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Comparing Steady State to Time Interval Measurements of Resting Metabolic Rate

Chelsea Jayne Irving

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

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ABSTRACT

Comparing Steady State to Time Interval Measurements of
Resting Metabolic Rate

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Master of Science

Background: The two most common methods to measure resting metabolic rate using indirect calorimetry are steady state or time interval. Steady state is commonly defined as the first five minutes in which oxygen consumption and carbon dioxide production vary by <10%. A time interval measurement generally lasts 20-60 minutes. Using steady state criteria is often harder to achieve, but many suggest it more accurately measures resting metabolic rate. Our objective was to determine if there were differences between steady state and time interval measurements in a healthy adult population.

Materials and Methods: Seventy seven subjects were measured for 45 minutes. Inclusion criteria included healthy subjects ages 18-65, excluding pregnant and lactating women. Paired t-tests analyzed differences between measures, and Bland-Altman plots evaluated bias, precision, and accuracy

Results: Of 77 subjects, 84% achieved steady state, and 95% achieved SS by minute 30. Most differences between steady state and time intervals were statistically but not practically significant. Bland-Altman plots showed steady state measurements were generally lower indicating that steady state is more indicative of resting metabolic rate. Minutes 6-25 were most precise, accurate and fairly unbiased compared to steady state.

Conclusions: We recommend measuring a subject for 30 minutes and using steady state criteria of <10% variation of oxygen consumption and carbon dioxide production for five minutes if a subject is able to achieve it. However, if a subject cannot achieve steady state, we recommend averaging minutes 6-25.

Keywords: steady state, indirect calorimetry, resting metabolic rate, time interval, Bland-Altman plots

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I have been blessed with wonderful friends who understand my own brand of crazy and have helped me channel that to achieve my goals. My boyfriend, Derek, has been almost overly-supportive and encouraging during my entire graduate degree. Even when I felt like I couldn't continue studying, conducting research, writing, etc., he told me to keep going. I am so grateful for a man who inspires me to pursue my dreams and isn't afraid of my accomplishments.

My family has always been my rock, and they are my entire life. My siblings and their families have taught me a new meaning of love. They always have my back and listen without judgment, and I value their advice and commitment to each other and God. Finally, my parents have been my biggest cheerleaders for as long as I can remember. I've always had big dreams, and they have done everything in their power to help me in any way possible. I can't thank them enough for their love, support, advice, and belief in me since I was a little kid. They are the people I turn to first, during both good and bad times, and I only hope that I can continue to make them proud.

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INTRODUCTION

Indirect calorimetry (IC) is the most widely-used technique to measure resting metabolic rate (RMR) in both clinical and research settings. IC measures energy expenditure by analyzing oxygen consumption (VO_2) and carbon dioxide production (VCO_2) using a metabolic gas monitor. This technique of measurement more accurately identifies RMR, whereas prediction equations estimate RMR (1).

Determining RMR from an IC test has been determined two ways. The first uses steady state (SS) criteria often defined as the first five minutes in which VO_2 and VCO_2 vary by less than a predetermined percentage, usually 10%. The second uses a time interval approach where values are averaged over a predetermined time interval (2). Time intervals can range from five minutes to several hours, although most practitioners generally use an interval of 20-60 minutes (3,4).

The Evidence Analysis Library (EAL) of the Academy of Nutrition and Dietetics published best practice recommendations for how to perform indirect calorimetry in a healthy population (5). They recommended using SS criteria rather than a time interval because there were no studies rigorous enough to provide other recommendations. Specifically, they recommended discarding the first five minutes of data and then use a validated SS definition to determine the duration of the remainder of the measurement (5). This recommendation was considered weak and conditional due to a lack of studies. Additionally, due to a lack of adequate studies the EAL was unable to make a recommendation in the event a subject is unable to achieve SS. The reviewers concluded that more studies were needed to determine if SS is a better measurement than a time interval and to also provide a recommendation for subjects who do not achieve SS.

We identified only two studies comparing SS to time interval measurements, one in the critically ill and the other in postmenopausal women (2,6). McClave et al (2) found that SS was more highly correlated to total energy expenditure than time intervals in critically ill patients. Horner et al (6) measured post-menopausal women and found a statistically significant difference between multiple time intervals and SS, but because there was a high correlation (>0.9) they suggested using minutes 6-10 to minimize subject burden.

A potential weakness of these studies is the use of correlation coefficients and t-tests because they only measure correlation and differences between means which do not consider bias, precision, or accuracy. Bland and Altman (7) introduced a statistical method to more rigorously determine the difference between two measurement techniques. A Bland-Altman plot plots the average of the two values on the x-axis and the difference between the averages of those values on the y-axis. This plot can identify a relationship between measurement error and the true value (7), and is most often used to compare two devices or measurements. Limits of agreement (generally defined as ± two standard deviations) are also calculated, identifying where 95% of the values should fall if there is precision between the two measurements.

To the best of our knowledge, there are no studies comparing SS to time interval measurements using Bland-Altman plots. The purpose of this study was to use Bland-Altman plots to compare SS and various time interval measurements of RMR by IC in a healthy, cross-sectional adult population. Answering this question will help those who perform IC choose the method that imposes the least burden on the subject while still providing an accurate measurement.

METHODS

Study Design

A cross-sectional study using a convenience sample of 77 male and female subjects was recruited from the faculty, staff, and students at our institution and the surrounding area. Recruitment efforts involved flyers sent to all faculty and staff and displayed throughout campus, announcements in classes, and by word of mouth. Inclusion criteria were healthy subjects between the ages of 18-65 years. Pregnant and lactating women were excluded. The Institutional Review Board approved the study protocol, the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation, and all participants provided written informed consent.

Procedures

Following recruitment, subjects came in for one visit. The study procedures were explained, and informed consent was given. Height was measured prior to the IC measurement using a professional grade stadiometer, Model PE-WM-60-76-BRG2 (Perspective Enterprises, Portage, MI). Subjects were measured to the nearest centimeter while standing without shoes with weight evenly distributed. A Tanita 310 electronic scale (Arlington Heights, IL) was used to assess weight. Subjects were weighed to the nearest kilogram with minimal clothing and without shoes.

Indirect Calorimetry Measurements

IC tests followed current protocols (5) including: starting the test between 6:00 am and 9:00 am, following an overnight fast, and no showering the morning of the test. Subjects were asked to park close to the building and abstain from any stimulants and exercise for 24 hours before measurement. Upon arrival, subjects rested for 30 minutes in a reclined position prior to data collection. All measurements were taken in a quiet, thermoneutral room with dimmed lights to avoid any environmental influences on RMR. A blanket was provided if requested.

RMR was measured using a Quark RMR indirect calorimeter (COSMED, Rome, Italy). Calibrations on the mass flow sensor and gas analyzers were performed prior to each study. The gas used for calibrations was 16% O_2/5% CO_2. A clear canopy was placed over the subjects' heads to collect gas samples.

Once data collection began, RMR was measured for 45 minutes with the first five minutes being discarded for acclimatization purposes (5). SS was defined as the first five minutes when both VO_2 and VCO_2 varied by <10%. If subjects failed to achieve SS during the 45 minute study, their results were discarded and not included in the final SS statistical analysis. Periods of SS were then compared to different time intervals (minutes 6-10, minutes 6-15, minutes 6-20, minutes 6-25, minutes 6-30, minutes 6-45).

Statistical Analysis

A power analysis based on a 75 calories ± standard deviation of 160 with 80% power and a p-value of 0.05 suggested 75 subjects. The differences between measures were determined with paired-T-tests, and demographic information was analyzed using Statistical Analysis Software (Version 9.5, SAS Institute Inc., Cary, NC). Results are reported as means ± standard deviations and ranges. The limits of agreement between measures were analyzed with Bland-Altman plots using GraphPad Prism (Version 6.02, GraphPad Software Inc., San Diego, CA). The SS measurements were subtracted from the time interval measurements, so a positive difference indicates that SS was lower than the time interval. A lack of bias was defined when the differences between measurements were evenly distributed along the 0 line (i.e.50% of differences are greater than 0 and 50% less than 0). Precision was determined by the number of differences that fell outside of the 95% limits of agreement. Accuracy was determined by counting the number of absolute differences that were greater than 75 calories.

RESULTS

Patient Characteristics

Of the 166 subjects who were screened, 78 started the study and 77 completed the study. Subject characteristics are found in Table 1. Males and females were equally represented, and the age range was broad, from 18-65 years. The majority of subjects (88%) had a normal BMI (mean 24.2 $kg/m^2 \pm 4.7\ kg/m^2$), five subjects (7%) had a BMI >30 kg/m^2 and four subjects (5%) had a BMI <18.5 kg/m^2. The mean RMR for the study population was 1,570 calories (± 281 calories).

Time to Steady State

Of the 77 participants, 84% (n = 65) achieved SS; the remaining 16% of subjects never achieved SS during the 45 minute study period. The median time to SS was minute 11 (n = 32), and 38% of subjects achieved SS by minute 10 (n = 24) and 72% by minute 15 (n = 47) (Figure 1). After minute 15, some participants continued to achieve SS, but no consistent patterns emerged as to when another large group of participants achieved SS. Of those subjects who achieved SS, 95% did so by minute 30. Only two subjects achieved SS between minutes 30-45. It is important to note that we only determined when subjects first achieved SS, not how long they remained in SS.

Steady State vs Time Intervals

Various time intervals were compared to SS. When comparing minutes 6-10 to SS, there was a statistically significant difference in the means of VCO_2 and calories ($p = 0.0025$, $p = 0.0439$; respectively) (Table 2). However, the average calorie difference was only 12 which is not practically significant. The Bland-Altman plot comparing minutes 6-10 to SS is shown in Figure 2. Because many subjects achieved SS during this time period, 37% of the differences between measurements were zero. 75% of the differences favored SS or were not different than the 6-10 minute time interval (Table 3); additionally, 6% of the differences fell outside of the 95% limits of agreement. 20% of absolute differences were greater than 75 calories (Table 3).

