202 **Statistical analysis**

203 Gas exchange parameters including VO₂, VCO₂, VE, RMR, and RER were averaged each
204 minute with the Breeze Suite (8.1.0.54 SP7, MGCDiagnostic®) software and downloaded
205 to an Excel spreadsheet where the CVs and outputs of the different methods for data
206 analysis were calculated. Results are presented as means ± standard deviation, unless
207 otherwise stated. The analyses were conducted using the Statistical Package for Social
208 Sciences (SPSS, v. 21.0, IBM SPSS Statistics, IBM Corporation), and the level of
209 significance was set to <0.05.

210 *Impact of methods for data analysis on RMR and RER measurements*

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

215 *Impact of methods for data analysis on inter-day reliability*

216 We compared the absolute value of inter-day differences in RMR and RER values (e.g.
217 $|\text{RMR Day1} - \text{RMR Day2}|$) with every method for data analysis using repeated-measures
218 ANOVA for both CCM and MGU metabolic carts. Inter-day RMR and RER reliability
219 for every method of data analysis was also assessed using the Bland-Altman method [20].
220 Day 1 measurements were subtracted from day 2 measurements, so a positive difference
221 indicates that day 2 measurements were higher than day 1. Bias was measured by using a
222 2-sided *t*-test to determine if there was a significant difference between RMR and RER
223 measures on day 2 vs. day 1.

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

226 The absolute value of inter-day differences in RMR and RER (e.g. $|\text{RMR Day1} - \text{RMR}$
227 $\text{Day2}|$) in each method for data analysis and for both CCM and MGU metabolic carts
228 were compared between those participants who achieved the SS criteria and those who
229 did not, using independent sample *t*-tests.

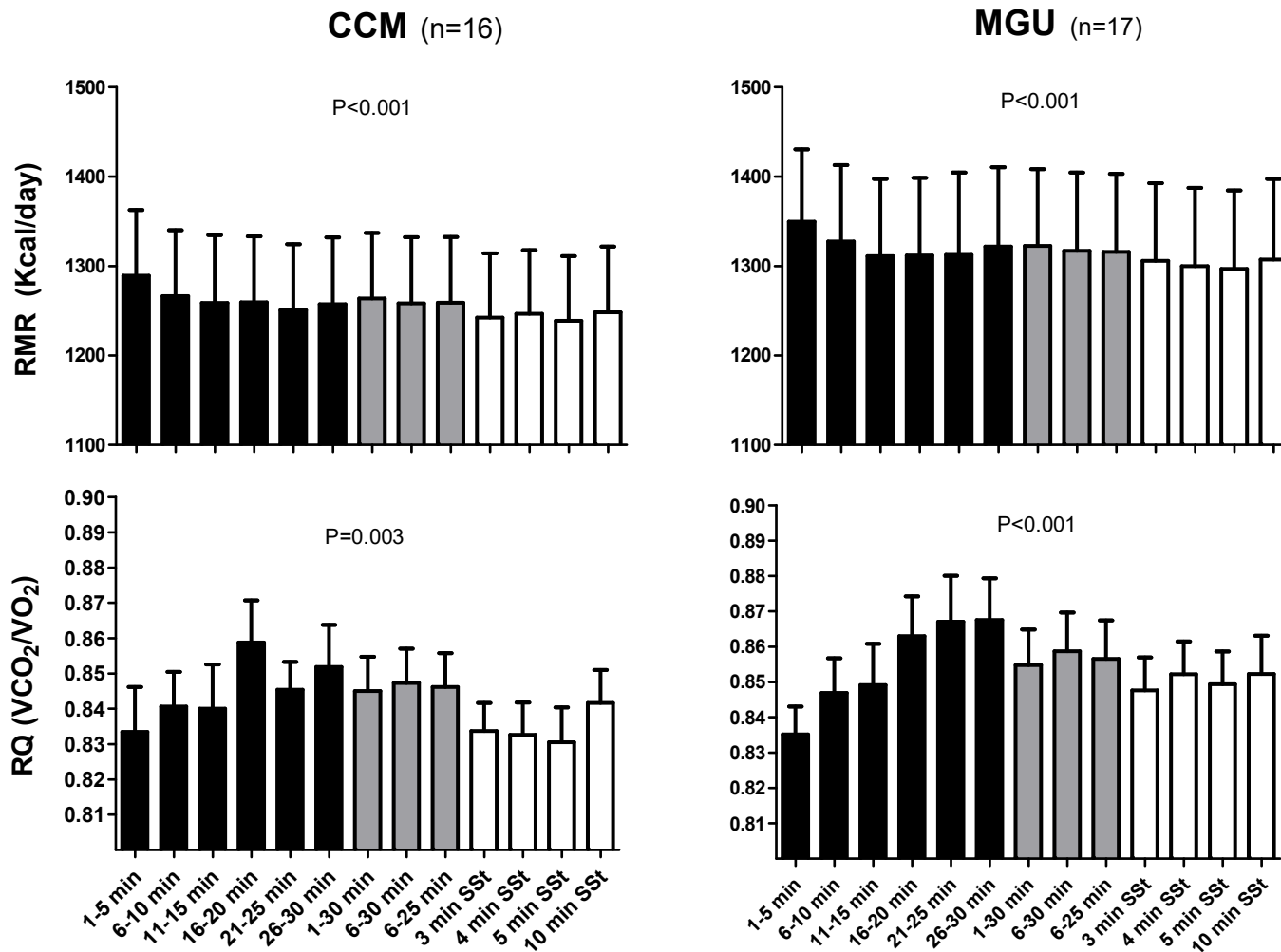
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231 RESULTS

