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Methods for data analysis of resting energy expenditure measured using indirect calorimetry

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ABSTRACT

Objectives: The aim of this study was to test the accuracy of different methods of resting energy expenditure (REE) data analysis using indirect calorimetry (IC) during traditional (30 min) and abbreviated (10 min) protocols.

Methods: Fifteen women and 15 men (21–34 y of age) completed two consecutive 30-min IC measurements. Body composition was measured using dual-energy x-ray absorptiometry. The reference method for REE analysis was 5 min in steady state (SS) during 30 min (first 5 min discarded). REE measurements were randomized to define a reference or testing method. An interval method was defined using 25, 20, and 15 min (with first 5, 10, and 15 min discarded, respectively), during 30 min, and 5 min (first 5 min discarded) during 10-min intervals. The SS method was defined using 5 min in SS (first 5 min discarded) during 30 min, 5, 4, and 3 min in SS during 10-min (first 5 min discarded) intervals.

Results: Interval methods during 30 min and SS and interval methods during 10 min demonstrated large bias with significantly high REEs compared to the reference method (78.8–109.0 kcal/d, all $P < 0.001$). Testing methods demonstrated large upper limits of agreement between 225.2 and 322.8 kcal/d. No mean differences ($P > 0.05$), small bias (14.3 kcal/d), and narrow limits of agreement (−125.8 to 154.4 kcal/d) were observed between 5-min SS during 30 min and the reference method.

Conclusions: All interval methods and SS methods during 10 min overestimated REE. We recommend using 5-min SS during 30 min. The measurement may be repeated until all participants achieve SS.

Introduction

Indirect calorimetry (IC) is the most commonly used method to measure energy expenditure in the research setting when evaluating resting and physical activity energy expenditure and to support understanding of energy homeostasis (i.e., mechanisms that regulate weight loss or weight gain) [1]. Despite resting energy expenditure (REE) representing between 60% and 70% of daily energy expenditure [1], IC measurements usually last between 30 and 60 min to estimate the daily REE [2], using volume of oxygen consumption (VO_2) and volume of carbon dioxide production (VCO_2) equations [3]. Aside from an abbreviated IC measurement protocol being used to estimate the REE, reduced intervals may be selected for data analysis. Therefore, an REE can be analyzed according to steady state (SS) or interval methods [4,5].

SS is considered the standard method for REE analysis, and is defined as 5 min of coefficient of variation (CV) <10% for VO_2 and VCO_2 [2,6]. However, it is suggested that patients take a longer time or do not achieve a 5-min SS during abbreviated periods of IC measurements; therefore, a reduced SS during a 30- to 60-min interval, with a reduced error to REE estimation has been proposed [7–10]. A reduced SS, such as 4 and 3 min, greatly increased the proportion of participants who satisfied the SS [9]. Moreover, a 4-min SS has been considered clinically acceptable to estimate the REE in patients [7–9,11]. Reeves et al. [9] showed a higher variation of REE when a 3-min SS was used. Moreover, the possibility of using a 3-min SS in critically ill children [8] and in spontaneously breathing patients with traumatic brain injury [7] has been demonstrated.

The interval approach is another strategy used to avoid participants repeating IC measurements in cases of SS failure. This method of data analysis uses averaged values over a predetermined time

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interval [4]. Abbreviated IC measurements, and reduced SS and interval methods of data analysis may be alternative strategies for sports physiologists, dietitians, or researchers when there is a requirement to perform many IC measurements in a day. It has previously been shown that an REE can be obtained during a 10-min protocol with healthy participants, discarding the first 5 min and selecting data from the remaining 5 min [12,13], with a CV of VO_2 and VCO_2 of ≤10% [6,14]. Irving et al. [4] reported that 38% of participants achieved an SS at 10 min, 72% at 15 min, and 95% at 30 min. These findings show that it is possible to achieve an SS of <30 min, as well as during 10 min, but probably not in all participants of a sample. However, the use of a 5-min SS during a 10-min IC measurement may induce a substantial failure in participants aiming to achieve an SS, which raises questions as to whether using reduced methods for data analysis is merited.