Comparing SS to minutes 6-15 showed a statistically significant difference in VCO_2 and calories ($p = 0.0174$, $p = 0.0222$; respectively) but not a practical one, with an average difference of only 13 calories (Table 4). The Bland-Altman plot showed that the difference between these measures was fairly unbiased with 57% of the values being lower in SS (Figure 3). Only 3% of the differences fell outside of the 95% limits of agreement; 17% of absolute differences were greater than 75 calories (Table 3).

Minutes 6-20 compared to SS showed a statistically significant difference in VCO_2 and calories ($p = 0.0128$, $p = 0.0219$; respectively) but only an average difference of 12 calories (Table 5). Minutes 6-20 showed the least bias with 52% of differences being lower in SS (Figure 4). Only 3% of the differences fell outside of the 95% limits of agreement, and only 9% of absolute differences were greater than 75 calories (Table 3).

When comparing minutes 6-25 to SS, all variables (VO_2, VCO_2, and calories) were statistically different from one another ($p = 0.0434$, $p = 0.0083$, $p = 0.0095$; respectively) with an average difference of 12 calories (Table 6). Minutes 6-25 were very precise with only 1.5% of differences falling outside the 95% limits of agreement (Figure 5). The differences between measurements were fairly unbiased with 62% of differences favoring SS. However, only 6% of absolute differences in measurements were greater than 75 calories (Table 3).

All variables were again statistically significant for minutes 6-30 compared to SS ($p = 0.0170$, $p = 0.0036$, $p = 0.0033$; respectively) with an average difference of 15 calories (Table 7). 66% of measurements were lower for SS than minutes 6-30, and 3% of the differences between

SS and minutes 6-30 fell outside the 95% limits of agreement (Figure 6). During minutes 6-30 compared to SS, 11% of absolute differences were greater than 75 calories (Table 3).

Finally, there were statistically significant differences between SS and minutes 6-45 for all variables ($p = 0.0141$, $p = 0.0006$, $p = 0.0018$; respectively) (Table 8). Differences between measures were biased with 67% of values favoring SS (Table 3), and 6% of the differences fell outside of the 95% limits of agreement (Figure 7). Also, 9% of absolute differences between measurements fell outside of the 75 calorie limit (Table 3).

Demographics and Time to Steady State

Demographic characteristics were analyzed post-hoc to determine if they were related to time to SS. Backward elimination found no relationships when using age, weight, and BMI with time to SS. When gender (female) and height were assessed as individual predictor variables, each was slightly statistically significant ($p = 0.02$, $p = 0.04$; respectively). However, when these variables were analyzed together, each became insignificant ($p = 0.12$, $p = 0.50$; respectively). There is likely no relationship between gender and height in regards to how quickly SS is achieved.

DISCUSSION

IC is often performed in both clinical and research settings in order to accurately measure RMR. Based on the results of our study, we have two primary conclusions. First, when comparing SS to the various time intervals, SS measurements were consistently lower than any time interval. If RMR is defined as the lowest measurement possible in an awake person, we suggest that SS is more representative of RMR than any time interval we measured. Secondly, if SS is not achieved or is not practical to calculate, we recommend using minutes 6-25 after discarding the first five minutes.

McClave et al (2) also suggested that SS is more representative of RMR than a time interval in critically ill patients. They compared various SS criteria and time intervals to 24-hour total energy expenditure (TEE) in mechanically-ventilated and sedated patients. They found that the most stringent SS criteria (<10% variation in VO_2 and VCO_2 for five minutes) was most representative of TEE by correlation coefficient. If a subject was not able to meet SS, they recommended measuring subjects for 30-60 minutes. Also, they did not use Bland-Altman plots to determine agreement between measures, and the study population was critically ill.

In contrast, Horner et al (6) recommended using minutes 6-10 despite finding a significant difference between SS (<10% variation in VO_2, minute ventilation, and respiratory quotient) and various time intervals based on high correlation coefficients. They did so because the two values had a high correlation coefficient (>0.9) and a time interval measurement would be least burdensome. They also did not use Bland-Altman plots to evaluate bias and agreement, and the study population was narrow.

Reeves et al (8) compared various time intervals of SS (five minutes vs four minutes vs three minutes) to determine if a shorter time is still an accurate measurement of RMR. They found no significant differences between any measures, but Bland-Altman plots showed that while four minutes was not different than five, three minute SS underestimated RMR.

Unfortunately, not all subjects can achieve SS. The percentage of our subjects who did not achieve SS (16%) was similar to other studies (average 15-20%, full range 4-41%) (2,6,8-14) However, it is important to note that SS is defined differently across studies, some including respiratory quotient (RQ) or minute ventilation (V_E) or having more stringent variation criteria (i.e. percent difference of values).

Cunha et al (15) proposed measuring a subject for at least 30 minutes after which a 5-minute SS could be used for RMR. Based on our findings, this recommendation is longer than necessary because 95% of our subjects who achieved SS did so by minute 30. This difference is likely due to the fact that they only rested their subjects for 10 minutes, not 30, as is recommended (7).

Our second recommendation is that if SS is not achieved or able to be calculated, minutes 6-25 are preferred. Minutes 6-25 were most precise with only 1.5% of the differences outside of the 95% limits of agreement, and they were also fairly unbiased with 62% of differences in measurement lower for SS. Minutes 6-25 was also most accurate with the least number of absolute differences that were greater than 75 calories (6%). Other time intervals were less precise, having 3-6% of measurements outside the 95% limits of agreement. Minutes 6-15 and 6-20 compared to SS had differences that were more evenly distributed around the mean, with 57% and 52% of differences favoring SS respectively, but overall, minutes 6-25 are most similar to SS. Therefore, using minutes 6-25 is the most accurate and a fairly unbiased alternative to SS.

There were a few limitations to our study and avenues for future research. The sample size was adequate to determine differences in RMR; however, a larger sample size with a more varied population (i.e. range of BMIs, ethnicity, etc.) may be necessary to confirm our findings. Of the 12 subjects who were unable to achieve SS during this study, two (17%) were underweight or obese. One subject had a BMI of 17.0, and the other had a BMI of 48.5. While it is possible that extreme BMIs affect the ability to achieve SS, our regression analysis did not find weight to be a predictor variable. Future research could elucidate if there are statistical and/or practical differences in RMR between those populations.

CONCLUSION

From these results, we conclude that using SS criteria with <10% variation for VO_2 and VCO_2 for five minutes is likely more indicative of RMR than using any time interval. However, if SS cannot be achieved or is not calculated, we recommend using minutes 6-25 for consistency and to reduce the burden on the subject.

REFERENCES

1. Mahan LK, Escott-Stump S, Raymond JL. Krause's Food and the Nutrition Care Process. 13th ed. St. Louis: Saunders Elsevier; 2012.
2. McClave SA, Spain DA, Skolnick JL, et al. Achievement of steady state optimizes results when performing indirect calorimetry. *JPEN J Parenter Enteral Nutr*. 2003;27:16-20.
3. Leff ML, Hill JO, Yates AA, Cotsonis GA, Heymsfield SB. Resting metabolic rate: measurement reliability. *JPEN J Parenter Enteral Nutr*. 1987;11:354-359.
4. Long CL, Schaffel N, Geiger JW, Schiller WR, Blakemore WS. Metabolic response to injury and illness: estimation of energy and protein needs from indirect calorimetry and nitrogen balance. *JPEN J Parenter Enteral Nutr*. 1979;3:452-456.
5. Fullmer S, Benson-Davies S, Earthman CP, et al. Evidence analysis library review of best practices for performing indirect calorimetry in healthy and non-critically ill individuals. *J Acad Nutr Diet*. 2015;115:1417-1446.
6. Horner NK, Lampe JW, Patterson RE, Neuhouser ML, Beresford SA, Prentice RL. Indirect calorimetry protocol development for measuring resting metabolic rate as a component of total energy expenditure in free-living postmenopausal women. *J Nutr*. 2001;131:2215-2218.
7. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986;1(8476):307-310.
8. Reeves MM, Davies PSW, Bauer J, Battistutta D. Reducing the time period of steady state does not affect the accuracy of energy expenditure measurements by indirect calorimetry. *J Appl Physiol*. 2004;97:130-134.
9. Dickerson RN, Brown RO, Gervasio JG, Hak EB, Hak LJ, Williams JE. Measured energy expenditure of tube-fed patients with severe neurodevelopmental disabilities. *J Am Coll Nutr*. 1999;18:61-68.
10. Frankenfield DC, Sarson GY, Blosser SA, Cooney RN, Smith JS. Validation of a 5-minute steady state indirect calorimetry protocol for resting energy expenditure in critically ill patients. *J Am Coll Nutr*. 1996;15:397-402.
11. Reidlinger DP, Willis JM, Whelan K. Resting metabolic rate and anthropometry in older people: a comparison of measured and calculated values. *J Hum Nutr Diet*. 2015;28:72-84.
12. McEvoy C, Cooke SR, Young IS. A reduced abbreviated indirect calorimetry protocol is clinically acceptable for use in spontaneously breathing patients with traumatic brain injury. *Nutr Clin Pract*. 2009;24:513-519.
13. Dickerson RN, Gervasio JM, Riley ML, et al. Accuracy of predictive methods to estimate resting energy expenditure of thermally-injured patients. *JPEN J Parenter Enteral Nutr*. 2002;26:17-29.
14. Petros S, Engelmann L. Validity of an abbreviated indirect calorimetry protocol for measurement of resting energy expenditure in mechanically ventilated and spontaneously breathing critically ill patients. *Intensive Care Med*. 2001;27:1164-1168.
15. Cunha FA, Midgley AW, Monteiro W, Freire R, Lima T, Farinatii PTV. How long does it take to achieve steady state for an accurate assessment of resting VO_2 in healthy men? *Eur J Appl Physiol*. 2013;113:1441-1447.