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

236 *Impact of methods for data analysis on RMR and RER measurements*

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



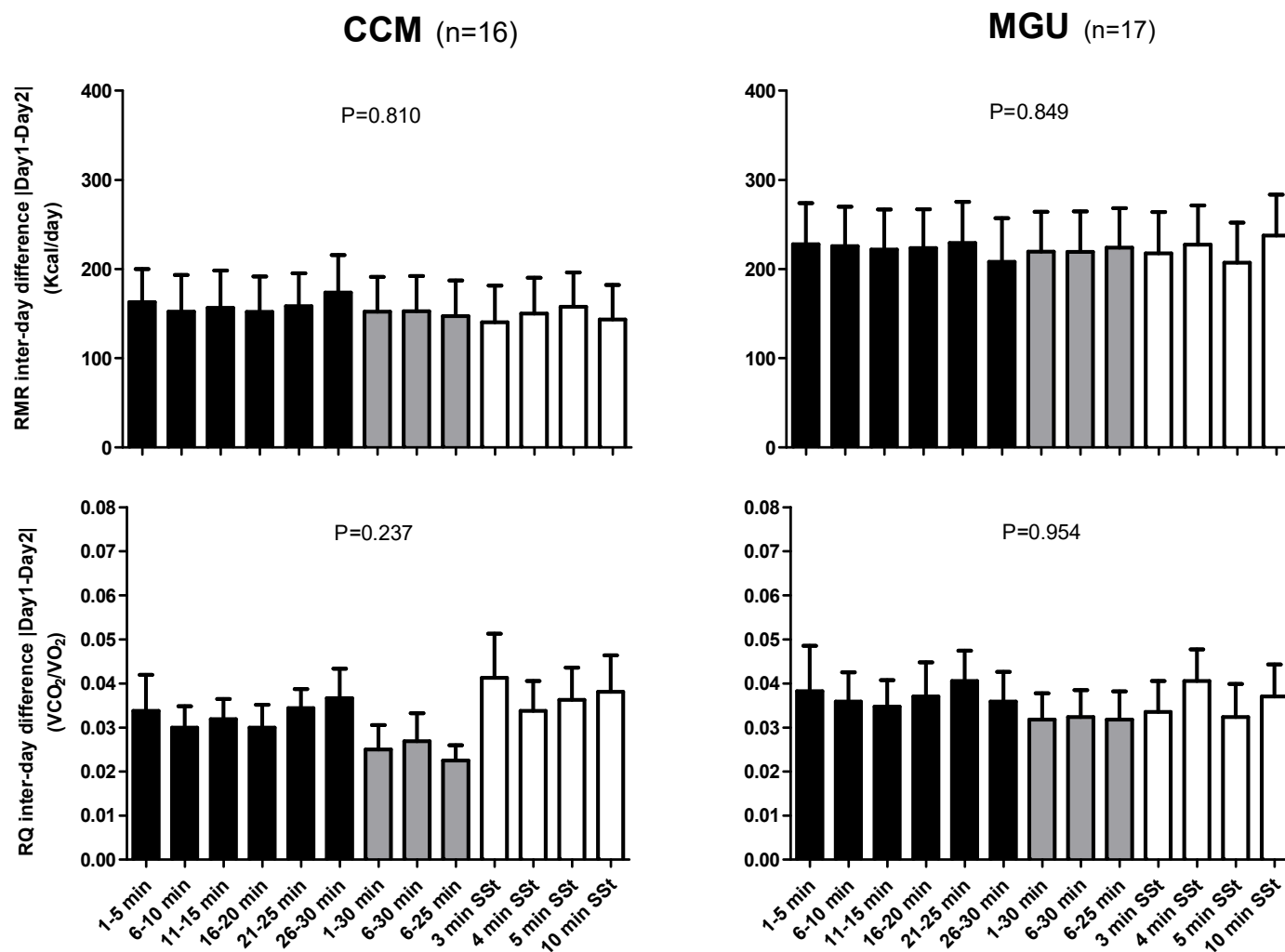
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249 **Figure 2.** Day 1 resting metabolic rate (RMR) and respiratory exchange ratio (RER) measurements across methods for data analysis (steady state
 250 time and time interval) in the CCM and MGU metabolic carts. Black columns represent time intervals of 5 minutes; Grey columns represent time
 251 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)
 252 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.