Therefore, it is important to investigate the best method for data analysis during 30 and 10 min of IC measurements to estimate the REE accurately. The purpose of this study was to test the accuracy of different methods for data analysis of the REE measured using IC during a traditional protocol (30 min) and an abbreviated protocol (10 min).

Materials and methods

Participants

Fifteen woman and 15 men between 21 and 34 y of age participated in this study. Participants who had not had >10% change in their body weight within the past year, no present acute or chronic illness, who were not pregnant, and who were not taking medication known to affect metabolic rates were invited to participate in the study. Measurements were performed at the Center for Investigations in Pediatrics of the University of Campinas, at Campinas, SP, Brazil. Body composition and REE were measured twice consecutively on the same morning. Procedures were approved by the Research Ethics Committee of the University of Campinas and conformed to the declaration of Helsinki for studies involving humans. All participants provided their informed consent.

Procedures

Indirect calorimetry

A metabolic cart Vmax Encore 29 n (CareFusion Corp., San Diego, CA, USA) was used to measure the REE, and procedures were performed according to Compher

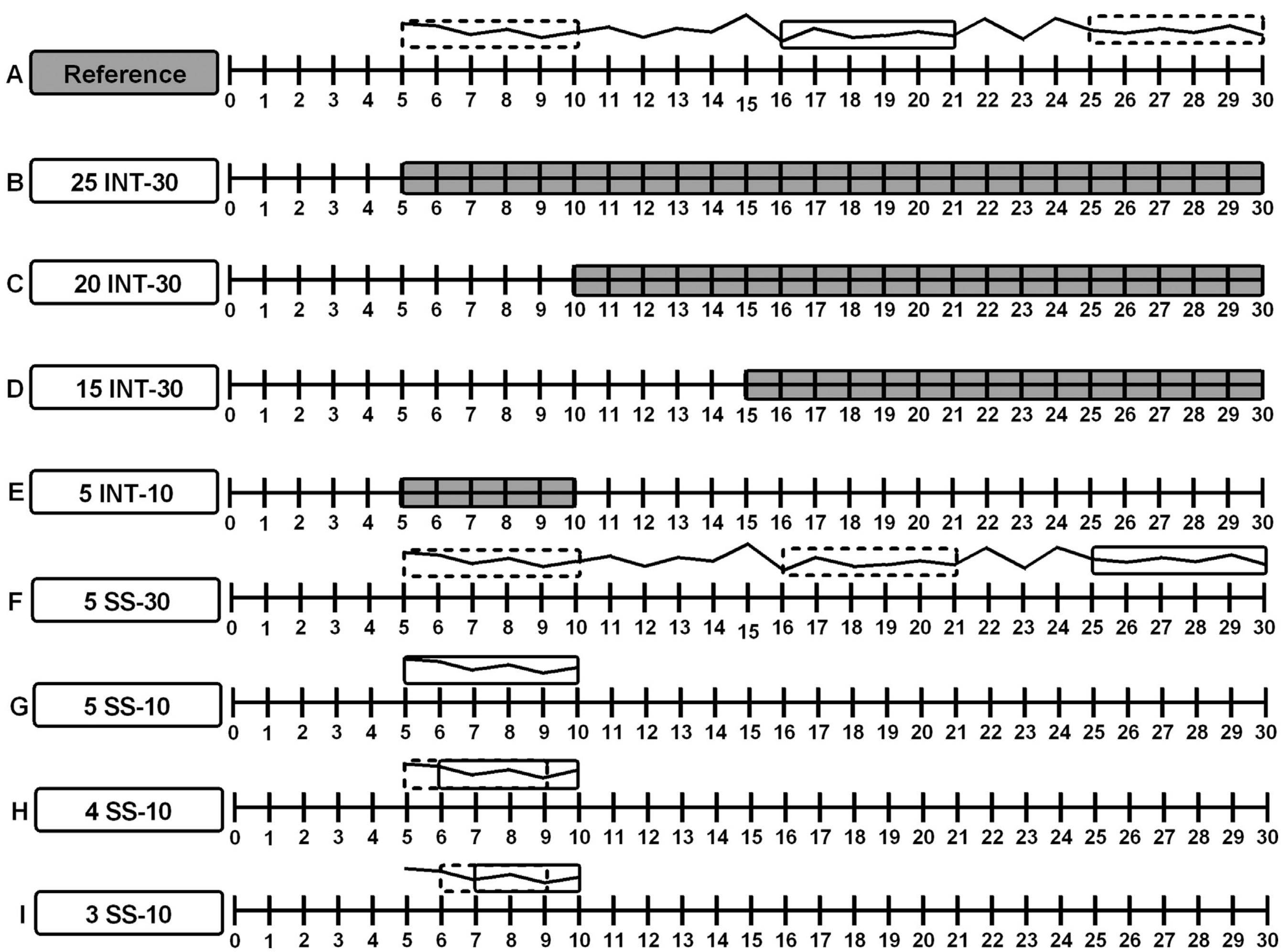

Fig. 1. Simulated models of reference and testing methods for data analysis of resting energy expenditure (REE). (**A**) The reference method used 5 min in steady state (SS) during 30 min, and the first 5 min was discarded. (**B**) The testing method used an interval of 25 min during 30 min, and the first 5 min was discarded (25 INT-30). (**C**) The testing method used an interval of 20 min during 30 min, and the first 10 min was discarded (20 INT-30). (**D**) The testing method used an interval of 15 min during 30 min, and the first 15 min was discarded (15 INT-30). (**E**) The testing method used an