Tables and Figures

Table 1. Subject Characteristics (n = 77).

Variable	Mean ± SD	Range	Median
Age (years)	32.8 ± 16.5	18 to 65	23
Height (cm)	170.6 ± 15.3	67 to 191	170
Weight (kg)	73.0 ± 18.9	46 to 166	68
BMI (kg/m^2)	24.2 ± 4.7	16.5 to 48.5	23.5
Gender (%F)	49		
VO_2 (ml/min)	229.3 ± 41.1	160.2 to 332.0	225.4
VCO_2 (ml/min)	177.7 ± 33.6	123.9 to 289.8	178.2
RMR (calories)	1,570 ± 281	1,102 to 2324	1549

BMI, body mass index; VO_2, oxygen consumption; VCO_2, carbon dioxide production; RMR, resting metabolic rate.

Table 2. Differences between minutes 6-10 and steady state (n = 65).

Measure	SS Mean	Minutes 6-10 Mean	Mean Difference ± SD	Range	P-value
VO_2 (ml/min)	225.20	226.47	1.27 ± 7.14	-18.37 to 17.50	0.1577
VCO_2 (ml/min)	175.91	175.91	3.34 ± 11.53	-20.03 to 67.10	0.0225*
RMR (calories)	1,538.12	1,550.47	12.35 ± 48.43	-101.27 to 121.43	0.0439*

VO_2, oxygen consumption; VCO_2, carbon dioxide production; RMR, resting metabolic rate.

Steady state defined as the first 5 minutes where VO_2 and VCO_2 vary by <10%.

*P-value <0.05 classified as significant.

Table 3. Measures of bias, precision, and accuracy for steady state vs all time intervals using Bland-Altman plots.

Time Intervals (minutes)	**Percent of Differences >0[ǂ] (%)**	**Percent of Measures Outside the Limits of Agreement[†] (%)**	**Percent of Differences Greater than 75 Calories[∫] (%)**
6-10	75%	6%	20%
6-15	57%	3%	17%
6-20	52%*	3%	9%
6-25	62%	1.5%*	6%*
6-30	66%	3%	11%
6-45	67%	6%	9%

*Indicates the best percentage for each variable

ǂ Measure of bias

† Measure of precision

∫ Measure of accuracy

Table 4. Differences between minutes 6-15 and steady state (n = 65).

Measure	**SS Mean**	**Minutes 6-15 Mean**	**Mean Difference ± SD**	**Range**	**P-value**
VO2 (ml/min)	225.20	226.55	1.35 ± 6.01	-13.17 to 16.03	0.0750
VCO2 (ml/min)	175.91	175.98	3.41 ± 11.28	-11.82 to 70.25	0.0174*
RMR (calories)	1,538.12	1,551.10	12.98 ± 44.66	-78.77 to 131.57	0.0222*

VO_2, oxygen consumption; VCO_2, carbon dioxide production; RMR, resting metabolic rate.

Steady state defined as the first 5 minutes where VO_2 and VCO_2 vary by <10%.

*P-value <0.05 classified as significant.

Table 5. Differences between minutes 6-20 and steady state (n = 65).

Measure	SS Mean	Minutes 6-20 Mean	Mean Difference ± SD	Range	P-value
VO_2 (ml/min)	225.20	226.43	1.22 ± 5.83	-14.02 to 14.87	0.0956
VCO_2 (ml/min)	175.91	175.55	2.99 ± 9.41	-14.38 to 49.53	0.0128*
RMR (calories)	1,538.12	1,549.71	11.59 ± 39.76	-61.72 to 97.36	0.0219*

VO_2, oxygen consumption; VCO_2, carbon dioxide production; RMR, resting metabolic rate.

Steady state defined as the first 5 minutes where VO_2 and VCO_2 vary by <10%.

*P-value <0.05 classified as significant.

Table 6. Differences between minutes 6-25 and steady state (n = 65).

Measure	SS Mean	Minutes 6-25 Mean	Mean Difference ± SD	Range	P-value
VO_2 (ml/min)	225.20	226.56	1.36 ± 5.32	-9.58 to 11.51	0.0434*
VCO_2 (ml/min)	175.91	175.53	2.97 ± 8.80	-16.04 to 48.23	0.0083*
RMR (calories)	1,538.12	1,550.45	12.33 ± 37.19	-65.16 to 80.63	0.0095*

VO_2, oxygen consumption; VCO_2, carbon dioxide production; RMR, resting metabolic rate.

Steady state defined as the first 5 minutes where VO_2 and VCO_2 vary by <10%.

*P-value <0.05 classified as significant.

Table 7. Differences between minutes 6-30 and steady state (n = 65).

Measure	SS Mean	Minutes 6-30 Mean	Mean Difference ± SD	Range	P-value
VO_2 (ml/min)	225.20	226.99	1.79 ± 5.87	-11.51 to 14.49	0.0170*
VCO_2 (ml/min)	175.91	175.85	3.29 ± 8.78	-18.27 to 48.65	0.0036*
RMR (calories)	1,538.12	1,553.34	15.22 ± 40.18	-78.67 to 101.12	0.0033*

VO_2, oxygen consumption; VCO_2, carbon dioxide production; RMR, resting metabolic rate.

Steady state defined as the first 5 minutes where VO_2 and VCO_2 vary by <10%.

*P-value <0.05 classified as significant.

Table 8. Differences between minutes 6-45 and steady state (n = 65).

Measure	SS Mean	Minutes 6-45 Mean	Mean Difference ± SD	Range	P-value
VO_2 (ml/min)	225.20	227.23	2.03 ± 6.49	-11.56 to 18.80	0.0141*
VCO_2 (ml/min)	175.91	176.15	3.58 ± 8.00	-20.70 to 34.43	0.0006*
RMR (calories)	1,538.12	1,555.20	17.08 ± 42.15	-74.11 to 116.48	0.0018*

VO_2, oxygen consumption; VCO_2, carbon dioxide production; RMR, resting metabolic rate.

Steady state defined as the first 5 minutes where VO_2 and VCO_2 vary by <10%.

*P-value <0.05 classified as significant.

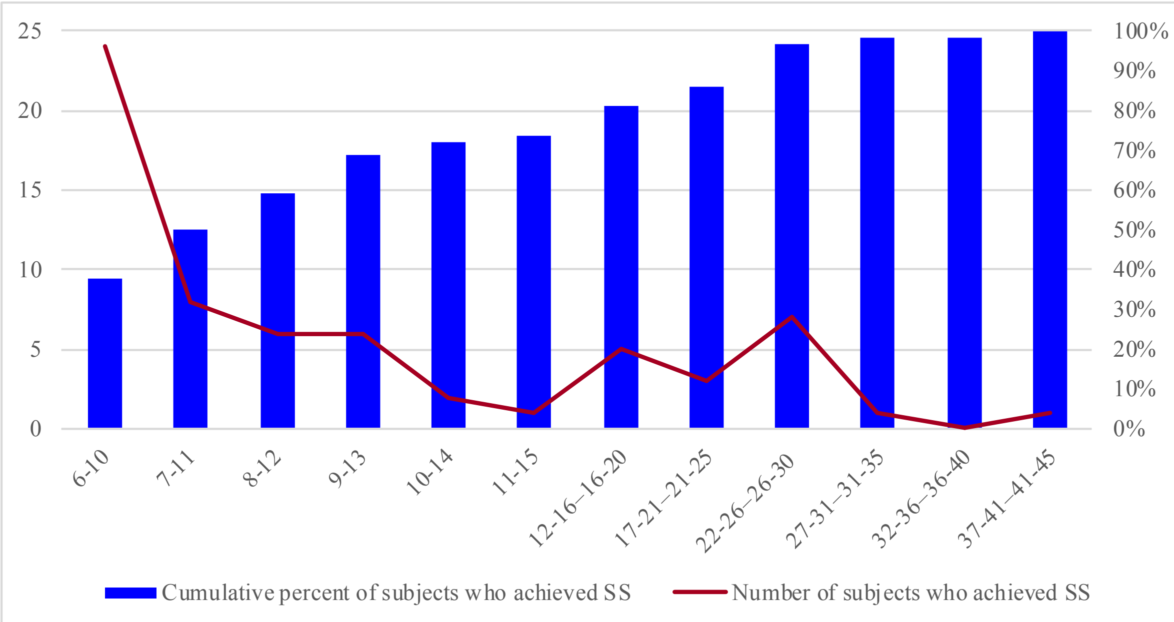

Figure 1. Time to steady state (N = 65). The x-axis shows minute intervals during which SS was achieved. The left y-axis shows number of individuals who achieved SS. The right y-axis shows cumulative percent of subjects who achieved SS.

*12 out of 77 subject did not achieve steady state.