253 *Impact of methods for data analysis on inter-day reliability*

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

258 Table 1 shows inter-day mean bias (Day2 - Day1) and the 95% limit of agreement (mean
259 difference $\pm$ 1.96 standard deviation of the difference) for every method for data analysis
260 with the CCM and MGU metabolic carts. Paired *t*-test showed no significant RMR or
261 RER inter-day mean differences in any of the methods for data analysis (all $P>0.1$). The
262 limits of agreement were quite similar across methods, and no method presented the
263 narrowest limits of agreement for all analysis. We observed that *10 min SSt*, *5 min SSt*, *6-*
264 *25 min*, and *10 min SSt* presented the narrowest limits of agreement for RMR-CCM,
265 RMR-MGU, RER -CCM, and RER -MGU, respectively.



266

267 **Figure 3.** Inter-day reliability of resting metabolic rate (RMR) and respiratory exchange ratio (RER) across methods for data analysis (steady
 268 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 –
 269 RMR Day2|). Black columns represent time intervals of 5 minutes; Grey columns represent time intervals for longer time periods; and White
 270 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
 271 VO₂, VCO₂, RER, and VE (i.e. the most stable period of *n* minutes). P from analysis of variance.

272 **Table 1.** Resting metabolic rate (RMR) and respiratory exchange ratio (RER) inter-day reliability across methods for data analysis by metabolic cart (CCM
273 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

274 Data are mean bias (Day2 - Day1) and the 95% limits of agreement (mean difference $\pm$ 1.96 standard deviation of the difference). P from paired T-test for
275 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,
276 and VE (i.e. the most stable period of *n* minutes).

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

279 Table 2 shows the comparisons between participants who achieved SS and those who did
280 not on inter-day differences in RMR and RER in each method for data analysis and for
281 both CCM and MGU metabolic carts. All participants, except one, achieved the SS
282 criteria when following the 3, 4, and 5 min *SS* method for data analysis. There were no
283 significant mean differences between participants who achieved the SS criteria and those
284 who did not, except in RMR-MGU following the 6-10 min method (98 ± 108 vs. 296 ± 180
285 Kcal/day, respectively, $P=0.027$). Results were similar when the SS criteria were based
286 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).