interval of 5 min during 10 min, and the first 5 min was discarded (5 INT-10). (**F**) The testing method used 5 min in SS during 30 min, and the first 5 min was discarded (5 SS-30). (**G**) The testing method used 5 min in SS during 10 min, and the first 5 min was discarded (5 SS-10). (**H**) The testing method used 4 min in SS during 10 min, and the first 5 min was discarded (4 SS-10). (**I**) The testing method used 3 min in SS during 10 min, and the first 5 min was discarded (3 SS-10). The solid lined blocks represent the period of SS with the lowest REE from the mean of volume of oxygen consumption and volume of carbon dioxide production. The dashed lined blocks represent SS periods with a higher REE estimation. The pointed blocks represent the interval period used to estimate the REE.

Table 1
Characteristics of the participants (mean ± SD)

	All (N = 30)	Women (n = 15)	Men (n = 15)	*P*-value*
Age (y)	28.2 ± 3.5	27.5 ± 2.8	28.9 ± 4.0	0.293
Height (cm)	171.8 ± 7.3	168.9 ± 8.5	174.7 ± 4.8	**0.033**
Weight (kg)	69.1 ± 11.9	62.9 ± 8.0	75.3 ± 12.1	**0.003**
BMI (kg/m^2)	23.4 ± 3.5	22.0 ± 2.4	24.7 ± 3.9	**0.037**
% FM	25.7 ± 8.2	31.5 ± 5.1	20.0 ± 6.4	<**0.001**
Body composition				
BMC (kg)	2.9 ± 0.5	2.6 ± 0.4	3.2 ± 0.4	<**0.001**
FM (kg)	17.7 ± 6.2	19.9 ± 4.7	15.6 ± 7.0	0.058
LST (kg)	48.5 ± 10.3	40.3 ± 5.1	56.7 ± 7.0	<**0.001**
Reference method				
REE (kcal/d)	1284.1 ± 172.9	1173.4 ± 129.3	1394.7 ± 137.6	<**0.001**
VO_2 (L/min)	0.186 ± 0.024	0.170 ± 0.018	0.201 ± 0.020	<**0.001**
VCO_2 (L/min)	0.152 ± 0.023	0.137 ± 0.019	0.166 ± 0.019	<**0.001**
RQ (VCO_2/VO_2)	0.81 ± 0.04	0.81 ± 0.05	0.82 ± 0.040	0.335

BMC, bone mineral content; BMI, body mass index; FM, fat mass; LST, lean soft tissue; REE, resting energy expenditure; VO_2, volume of oxygen consumption; VCO_2, volume of carbon dioxide production; RQ, respiratory quotient; SD, standard deviation; %FM, percentage of fat mass.
*Values in boldface indicate significant differences ($P < 0.05$).