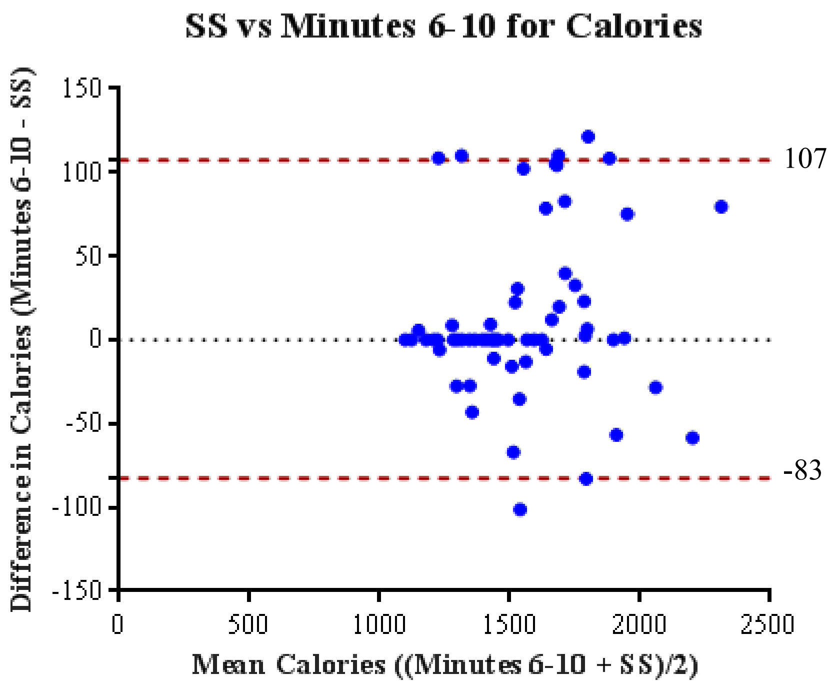

Figure 2. Bland-Altman plot of differences between steady state and minutes 6-10 plotted against the mean of the two measures. The 95% limits of agreement were -83 to 107 (dashed lines). Dots above the 0 line indicates steady state was lower.

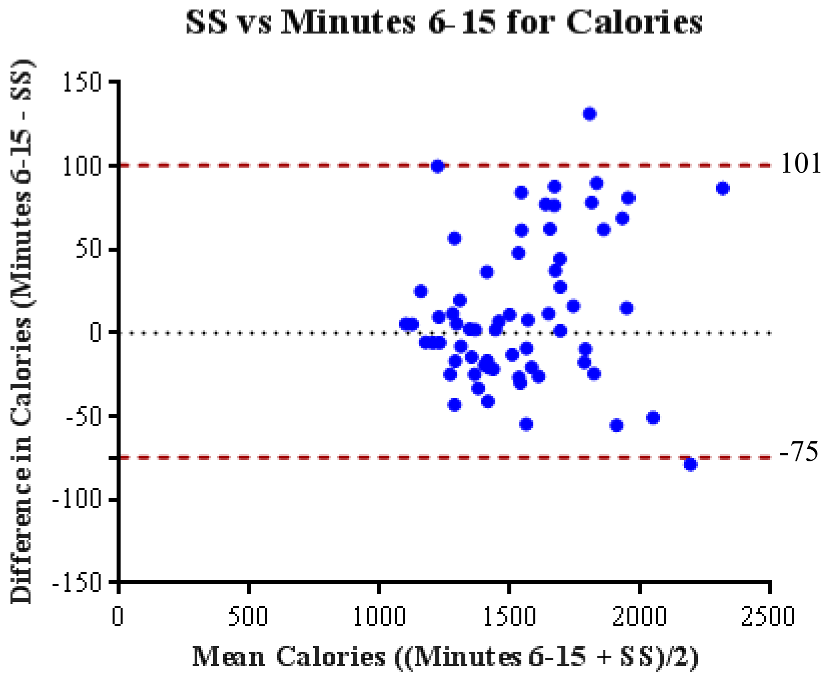

Figure 3. Bland-Altman plot of differences between steady state and minutes 6-15 plotted against the mean of the two measures. The 95% limits of agreement were -75 to 101 (dashed lines) Dots above the 0 line indicates steady state was lower.

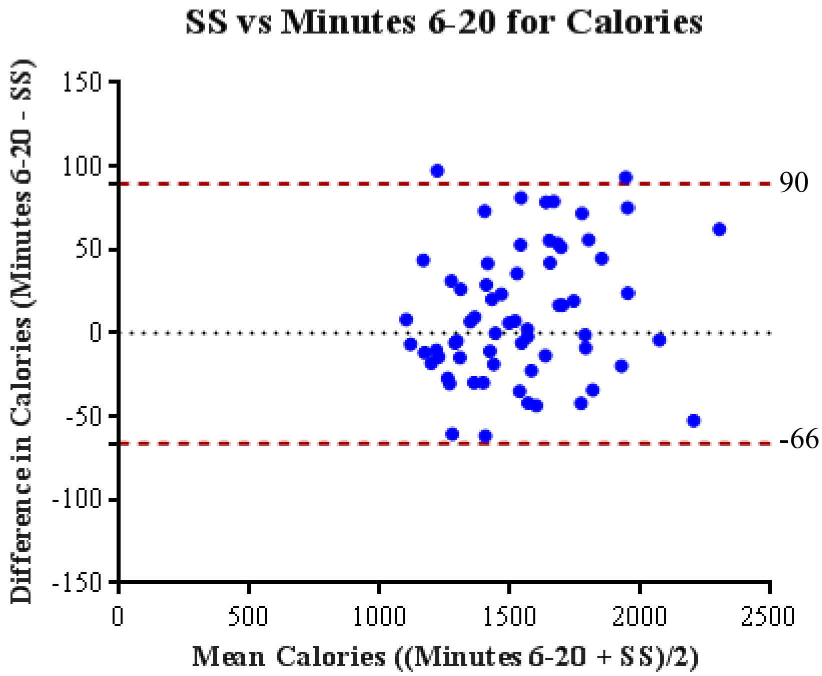

Figure 4. Bland-Altman plot of differences between steady state and minutes 6-20 plotted against the mean of the two measures. The 95% limits of agreement were -66 to 90 (dashed lines). Dots above the 0 line indicates steady state was lower.

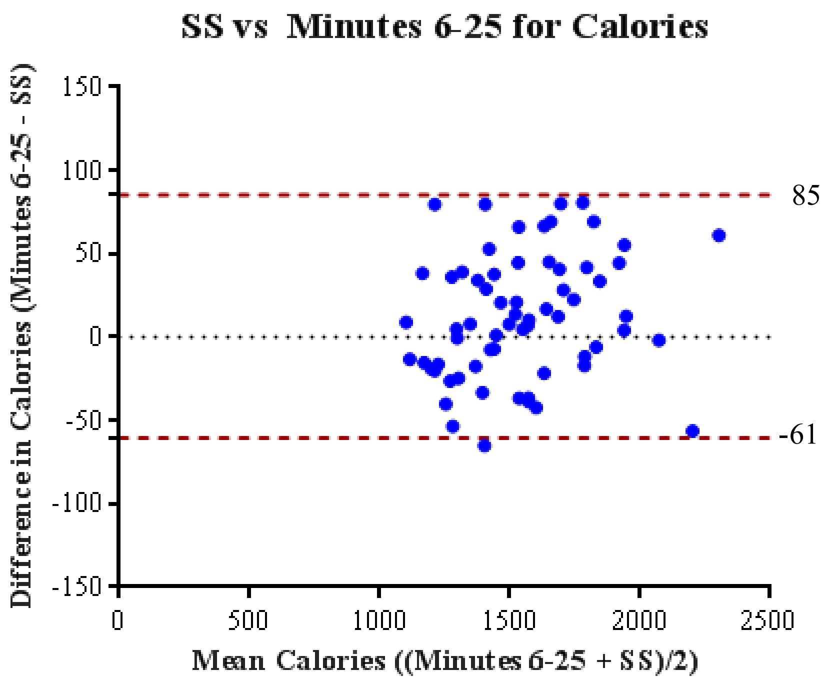

Figure 5. Bland-Altman plot of differences between steady state and minutes 6-25 plotted against the mean of the two measures. The 95% limits of agreement were -61 to 85 (dashed lines). Dots above the 0 line indicates steady state was lower.

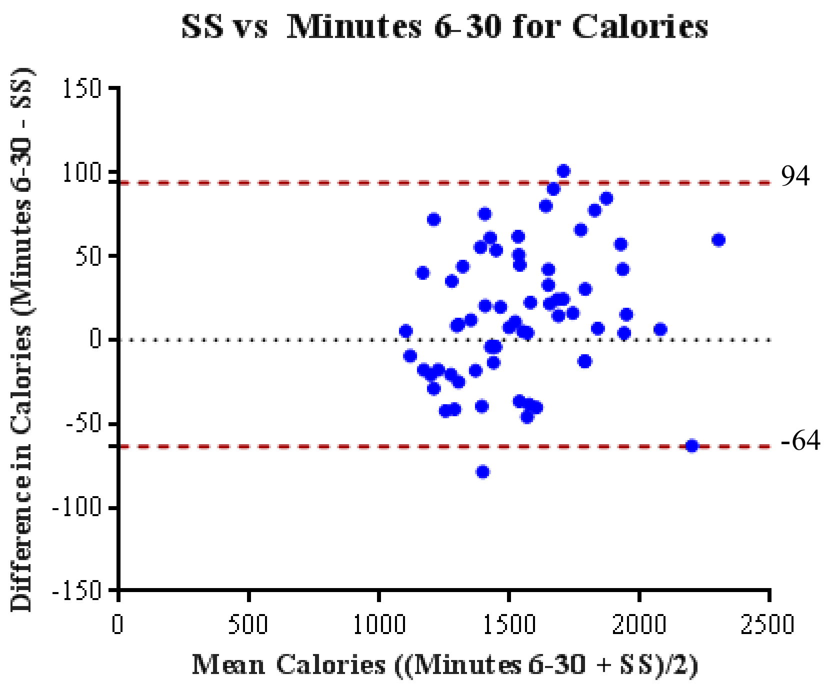

Figure 6. Bland-Altman plot of differences between steady state and minutes 6-30 plotted against the mean of the two measures. The 95% limits of agreement were -64 to 94 (dashed lines). Dots above the 0 line indicates steady state was lower.