294 **DISCUSSION**

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

307 We observed that *5 min SSt* was the method which obtained the lowest RMR value in
308 both metabolic carts, and the lowest RER in one of the metabolic carts (CCM). These
309 results concur with those reported by Irving et al. [10]. They reported that RMR values
310 obtained by *5 min SSt* method was lower than values obtained with TI methods of several
311 lengths. However, there are some differences between our study and the one by Irving et
312 al. [10]: (i) they excluded participants who did not achieve the SS, (ii) they did not include
313 other SSt periods in their analysis, and (iii) they did not compare RER values between
314 methods for data analysis. Nevertheless, as RMR is considered to be the lowest energy
315 expenditure on an awake person, our results also suggest that *5 min SSt* may provide the
316 most valid RMR measurement. Nonetheless, it should be noted that when comparing
317 mean RMR measurements between different SSt methods, maximum differences were of
318 20 Kcal/day, which may not be of clinical relevance, and no statistical differences were

319 found. Reeves et al. [11] also showed similar RMR differences when comparing 3 min
320 SS*t*, 4 min SS*t*, and 5 min SS*t*, which also concur with our results.

321 Interestingly, Reeves et al. [11] showed that only 54% of the participants were able to
322 achieve SS on 5 min SS*t*, while Irving et al. [10] reported that 84% of participants achieved
323 SS on 5 min SS*t*. Horner et al. [16] observed that 93% of participants achieved SS on 5
324 min SS*t* (considering a CV<10% for VO₂, RER, and VE) and that 47% of participants
325 achieved the SS on 10 SS*t* in 30 min of measuring. These results are in agreement with
326 our study. We observed that 93.7% and 94.1% (CCM and MGU, respectively) of
327 participants achieved the SS on 5 min SS*t* on both testing days, and only 68.7% and 82.3%
328 (CCM and MGU, respectively) achieved the SS on 10 min SS*t* on both testing days.
329 Surprisingly, we found a higher percentage of participants that achieved SS than most of
330 previous studies, except for Horner et al. [16]. It is to note that Reeves et al. [11] included
331 patients with cancer, and that the participants in Irving et al. [10] study were considerably
332 older than those taking part in our study. Thus, it is plausible that both health status [9],
333 sex [11], and age influence the ability to achieve SS. Further studies are needed to confirm
334 this hypothesis.

335 A high RMR reliability is important in order to be able to detect changes resulting from
336 an intervention or for between-individual comparisons on a cross-sectional study [12, 13].
337 Haugen et al. [21] reported 79±11 Kcal/day absolute inter-day RMR differences when
338 measuring healthy individuals with a canopy system (model 2900 Metabolic Cart;
339 SensorMedics). Cooper et al. [19] showed a 10.9% mean inter-day variance of RMR
340 measured with an older MGU model. In our study, the inter-day variability following the
341 5 min SS*t* method was 158±154 Kcal/day (13.5±15.3%) for the CCM, and 219±185
342 Kcal/day (18.3±17.2%) for the MGU (Figure 3). Of note is that the reliability was similar
343 when using different methods for data analysis (TI and SS*t*). 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 that 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

369 results suggest that achieving SS does not improve inter-day reliability. These findings
370 concur with those by Horner et al. [16]. They showed no higher repeatability on
371 participants achieving SS than in those not achieving SS when analyzing data following
372 the TI methods. If confirmed, these results should be considered in future IC studies, as
373 it might be that excluding participants who do not achieve SS, and consequently losing
374 statistical power, has no advantage in terms of inter-day reliability.

375 The results of this study should be considered with caution as there are some limitations.
376 Participants were healthy young adults, and we do not know if these findings can be
377 extended to older or unhealthy people. We strictly controlled the fasting time (8 hours)
378 prior to IC measurements, which is considered a mandatory condition to measure RER
379 [22]. However, the composition of previous meals was not standardized, which affects the
380 RER measurements [23]. Whereas our results are similar when using the CCM and MGU
381 metabolic carts, we do not know if our findings apply to other metabolic carts, or even to
382 other gases collection systems such as canopy which may affect the RMR estimation (e.g.
383 canopy) [24, 25]. Due to the relatively low sample size, we were not able to analyze the
384 data in men and women separately. Further studies with larger sample sizes are needed to
385 confirm the impact of achieving SS on inter-day RMR and RER reliability.

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

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

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406 **AUTHOR'S CONTRIBUTION**

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

410 **CONFLICT OF INTEREST SOURCES**

411 The authors confirm that there are no conflicts of interest.

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