et al. [6]. The REE was measured between 0730 and 1000, after 12 h of fasting, and participants were instructed to refrain from physical activity the day before the measurements. The temperature and humidity of the room was approximately 23°C and 54%, respectively. The participants were awake, and their heads were positioned within a ventilated canopy. A resting period of 10 min was undertaken before initiating the REE measurements. The procedures of calibration, and the REE measurements were performed twice consecutively. The flow and gas analyzers were calibrated before each test using a 3-L syringe and standard gas concentrations (16% O_2, 4% CO_2; 26% O_2, and 0% CO_2; room air, 20.94% O_2 and 0.05% CO_2), respectively. VO_2, VCO_2, and respiratory quotient (RQ) data were collected every minute. Thirty minutes of data were collected for the analysis. The CV of the REE for the repeated measurements involving the participants was 2.9% ± 2.5%.

Dual-energy x-ray absorptiometry

Whole bone mineral content (BMC), fat mass (FM), and lean soft tissue (LST) were estimated using the iDXA with software enCORE 2011 version 13.60 (GE Healthcare Lunar, Madison, WI, USA). Assessments were performed twice consecutively and the mean between both measurements was used to estimate the body composition. The CV of BMC, FM, and LST for the repeated measurements of the participants was 0.3% ± 0.3%, 0.9% ± 0.9%, and 0.4% ± 0.3%, respectively.

Methods for data analysis of REE

A randomization trial was performed for each participant to define which REE measure was to be considered as the reference or testing method (e.g., the first measure of REE was defined for testing methods and the second measure was defined as the reference method for one participant, and subsequent measures were randomly defined for each participant).

The reference method was defined as 5 min in an SS during 30 min (discarding the first 5 min). Testing method was defined as an interval approach using 25 min (discarding the first 5 min; 25 INT-30), 20 min (discarding the first 10 min; 20 INT-30), and 15 min (discarding the first 15 min; 15 INT-30) during 30 min, and 5 min (discarding the first 5 min; 5 INT-10) during 10 min; SS method using 5 min in SS (discarding the first 5 min; 5 SS-30) during 30 min, 5 min in SS (5 SS-10), 4 min in SS (4 SS-10), and 3 min in SS (3 SS-10) during 10 min (discarding the first 5 min). The reference and testing methods are detailed and described in Figure 1.

Interval methods were calculated using the mean of VO_2 and VCO_2 from a predetermined period to estimate the REE. The SS methods were achieved when the CV was ≤10% for VO_2 and VCO_2. Therefore, we used the lowest REE [15] from the mean of VO_2 and VCO_2 during the SS periods. Only VO_2 and VCO_2 values with an RQ between 0.7 and 1.0 were considered for analysis [6]. The REE (kcal/d) was calculated using the Weir equation [3]:

$$\text{REE (kcal/d)} = [3.941 \times VO_2(\text{L/min}) + 1.106 \times VCO_2(\text{L/min})] \times 1440 \quad (1)$$

Statistical analysis

Mean ± standard deviation (SD) was used for descriptive analysis, and the Shapiro–Wilk test was used to verify data normality. The unpaired Student's *t* test or the Mann–Whitney U test were used to compare differences between women and men related to body composition, REE, VO_2, VCO_2, and RQ. IC variables used values from the measurement related to the reference method. The paired Student's *t* test or the Wilcoxon test was used to compare REE differences between the reference and testing methods. Simple linear regression analysis was used to estimate the coefficient of determination (r^2). We used the Bland–Altman analysis [16] and a two-way mixed intraclass coefficient of correlation (ICC) to verify agreement [17]. In addition, bivariate Pearson's coefficient of correlation (*r*) was applied between the mean and differences of the reference and testing methods to verify trends in the REE estimation. SPSS version 16.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. The significance level was set at $\alpha \leq 0.05$.

Results

Table 1 shows the characteristics, body composition, and IC approach of the participants. Significantly higher height, weight, body mass index, BMC, LST, REE, VO_2, and VCO_2 were observed for men compared with women. However, only percentage of fat mass (%FM) demonstrated higher values for women than for men. No significant differences between women and men were observed for age, FM, and RQ.