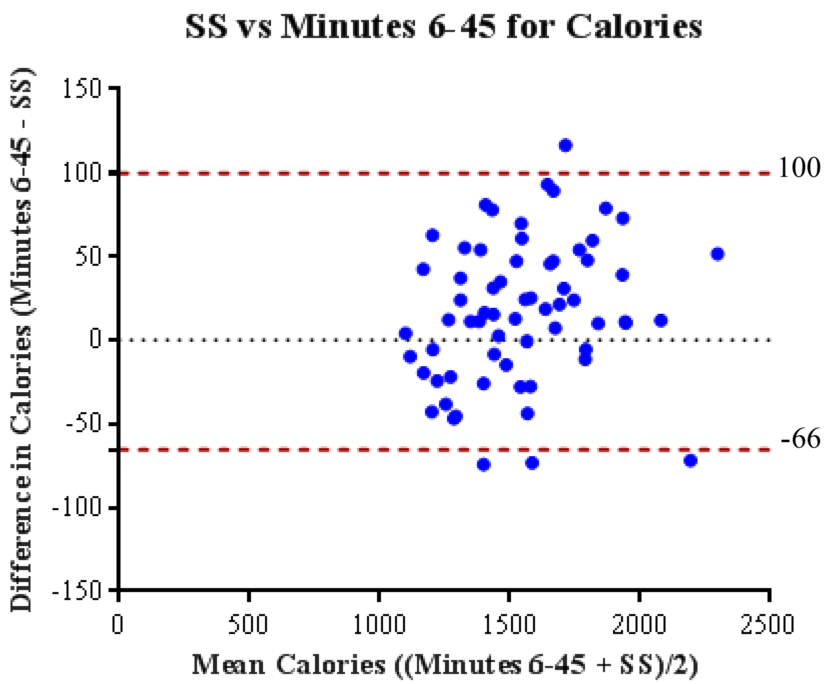

Figure 7. Bland-Altman plot of differences between steady state and minutes 6-45 plotted against the mean of the two measures. The 95% limits of agreement were -66 to 100 (dashed lines). Dots above the 0 line indicates steady state was lower.

APPENDIX A

Problem Statement

When determining a person's energy needs, a practitioner needs a reference of that person's metabolic rate. There are several terms that can be used when defining a person's metabolic rate, but most sources use resting metabolic rate (RMR). RMR is the energy expended for maintenance of normal body functions and homeostasis at rest. It is measured in calories per day (1-2).

There are several ways RMR can be measured. The most widely used technique is indirect calorimetry. Indirect calorimetry measures energy expenditure by determining the amount of oxygen consumption (VO_2) and carbon dioxide (VCO_2) production of the body by using a metabolic gas monitor. This is the most preferred method due to its low cost and mobility of equipment (1-2).

There are two main ways that an IC RMR measurement may be considered a true and accurate RMR (3). First, the practitioner may use steady state (SS) criteria which are generally defined as the first five minutes in which VO_2 and VCO_2 change by less than a predetermined percentage. The second way is by using a pre-set time interval. This interval can vary from five minutes to over 2 hours, but most practitioners generally use an interval between 20-60 minutes (3).

Using SS criteria is often harder and more inconvenient to achieve than a time interval measurement, but some practitioners suggest that SS is more accurate than time interval measurements (3). However, research showing that SS measurement and time interval measurement are statistically and practically significantly different from each other in healthy adults has not yet been settled.

Purpose

The purpose of this research is to determine:

(1) If there is a statistically and practically significant difference between SS and time interval measurements of RMR in healthy adults.

APPENDIX B

Literature Review

Energy Expenditure

Energy is defined as the capacity to do work, and energy expenditure (EE) is defined as the energy needed to maintain body functions and other functions of daily living (1). Several components involved in measuring EE are described below.

Basal metabolic rate (BMR) is “the energy needed to sustain the metabolic activities of cells and tissues and to maintain circulatory, respiratory, gastrointestinal, and renal processes.” (1) This is measured in kcal/hour. In order for a measurement to be classified as a person’s BMR, several assumptions must be met. BMR must be measured early in the morning before any physical activity has been performed and 10-12 hours after the consumption of food, alcohol, or nicotine (1-2). The person must also be lying down and not performing mentally strenuous activities in a thermoneutral environment (1). BMR represents about 60-70% of total energy expenditure (TEE) (1). If this measurement is generalized to represent the amount of energy used in 24 hours, the measurement is then called the basal energy expenditure (BEE) (1-2). BMR is not usually measured, due to the strict nature of the requirements that need to be met. If any of those conditions are not met during measurement, the measurement is then called the resting metabolic rate (RMR), and it is also measured in kcal/hour (1-2). RMR is usually about 10-20% higher than BMR to account for the thermic effect of food or excess postexercise oxygen consumption (1). Resting energy expenditure (REE) is a measurement of the RMR over a 24 hour period and is expressed as calories per day (1-2).

The major component of EE is usually RMR. Accurate identification of RMR is important in both clinical and research settings (2).

Factors affecting Resting Metabolic Rate

Several factors affect RMR measurements. One of the biggest variables affecting RMR is body size. Larger people generally have a higher RMR, but tall and thin people have higher RMRs than short, wide people (1). Body composition is the other major variable in RMR. Fat-free mass (FFM) is the most metabolically active tissue in the body, and the more FFM a person has, the higher the RMR will be (1).

Age also affects RMR. RMR is highest during periods of growth, and once early adulthood is reached, RMR declines at about 1%-2% per kg of FFM per decade (1-2). This is due to loss of muscle mass, hormonal changes, and lost efficiency of organs (1-2). Gender also affects RMR, largely due to the amount of FFM in each gender. In general, women have more fat and less FFM which contributes to a lower RMR (1-2). Caffeine, nicotine, and alcohol also raise RMR because they are stimulants (1).

Measuring Resting Metabolic Rate

RMR is measured in three ways. Direct calorimetry measures the amount of heat produced by a person inside a metabolic chamber which determines how much energy they are producing. This method is very expensive and is rarely used (1-2). Doubly labeled water measures the amount of water turnover in the body. It is based on the concept that carbon dioxide production can be estimated from the difference in elimination rates of hydrogen and oxygen. The person consumes water that is labeled with isotopes, and the elimination rates of the two are measured for 10-14 days. The difference in their elimination is determined which then estimates carbon dioxide production which can then be converted to TEE (1-2). This method is expensive and impractical for daily use (1-2).

The most widely used technique is indirect calorimetry (IC). IC estimates energy expenditure by determining the amount of oxygen consumed and the amount of carbon dioxide produced, and based on the assumption that the oxygen is used to degrade fuels and all the carbon dioxide that is produced is recovered, the total amount of energy produced can be calculated (4,5). This is usually measured using a mouthpiece or ventilated hood into which the person breathes. The indirect calorimeter uses the Weir equation (below) to convert expired carbon dioxide (VCO_2) and inspired oxygen (VO_2) to determine RMR (Figure 1) (1-2).

Figure 1. The Weir Equation (6)

Complete Weir Equation

$$RMR = [3.9(VO_2) + 1.1(VCO_2)]\ 1.44 - 2.17\ UN$$

Abbreviated Weir Equation

$$RMR = [3.9(VO_2) + 1.1(VCO_2)]\ 1.44$$

There are three main types of collection devices when using an indirect calorimeter. They include rigid canopies, facemasks, and mouthpieces with nose clips. As long as the collection device is monitored and no air leaks occur, measurements are comparable among the devices (7).

Current Practice Recommendations

In order to record an accurate measurement of RMR, several protocols are recommended in order to increase the accuracy of the measurement. They include:

- Fasting for a minimum of 7 hours after meals and snacks
- Abstaining from alcohol and nicotine for a minimum of 2 hours
- Restricting resistance exercise for 48 hours before measurement
- Resting for 30 minutes before initiating a measurement
- Laying in a supine position while avoiding fidgeting during measurement
- Abstaining from reading, listening to music, or other activities during measurement
- Keeping the measurement room quiet, dimly lit, and between 72^{0}F and 75^{0}F (7)

Methods to Measure Resting Metabolic Rate Using Indirect Calorimetry

There are two main ways in which an IC RMR measurement is considered valid. The first is by using steady state (SS) criteria, which is defined differently between researchers and practitioners. McClave, et al (3) defines SS as "a single 5-minute period during which average VO_2, VCO_2, and respiratory quotient (RQ) change by less than a predetermined percentage range." However, SS is not used in all clinical and research settings due to the difficult nature of achieving SS in some subjects. Those clinicians instead choose to perform IC testing using a pre-determined time interval, generally between 20-60 minutes (3).

The first indirect calorimeter was made by Atwater and Rosa in 1892 (8). Francis Benedict then came on as an assistant to Atwater, and he made the first portable indirect calorimeter that was used in doctors' offices for metabolic studies in 1918 (9). Benedict published over 20 studies dealing with basal metabolism in the 1910s and 1920s as part of first studies using IC, and he used time interval measurements in his work (9). This method of performing IC was carried on until the mid-1980s.

The first publication that used SS as a measure of RMR was published in 1984. Feurer, Crosby, and Mullen (10) compared bedside IC measurements to the Harris-Benedict and Kleiber equations in hospitalized and control subjects. Their criteria for a valid SS measurement was "five consecutive, stable 1-minute measurements of VO_2 and VCO_2 which were within a range of 5%." (10) However, the authors provided no justification or reference as to why using SS criteria is a more accurate reflection of RMR compared to time interval measurements. A few other studies in the late 1980s and early 1990s also used SS criteria and made the practice of using SS to measure RMR more widely accepted and even promoted in manuals about IC measurement (11-16). The Academy of Nutrition and Dietetics released recommendations in 2006 which supported using SS criteria when measuring RMR by IC in both healthy and critically ill individuals (17). The newest guidelines released by the Academy in 2015 also recommend SS when measuring RMR, but this recommendation received a Grade III recommendation, meaning the evidence to support this is weak and conditional (7).