Differences and correlations between reference and testing methods are shown in Table 2. A relatively high number of participants who achieved SS demonstrated 5 SS-30 (100%), 5 SS-10 (80%), 3 SS-10 (93.3%), and 4 SS-10 (76.7%). The 5 SS-30 was the only testing method that showed no mean difference in REE compared to the reference method. However, 25 INT-30, 20 INT-30, 15 INT-30, 5 INT-10, 5 SS-10, 4 SS-10, and 3 SS-10 showed a significantly higher REE (between 6% and 8.5%) when compared with the reference method. The 25 INT-30, 20 INT-30, 15 INT-30, 5 SS-30, and 5 SS-10 were the methods that mostly explained the REE variance of the reference method (r^2 between 81% and 88%, all $P < 0.001$), and also demonstrated an ICC between 0.77 and 0.92 (all, $P < 0.001$). However, 5 INT-10, 4 SS-10, and 3 SS-10 demonstrated lower r^2 and ICC, between 67% and 77% (all, $P < 0.001$) and 0.69 to 0.75 (all, $P < 0.001$), respectively.

A Bland–Altman analysis demonstrated small bias, narrow limits of agreement, and no significant trends for REE when 5 SS-30 and the reference method were compared (Fig. 2E). However, 25 INT-30, 20 INT-30, 15 INT-30, 5 INT-10 (Figs. 2A to D), and 5 SS-10, 4 SS-10, and 3 SS-10 (Figs. 2F to H) showed large bias (between 78.8 and 109 kcal/d) and a large upper limit of agreement (between 225.2 and 322.8 kcal/d). In addition, a significant correlation between differences and mean was observed for 25 INT-30, 20 INT-30, 15 INT-30, 5 SS-10, and 3 SS-10, and an almost significant difference for 5 INT-10 and 4 SS-10, demonstrating trends of REE overestimation for those participants with a high REE.

Figure 3 shows the descriptive analysis of CV (%) from VO_2 and VCO_2 according to the reference and testing methods. We observed mean CV values for VO_2 and VCO_2 of <10% when SS criteria were applied. Although the mean CV for VO_2 and VCO_2 was

Table 2
Differences, linear regression, and intraclass coefficient of correlation between the reference and testing methods for data analysis of resting energy expenditure (mean ± SD)

Testing	%SS	REE (kcal/d)	Δ%	Δkcal/d	*P*-value*	Model r^2	*P*-value*	ICC	*P*-value*
25 INT-30	–	1370.3 ± 201.9	6.7 ± 5.3	86.2 ± 71.9	<**0.001**	0.88	<**0.001**	0.84	<**0.001**
20 INT-30	–	1364.6 ± 204.1	6.2 ± 5.4	80.5 ± 73.8	<**0.001**	0.88	<**0.001**	0.85	<**0.001**
15 INT-30	–	1362.9 ± 209.3	6.0 ± 5.9	78.8 ± 82.8	<**0.001**	0.85	<**0.001**	0.84	<**0.001**
5 INT-10	–	1393.1 ± 207.1	8.5 ± 7.5	109.0 ± 99.2	<**0.001**	0.77	<**0.001**	0.75	<**0.001**
5 SS-30	100	1298.4 ± 184.2	1.2 ± 5.4	14.3 ± 71.5	0.282	0.85	<**0.001**	0.92	<**0.001**
5 SS-10	80	1401.8 ± 191.7	7.3 ± 6.4	96.0 ± 85.6	<**0.001**	0.81	<**0.001**	0.77	<**0.001**
4 SS-10	76.7	1404.5 ± 193.4	7.7 ± 8.5	100.2 ± 111.0	<**0.001**	0.67	<**0.001**	0.69	<**0.001**
3 SS-10	93.3	1398.2 ± 205.3	7.8 ± 8.5	102.0 ± 112.6	<**0.001**	0.70	<**0.001**	0.71	<**0.001**

ICC, intraclass coefficient of correlation; REE, resting energy expenditure; SS, steady state; 25 INT-30, testing method using an interval of 25 min during 30 min; 20 INT-30, testing method using an interval of 20 min during 30 min; 15 INT-30, testing method using an interval of 15 min during 30 min; 5 INT-10, testing method using an interval of 5 min during 10 min; 5 SS-30, testing method using 5 min in an SS during 30 min; 5 SS-10, testing method using 5 min in an SS during 10 min; 4 SS-10, testing method using 4 min in an SS during 10 min; 3 SS-10, testing method using 3 min in an SS during 10 min; %SS, relative number of participants who achieve a steady state; Δ%, relative difference between the testing method and the reference method; Δ, absolute difference between the testing method and the reference method; SD, standard deviation.
*Values in boldface indicate significant differences ($P < 0.05$).

<10% when the interval method was used, some participants presented CV values of >10%.

Discussion

The main findings of this study showed that 5 min in SS during 30 min is still considered the most accurate method for data analysis of REE. Reduced SS and interval methods during 10 and 30 min showed significant mean differences, high limits of agreement, and significant trends overestimating the REE.

As a consequence of ceasing production of the Deltatrac device that had been considered the gold standard metabolic monitor for decades, new-generation ICs have been increasing recently [18]. Therefore, accuracy testing using new devices is paramount in detecting high variability. Variability in the REE makes predicting calorie requirements challenging [19], previously having demonstrated a within-subject variability of between 5.4% and 12.2% over 2 d [20]. Thus, variability of a test-to-test has been described as involving biological variability, instrumental variability, and error [21]. Validation of protocols for IC measurements should consider within-subject variability. Only one measure is commonly used to test accuracy between different methods (interval or SS methods) for data analysis of REE [4,7–9,12,13]. A dependent effect between compared methods may be observed when only one measure is performed. We consider that a randomized trial using two measures may be the best design to demonstrate independent effects between compared methods; thus, the variability within-subject is considered in the REE analysis.

The reference method for data analysis of REE may be questionable, but an excellent review underlined the best practices for performing IC studies suggesting the 5 min in SS (CV <10% for VO_2 and VCO_2) as a good standard method to estimate the REE [6]. Another review suggested ≥4 min in SS [11] but still used the 5 min in SS as the reference method to validate the reduced SS [9]. In addition, it has been suggested that the first 5 min of measurement be discarded, that is, it is a period with high variability (>10%) [6,11–13,22]. Some studies selected the first 5 min found in SS during a predetermined time of IC measurement for data analysis [4,7,9]; or ceased the measurement once the SS had been achieved [7,9]; or averaged all periods of SS during a predetermined time [8]; or selected the lowest mean (5 min) of CV versus for VO_2 and VCO_2 during a predetermined time to estimate the REE [5]. In addition, the lowest REE during 5 min in SS from the mean of VO_2 and VCO_2 was measured during a predetermined period [15], which seems reasonable when considering an REE analysis closer to basal values. Cunha et al. [23] compared the REE mean during 5-min intervals over a period of 60 min and suggested ≥30 min of measurement to achieve an SS condition. Therefore, these previous findings support our reasoning for considering 5 min in SS during 30 min (discarding the first 5 min) as the reference method for this study.

Interval methods are commonly used in nutrition studies and may be considered an alternative option in clinical practice, mainly for patients in whom it has previously been suggested may take longer to achieve an SS [7–9]. Although interval methods showed an excellent ICC and high r^2 during 30 min of IC measurement in the present study, significant trends for participants with a high REE and an overestimation of the REE were observed (~80 kcal/d). Similar findings have been observed in other studies that have also demonstrated significantly higher REE values when interval methods were compared to the 5 min in SS [4,5].

A reduced SS is another strategy for IC measurements to help patients achieve the SS. Approximately 40% of healthy participants previously achieved 5 min in SS during between 6 and 10 min of measurement [4]. Thus, our hypothesis was that reducing the SS could help participants achieve the criteria for data analysis of REE during a period of 10 min. In addition, REE measurements during 10 min may support sports physiologists, nutritionists, or researchers who sometimes need to perform many IC measurements a day. Here, we observed a relatively high number of participants who achieved SS when different methods of reduced SS were applied during 10-min interval (between 76.7 and 93.3%). However, all testing methods during 10 min significantly overestimated the REE (~100 kcal/d), and also demonstrated significant trends for participants with a high REE.