When using the time interval method to measure RMR using IC, recommendations vary as to how long a subject should be measured. Most practitioners use a measure of 20-60 minutes, but some recommended intervals from 5 minutes to several hours (18,19). However, one aspect of time interval IC measurements that has been defined is that the first 5 minutes of data collection should be discarded due to acclimatization to testing conditions (7,20). Isbell (20) found that the first 5 minutes of data collection were much more variable compared to the rest of the testing period and should therefore be discarded. Schols (21) found that a 7 minute acclimatization period was needed, but the group RMR mean did not change between time intervals of 5, 10, 15, 20, 25, and 30 minutes. Therefore, there are many acceptable time intervals that may be used when measuring IC as long as the first 5-7 minutes of data collection is discarded.

Steady State Comparison to Resting Metabolic Rate

Many practitioners use SS criteria when performing RMR IC measurements. This is likely because they presume that this measure represents the body completely at rest and therefore RMR (3). A few researchers have tried to prove that SS measurements are in fact indicative of true RMR.

Daly (22) measured RMR in healthy adults to compare direct calorimetry and IC to the Harris-Benedict equation prediction of RMR. The publication states that VO_2, VCO_2, and respiratory exchange ratio were measured every 2 minutes until they were stable for 6 minutes, although it did not define the definition of stable, after which the three final results were averaged which provided a mean measurement of each variable. The researchers did find the

Harris-Benedict equation overestimated RMR, and there was a 3% difference between direct and indirect measurements of RMR (22).

Frankenfield (23) compared RMR to TEE in 33 patients in an intensive care unit. All patients were mechanically ventilated and either septic or had experienced recent trauma. The researchers measured RMR in these patients for 15-25 minutes while completely at rest without any interference which was then compared to a period of 12 hours where regular activity was allowed, which was termed as total energy expenditure. Any measure of VO_2, VCO_2, RQ, and RMR that was not within 2% was regarded as measurement error and automatically discarded; therefore, this study measured RMR using SS criteria. The researchers found that there was no statistically significant difference between RMR and TEE which would suggest that using SS criteria for RMR measurements accurately reflects RMR in this mechanically ventilated, critically ill population (23).

These two studies compare using SS criteria to see if it truly measures RMR, but neither conclusively prove that SS is the best method for measuring RMR.

Previous Research about Steady State in the Critically Ill

There have been many studies about using SS criteria when measuring RMR in the critically ill population. It is a known fact that RMR varies throughout the day, and this is amplified in critically ill patients due to their unstable nature (24). Hence, many practitioners that work in a critically ill population use SS criteria in order to assure that the patient's RMR measurement is as close to resting as possible (24).

Studies using SS criteria were conducted in tube-fed adult patients with severe neurodevelopmental disabilities (25,26), patients with severe burns (27), mechanically ventilated patients (3,28-32), patients with systemic inflammatory response syndrome/multiple organ dysfunction syndrome (23), patients with a traumatic brain injury (24), patients with cancer (33), and patients with respiratory distress syndrome (34). None of these studies proved that using SS criteria is better than using a time interval, but the researchers likely assume that SS represents baseline RMR.

There are a few studies that used SS criteria in a healthy population. Cunha (35) studied 30 healthy men in order to determine the time it takes to achieve steady state in a healthy population. However, the subjects only rested for 10 minutes before RMR measurements began, when the recommendation is generally 30 minutes. The researchers did find that it takes about 30 minutes for healthy men to achieve steady state. Reidlinger (36) studied 34 healthy, older men and women to compare their measured RMR to their calculated RMR using six commonly used equations. The researchers used SS criteria in order to determine the measured RMR of the subjects. They found that most equations are inaccurate, although the Mifflin-St. Jeor equation was the closest. These two studies are the few that use SS criteria when measuring RMR by IC in healthy subjects, and there has been no definitive research that using SS criteria in a healthy population is necessary.

24-Hour Resting Metabolic Rate Measurements

In order to determine which method is best at measuring true RMR, a study would need to be conducted which measures a person for 24 hours while lying completely at rest without eating or drinking the entire duration of the study. This study is very unlikely to be conducted due to the difficult nature of completing this study. However, there have been a few studies that measured 24-hour TEE and then compared it to RMR. Grunwald (37), Rasvussin (38), and Rumpler (39) all conducted 24-hour measurements of TEE and compared it to a shorter measure of RMR. For the 24-hour measurement, the subjects were able to walk around, read, write, watch TV, eat, etc.; therefore, this does not meet RMR criteria and is therefore considered TEE. As would be expected, the 24-hour TEE is significantly higher than RMR and cannot be used to compare to RMR.

There have also been some studies comparing a time interval of RMR to a daily RMR in mechanically ventilated, critically ill patients. Smyrnios (30) compared 24 hour RMR to different 30 minute time intervals, and he found that the time intervals were closely associated with the 24 hour RMR. Van Lanschot (31) compared 24 hour RMR to a 5 minute interval at 8 am, a 10 minute interval at 8 am, and two 5 minute intervals at 8 am and 5 pm. The researchers found that that the 2 RMR measurements at 8 am and 5 pm were closest to the 24 hour RMR. Weismann (32) compared 24 hour RMR to 8 hour RMR and found that there was no significant difference.

Overall, these studies give us a better picture that time interval measurement does correlate to a daily RMR, but they do not completely demonstrate that using a time interval measurement of RMR is the best method when measuring RMR in the healthy adult population.

Prior Research Comparing Steady State to Time Interval Measurements

There were two main studies that specifically compared time interval measurements of RMR to SS RMR. The first was published by McClave et al (3). McClave studied TEE and RMR in 22 patients with respiratory failure. The researchers measured RMR for 24 hours to obtain TEE. During the first hour of measurement, RMR was averaged over intervals of 20, 30, 40, and 60 minutes, and also for different definitions of SS where VO_2 and VCO_2 changed by <10%, <15%, and <20% for 5 consecutive minutes. The researchers then compared all of these various definitions of RMR to see which one most closely reflected true RMR measured over 24 hours. They found that the best correlation to 24 hour RMR was achieved when RMR was measured using SS criteria where VO_2 and VCO_2 varied by <10%. This study was performed in a critically ill population whose RMR is likely more variable than the average healthy person, and

SS criteria would likely most closely reflect RMR. But for a healthy population, this may not be the case.

The second study was performed by Horner et al (40). Horner measured the RMR of 102 postmenopausal women in order to compare different time intervals and SS criteria in order to determine which definition of RMR Is best to use in the postmenopausal women population. If the subject met SS criteria of 10 minutes with <10% variation in VO_2, RQ, and minute ventilation, then the test was stopped. If the subject did not meet that criteria by 30 minutes, the test was continued until that patient achieved 10 minutes with <10% variation or 45 minutes, whichever came first. The researchers then compared 0-5, 5-10, 5-15, 5-20, 5-25, 5-30, and 0-30 minutes along with different sets of SS criteria of <10% variation for 5 min, <10% variation for 10 minutes, <5% variation for 5 min, <5% variation for 10 min. The researchers found that SS criteria of <5% variation for 10 minutes was very hard to achieve and not practical to use. They also found that the first 5 minutes of the study was more reactive than the rest of the study and should therefore be discarded, as has been shown previously (18,19). The researchers did not find any practically significant differences in RMR when comparing any of the time intervals to the SS criteria and therefore recommended that practitioners use a time interval of 10 minutes to measure RMR in order to decrease subject burden. However, this is a very narrow patient population and does not use Bland-Altman plots in order to compare the agreement between the time interval and SS criteria method. We intend to improve upon this study by measuring a more varied subject population and using Bland-Altman plots to assess agreement between the methods of the study.

Bland-Altman Plots

Bland and Altman first designed a way to compare two methods of the same procedure in 1983, but their research was not published to the medical field until 1986 (41). The researchers suggest that using a correlation coefficient (r) when comparing two methods of performing the same procedure does not establish agreement between the two devices and can therefore be misleading. Bland and Altman then devised a way to compare two methods in order to show the agreement between the two by plotting the difference of the individual measures from the mean against the mean. This helps to determine if there is any relationship between the measurement error and the true value. Limits of agreement are also calculated to give a range in which we expect 95% of the samples to lie (41).

When reporting results of a study using a Bland-Altman plot, six items need to be reported (42):

1. Correct representation of x-axis on the Bland-Altman plot
2. Representation and correct definition of limits of agreement
3. Reporting of confidence interval of the limits of agreement
4. Comparison of the limits of agreement with *a priori* defined clinical criteria
5. Evaluation of the pattern of the relationship between difference (y-axis) and average (x-axis)
6. Measures of repeatability (42)

This statistical method is particularly useful for clinicians when comparing, for example, a new medical device or procedure to an established, proven device or procedure in order to assure that the new device or procedure is performing as expected and agrees with the standard. And as long as the results are reported correctly and appropriately, this statistical method can give insight.