The initial hypothesis was that reduced SS and interval methods could overestimate the REE. Interval methods used the REE, calculated from the mean of VO_2 and VCO_2, during a predetermined period, therefore accounting for values with high variability. Confirming this hypothesis, we observed high mean and individual values of CV for VO_2 and VCO_2 when interval methods were used (Fig. 3). A lower variability was observed when SS methods were used in the present study (i.e., a CV cutpoint of <10% for VO_2 and VCO_2). However, we consider that even when achieving an SS during 10 min, the REE may still decrease after this abbreviated period of IC measurement. Therefore, a period of 30 min may facilitate finding several periods of SS and selecting the lowest REE from the mean of VO_2 and VCO_2. On the other hand, 10 min allows for fewer periods of SS, decreasing the probability of finding the lowest accurate REE value. Thus, we recommend using 30 min of IC measurement selecting 5 min in SS to estimate the REE, and we suggest that participants who do not achieve SS repeat the procedures.

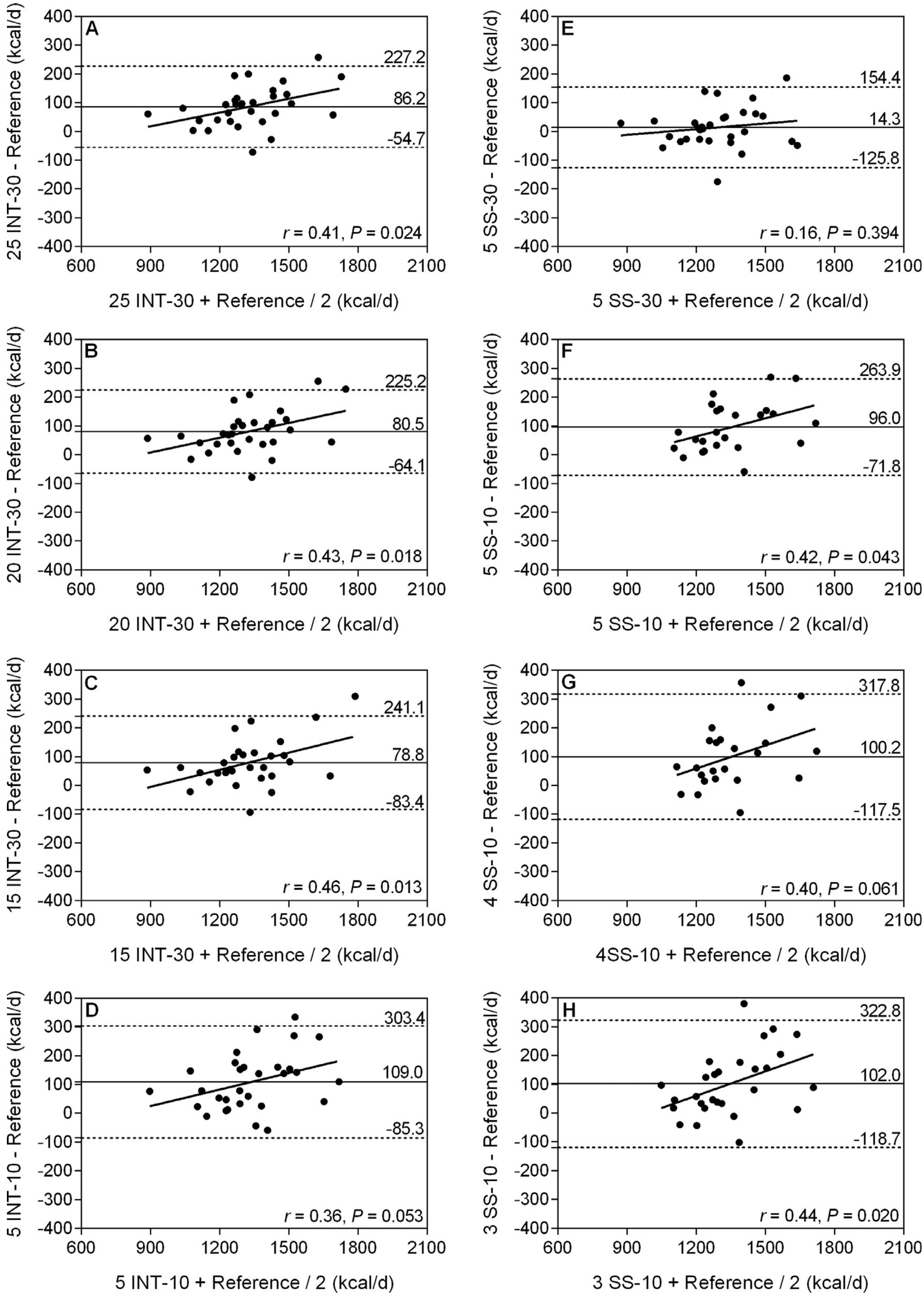

Fig. 2. Bland–Altman plots of agreement for resting energy expenditure between the reference method (5 min in a steady state [SS] during 30 min) and the testing method using an interval of 25 min during 30 min (25 INT-30) (**A**). Testing method using an interval of 20 min during 30 min (20 INT-30) (**B**). Testing method using an interval of 15 min during 30 min (15 INT-30) (**C**). Testing method using an interval of 5 min during 10 min (5 INT-10) (**D**). Testing method using 5 min in SS during 30 min (5 SS-30) (**E**). Testing method using 5 min in SS during 10 min (5 SS-10) (**F**). Testing method using 4 min in SS during 10 min (4 SS-10) (**G**). Testing method using 3 min in SS during 10 min (3 SS-10) (**H**). Solid line: mean of the differences; dashed line: limits of agreement of 95%; regression solid line: correlation between mean and differences of reference and testing methods.

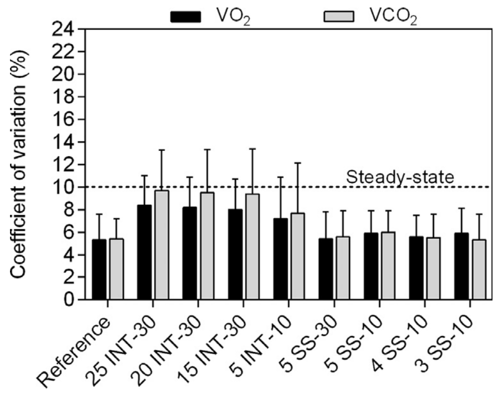

Fig. 3. Coefficient of variation for VO_2 and VCO_2 of the reference method; testing method using an interval of 25 min during 30 min (25 INT-30); testing method using an interval of 20 min during 30 min (20 INT-30); testing method using an interval of 15 min during 30 min (15 INT-30); testing method using an interval of 5 min during 10 min (5 INT-10); testing method using 5 min in a steady-state (SS) during 30 min (5 SS-30); testing method using 5 min in SS during 10 min (5 SS-10); testing method using 4 min in SS during 10 min (4 SS-10); testing method using 3 min in SS during 10 min (3 SS-10). SS, steady state; VCO_2, volume of carbon dioxide production; VO_2, volume of oxygen consumption.

The findings of this study may not extend to the intensive care unit setting. Using this methodology during a period of critical illness would be particularly challenging and may lead to errors [24]. Thus, our findings are likely to be limited to healthy participants. In addition, participants in the present study spent the night before the tests at home and needed to come to the laboratory facility. We consider this to be a further limitation to our study. Although no differences have been found previously between REE measured after participants had spent the night at home or at a clinic, it has been suggested that an overnight stay at a clinical facility may have resulted in an REE closer to basal values [25].

Conclusion

Interval and SS methods during 10 min and interval methods during 30 min overestimated the REE. Therefore, we recommend 30 min of IC measurement selecting the lowest REE from the mean of VO_2 and VCO_2 during 5 min in SS. If an SS cannot be achieved using this method, we recommend repeating the measurement at another time until the participant is able to achieve an SS.

Acknowledgments

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