Literature Review References

16. Mahan LK, Escott-Stump S, Raymond JL. Krause's Food and the Nutrition Care Process. 13th ed. St. Louis: Saunders Elsevier; 2012.
1. Fullmer S. Lecture notes. Clinical nutrition I, Brigham Young University, Sep. 20, 2010.
2. McClave SA, Spain DA, Skolnick JL, Lowen CC, Kleber MJ, Wickerham PS, Vogt JR, Looney SW. Achievement of steady state optimizes results when performing indirect calorimetry. *JPEN J Parenter Enteral Nutr*. 2003;27:16-20.
3. Ferranini E. The theoretical bases of indirect calorimetry: a review. *Metab.* 1988;37:287-301.
4. Head CA, McManus CB, Seitz S, Grossman GD, Staton Jr GW, Heymsfield SB. A simple and accurate indirect calorimetry system for assessment of resting energy expenditure. *JPEN J Parenter Enteral Nutr.* 1984;8:45-48.
5. Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol.* 1949;109:1-9.
6. Fullmer S, Benson-Davies S, Earthman C, Frankenfield D, Gladwell E, Lee P, Piemonte T, Trabulsi J. Evidence analysis library review of best practices for performing indirect calorimetry in healthy and non-critically ill individuals. *J Acad Nutr Diet.* 2015;115:1417-1446.
7. Maynard LA. Francis Gano Benedict – a biographical sketch. *J Nutr.* 1969;98:1-8.
8. Dubois EF, Riddle O. Francis Gano Benedict 1870-1957. *Natl Acad Sci.* 1958;67-99.
9. Feurer ID, Crosby LO, Mullen JL. Measured and predicted resting energy expenditure in clinically stable patients. *Clin Nutr.* 1984;3:27-34.
10. Feurer I, Mullen JL. Bedside measurement of resting energy expenditure and respiratory quotient via indirect calorimetry. *Nutr Clin Pract.* 1986;1:43-49.
11. Foster GD, Knox LS, Dempsey DT, Mullen JL. Calorie requirements in total parenteral nutrition. *J Am Coll Nutr.* 1987;6:231-253.
12. Makk LJK, McClave SA, Creech PW, Johnson DR, Short AF, Whitlow NL, Priddy FS, Sexton LK, Simpson P. Clinical application of the metabolic cart to the delivery of total parenteral nutrition. *Crit Care Med.* 1990;18:1320-1327.
13. Mullen JL. Indirect calorimetry in critical care. *Proc Nutr Soc.* 1991;50:239-244.
14. Zavala DC. *Nutritional assessment in critical care: a training handbook.* 1st ed. Iowa City, IA: University of Iowa Center for the Book; 1989.
15. Matarese LE. Indirect calorimetry: technical aspects. *J Am Diet Assoc.* 1997;97:S154-S160.
16. Compher C, Frankenfield D, Keim N, Roth-Yousey L. Best practice methods to apply to measurement of resting metabolic rate in adults: a systematic review. *J Am Diet Assoc.* 2006;106:881-903.
17. Leff ML, Hill JO, Yates AA, Cotsonis GA, Heymsfield SB. Resting metabolic rate: measurement reliability. *JPEN J Parenter Enteral Nutr*. 1987;11:354-359.
18. Long CL, Schaffel N, Geiger JW, Schiller WR, Blakemore WS. Metabolic response to injury and illness: estimation of energy and protein needs from indirect calorimetry and nitrogen balance. *JPEN J Parenter Enteral Nutr*. 1979;3:452-456.
19. Isbell TR, Klesges RC, Meyers AW, Klesges LM. Measurement reliability and reactivity using repeated measurements of resting energy expenditure with a face mask, mouthpiece, and ventilated canopy. *JPEN J Parenter Enteral Nutr.* 1991;15:165-168.

20. Schols AMWJ, Schoffelen PFM, Ceulemans H, Wouters EFM, Saris WHM. Measurement of resting energy expenditure in patients with chronic obstructive pulmonary disease in a clinical setting. *JPEN J Parenter Enteral Nutr.* 1992;16:364-368.
21. Daly JM, Heymsfield SB, Head CA, Harvey LP, Nixon DW, Katzeff H, Grossman GD. Human energy requirements: overestimation by widely used prediction equation. *Am J Clin Nutr.* 1985;42:1170-1174.
22. Frankenfield DC, Wiles III CE, Bagley S, Siegel JH. Relationships between resting and total energy expenditure in injured and septic patients. *Crit Care Med.* 1994;22:1796-1804.
23. McEvoy C, Cooke SR, Young IS. A reduced abbreviated indirect calorimetry protocol is clinically acceptable for use in spontaneously breathing patients with traumatic brain injury. *Nutr Clin Pract.* 2009;24:513-519.
24. Dickerson RN, Brown RO, Hanna DL, Williams JE. Effect of upper extremity posturing on measured resting energy expenditure of nonambulatory tube-fed adult patients with severe neurodevelopmental disabilities. *JPEN J Parenter Enteral Nutr.* 2002;26:278-284.
25. Dickerson RN, Brown RO, Gervasio JG, Hak EB, Hak LJ, Williams JE. Measured energy expenditure of tube-fed patients with severe neurodevelopmental disabilities. *J Am Coll Nutr.* 1999;18:61-68.
17. Dickerson RN, Gervasio JM, Riley ML, Murrell JE, Hickerson WL, Kudsk KA, Brown RO. Accuracy of predictive methods to estimate resting energy expenditure of thermally-injured patients. *JPEN J Parenter Enteral Nutr.* 2002;26:17-29.
26. Frankenfield DC, Sarson GY, Blosser SA, Cooney RN, Smith JS. Validation of a 5-minute steady state indirect calorimetry protocol for resting energy expenditure in critically ill patients. *J Am Coll Nutr.* 1996;15:397-402.
27. Petros S, Engelmann L. Validity of an abbreviated indirect calorimetry protocol for measurement of resting energy expenditure in mechanically ventilated and spontaneously breathing critically ill patients. *Intensive Care Med.* 2001;27:1164-1168.
28. Smyrnios NA, Curley FJ, Shaker KG. Accuracy of 30-minute indirect calorimetry studies in predicting 24-hour energy expenditure in mechanically ventilated, critically ill patients. *JPEN J Parenter Enteral Nutr.* 1997;21:168-174.
29. Van Lanschot JJB, Feenstra BWA, Vermeij CG, Bruining HA. Calculation versus measurement of total energy expenditure. *Crit Care Med.* 1986;14:981-985.
30. Weissman C, Kemper M, Elwyn DH, Askanazi J, Hyman AI, Kinney JM. The energy expenditure of the mechanically ventilated critically ill patient: an analysis. *Chest.* 1986;89:254-259.
31. Reeves MM, Davies PSW, Bauer J, Battistutta D. Reducing the time period of steady state does not affect the accuracy of energy expenditure measurements by indirect calorimetry. *J Appl Physiol.* 2004;97:130-134.
32. Ronco JJ, Phang PT, Walley KR, Wiggs B, Fenwick JC, Russell JA. Oxygen consumption is independent of changes in oxygen delivery in severe adult respiratory distress syndrome. *Am Rev Respir Dis.* 1991;143:1267-1273.
33. Cunha FA, Midgley AW, Monteiro W, Freire R, Lima T, Farinatii PTV. How long does it take to achieve steady state for an accurate assessment of resting VO_2 in healthy men? *Eur J Appl Physiol.* 2013;113:1441-1447.

34. Reidlinger DP, Willis JM, Whelan K. Resting metabolic rate and anthropometry in older people: a comparison of measured and calculated values. *J Hum Nutr Diet*. 2015;28:72-84.
35. Grunwald GK, Melanson EL, Forster JE, Seagle HM, Sharp TA, Hill JO. Comparison of methods for achieving 24-hour energy balance in a whole-room indirect calorimeter. *Obes Res*. 2003;11:752-759.
36. Rasvussin E, Burnand B, Schutz Y, Jequier E. Twenty-four-hour energy expenditure and resting metabolic rate in obese, moderately obese, and control subjects. *Am J Clin Nutr*. 1982;35:566-573.
37. Rumpler WV, Seale JL, Conway JM, Moe PW. Repeatability of 24-h energy expenditure measurements in humans by indirect calorimetry. *Am J Clin Nutr*. 1990;51:147-152.
38. Horner NK, Lampe JW, Patterson RE, Neuhouser ML, Beresford SA, Prentice RL. Indirect calorimetry protocol development for measuring resting metabolic rate as a component of total energy expenditure in free-living postmenopausal women. *J Nutr*. 2001;131:2215-2218.
39. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986;1(8476):307-310.
40. Chhapola V, Kanwal SK, Brar R. Reporting standards for Bland-Altman agreement analysis in laboratory research: a cross-sectional survey of current practice. *Ann Clin Biochem*. 2015;52:382-386.

APPENDIX C

Methods

Study Design

A cross-sectional study using a convenience sample of 77 male and female subjects was recruited from the faculty, staff, and students at our institution and the surrounding area. Recruitment efforts involved flyers sent to all faculty and staff and displayed throughout campus, announcements in classes, and by word of mouth. Inclusion criteria were healthy subjects between the ages of 18-65 years. Pregnant and lactating women were excluded. The Institutional Review Board approved the study protocol, the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation, and all participants provided written informed consent.

Procedures

Following recruitment, subjects came in for one visit. The study procedures were explained, and informed consent was given. Height was measured prior to the IC measurement using a professional grade stadiometer, Model PE-WM-60-76-BRG2 (Perspective Enterprises, Portage, MI). Subjects were measured to the nearest centimeter while standing without shoes with weight evenly distributed. A Tanita 310 electronic scale (Arlington Heights, IL) was used to assess weight. Subjects were weighed to the nearest kilogram with minimal clothing and without shoes.

Indirect Calorimetry Measurements

IC tests followed current protocols (5) including: starting the test between 6:00 am and 9:00 am, following an overnight fast, and no showering the morning of the test. Subjects were asked to park close to the building and abstain from any stimulants and exercise for 24 hours before measurement. Upon arrival, subjects rested for 30 minutes in a reclined position prior to data collection. All measurements were taken in a quiet, thermoneutral room with dimmed lights to avoid any environmental influences on RMR. A blanket was provided if requested.

RMR was measured using a Quark RMR indirect calorimeter (COSMED, Rome, Italy). Calibrations on the mass flow sensor and gas analyzers were performed prior to each study. The gas used for calibrations was 16% O_2/5% CO_2. A clear canopy was placed over the subjects' heads to collect gas samples.

Once data collection began, RMR was measured for 45 minutes with the first five minutes being discarded for acclimatization purposes (5). SS was defined as the first five minutes when both VO_2 and VCO_2 varied by <10%. If subjects failed to achieve SS during the 45 minute study, their results were discarded and not included in the final SS statistical analysis. Periods of SS were then compared to different time intervals (minutes 6-10, minutes 6-15, minutes 6-20, minutes 6-25, minutes 6-30, minutes 6-45).

Statistical Analysis

A power analysis based on a 75 calories ± standard deviation of 160 with 80% power and a p-value of 0.05 suggested 75 subjects. The differences between measures were determined with paired-T-tests, and demographic information was analyzed using Statistical Analysis Software (Version 9.5, SAS Institute Inc., Cary, NC). Results are reported as means ± standard deviations and ranges. The limits of agreement between measures were analyzed with Bland-Altman plots using GraphPad Prism (Version 6.02, GraphPad Software Inc., San Diego, CA). The SS measurements were subtracted from the time interval measurements, so a positive difference indicates that SS was lower than the time interval. A lack of bias was defined when the differences between measurements were evenly distributed along the 0 line (i.e.50% of differences are greater than 0 and 50% less than 0). Precision was determined by the number of differences that fell outside of the 95% limits of agreement. Accuracy was determined by counting the number of absolute differences that were greater than 75 calories.

APPENDIX D

Restatement of the Problem

When measuring a subject's resting metabolic rate (RMR) by indirect calorimetry (IC), there are two ways that a measurement is considered true and accurate. The first is by using steady state (SS) criteria, which is generally defined as the first five minutes in which VO_2 and VCO_2 change by less than a predetermined percentage. The second is by using a predetermined time interval, usually between 20-60 minutes. However, using SS criteria is often harder and more inconvenient to achieve than a time interval measurement, and there are no studies that show that there is a statistical and practical difference between SS and time interval measurements in healthy adults.

Purpose of the Study

The purpose of this study was to determine if there is a statistically and practically significant difference between SS and varying time interval measurements of RMR in healthy adults. The results of this study can then be used to provide further guidance as to which method of measuring RMR by IC is most accurate while also being least burdensome to the subject in both research and clinical settings.

A power analysis based on a 75 calories ± standard deviation of 160 with 80% power and a p-value of 0.05 suggested 75 subjects. The differences between treatments were determined with paired-T-tests using Statistical Analysis Software (Version 9.5, SAS Institute Inc.). Results are reported using means ± standard deviations and ranges. A p-value <0.05 was considered statistically significant. Demographic information was also analyzed using Statistical Analysis Software (Version 9.5, SAS Institute Inc.). Results are reported as means ± standard deviations and ranges. The agreement between measures was analyzed with Bland-Altman plots using GraphPad Prism (Version 6.02, GraphPad Software Inc.).

Results

Patient Characteristics

Of the 166 subjects who were screened, 78 started the study and 77 completed the study. Subject characteristics are found in Table 1. Males and females were equally represented, and the age range was broad, from 18-65 years. The majority of subjects (88%) had a normal BMI (mean 24.2 $kg/m^2 \pm 4.7 kg/m^2$), five subjects (7%) had a BMI >30 kg/m^2 and four subjects (5%) had a BMI <18.5 kg/m^2. The mean RMR for the study population was 1,570 calories (± 281 calories).

Time to Steady State

Of the 77 participants, 84% (n = 65) achieved SS; the remaining 16% of subjects never achieved SS during the 45 minute study period. The median time to SS was minute 11 (n = 32), and 38% of subjects achieved SS by minute 10 (n = 24) and 72% by minute 15 (n = 47) (Figure 1). After minute 15, some participants continued to achieve SS, but no consistent patterns emerged as to when another large group of participants achieved SS. Of those subjects who achieved SS, 95% did so by minute 30. Only two subjects achieved SS between minutes 30-45. It is important to note that we only determined when subjects first achieved SS, not how long they remained in SS.

Steady State vs Time Intervals

Various time intervals were compared to SS. When comparing minutes 6-10 to SS, there was a statistically significant difference in the means of VCO_2 and calories ($p = 0.0025$, $p = 0.0439$; respectively) (Table 2). However, the average calorie difference was only 12 which is not practically significant. The Bland-Altman plot comparing minutes 6-10 to SS is shown in Figure 2. Because many subjects achieved SS during this time period, 37% of the differences between measurements were zero. 75% of the differences favored SS or were not different than the 6-10 minute time interval (Table 3); additionally, 6% of the differences fell outside of the 95% limits of agreement. 20% of absolute differences were greater than 75 calories (Table 3).

Comparing SS to minutes 6-15 showed a statistically significant difference in VCO_2 and calories ($p = 0.0174$, $p = 0.0222$; respectively) but not a practical one, with an average difference of only 13 calories (Table 4). The Bland-Altman plot showed that the difference between these measures was fairly unbiased with 57% of the values being lower in SS (Figure 3). Only 3% of the differences fell outside of the 95% limits of agreement; 17% of absolute differences were greater than 75 calories (Table 3).

Minutes 6-20 compared to SS showed a statistically significant difference in VCO_2 and calories ($p = 0.0128$, $p = 0.0219$; respectively) but only an average difference of 12 calories (Table 5). Minutes 6-20 showed the least bias with 52% of differences being lower in SS (Figure 4). Only 3% of the differences fell outside of the 95% limits of agreement, and only 9% of absolute differences were greater than 75 calories (Table 3).

When comparing minutes 6-25 to SS, all variables (VO_2, VCO_2, and calories) were statistically different from one another ($p = 0.0434$, $p = 0.0083$, $p = 0.0095$; respectively) with an average difference of 12 calories (Table 6). Minutes 6-25 were very precise with only 1.5% of differences falling outside the 95% limits of agreement (Figure 5). The differences between measurements were fairly unbiased with 62% of differences favoring SS. However, only 6% of absolute differences in measurements were greater than 75 calories (Table 3).

All variables were again statistically significant for minutes 6-30 compared to SS ($p = 0.0170$, $p = 0.0036$, $p = 0.0033$; respectively) with an average difference of 15 calories (Table 7). 66% of measurements were lower for SS than minutes 6-30, and 3% of the differences between

SS and minutes 6-30 fell outside the 95% limits of agreement (Figure 6). During minutes 6-30 compared to SS, 11% of absolute differences were greater than 75 calories (Table 3).

Finally, there were statistically significant differences between SS and minutes 6-45 for all variables ($p = 0.0141$, $p = 0.0006$, $p = 0.0018$; respectively) (Table 8). Differences between measures were biased with 67% of values favoring SS (Table 3), and 6% of the differences fell outside of the 95% limits of agreement (Figure 7). Also, 9% of absolute differences between measurements fell outside of the 75 calorie limit (Table 3).

Demographics and Time to Steady State

Demographic characteristics were analyzed post-hoc to determine if they were related to time to SS. Backward elimination found no relationships when using age, weight, and BMI with time to SS. When gender (female) and height were assessed as individual predictor variables, each was slightly statistically significant ($p = 0.02$, $p = 0.04$; respectively). However, when these variables were analyzed together, each became insignificant ($p = 0.12$, $p = 0.50$; respectively). There is likely no relationship between gender and height in regards to how quickly SS is achieved.

APPENDIX E

Consent to be a Research Subject

Introduction

This research study is being conducted at Brigham Young University by Chelsea Irving, RD and Susan Fullmer, PhD, RD to determine if there is a statistically and practically significant difference between steady state and time interval measurement of resting metabolic rate (RMR).

Criteria for participation

- Healthy men and women 18-65 years of age

Conditions that exclude study participation

- Pregnancy or lactation

Procedures

If you agree to participate in this research study, you will be asked to come to the Nutrition Assessment Lab, S-288 of the Eyring Science Center at Brigham Young University for one visit.

On the day of your visit, we will orient you to the study, obtain your height and weight, and introduce to you the study procedures. This will last 10 minutes. To get a more accurate weight measurement, light clothing during this visit is encouraged.

Next, you will be seated in a reclining chair for approximately 30 minutes in order for you to rest. We will then measure your metabolic rate for 60 minutes using an indirect calorimeter which collects oxygen consumption and carbon dioxide production through a clear canopy that is placed over your head. Indirect calorimetry testing is done under the following conditions:

- Early morning (between 6:00 am and 8:00 am)
- No food or drink for 12 hours prior to measurement
- No caffeine intake 24 hours prior to measurement
- No exercise for 24 hours prior to measurement
- Unshowered (you may shower the night before but not the morning of)

After we have completed this measurement, you will be free to go. This visit will take about two hours.

Risks/Discomforts

There are few risks anticipated for this study. Hunger and discomfort may occur due to abstaining from food and water for up to 14 hours. Rarely individuals may also experience mild claustrophobia under the canopy. If any of the symptoms associated with claustrophobia, the test will be stopped immediately. You will then have the option of continuing with the test of withdrawing from the study.

Compensation

Participants will receive a report of their RMR and have the opportunity to discuss it with a registered dietitian.

Benefits

Benefits may include learning the best way to measure RMR which will help define acceptable protocols for measuring RMR in research and clinical settings.

Confidentiality

Strict confidentiality will be maintained. No individual identifying information will be disclosed. Your name will be on file for contact purposes only. All identifying references will be removed and replaced by control numbers. It is the intention of investigators to report and publish the mean values and other statistical reports of all subjects. Your personal information and the results of your individual measurement will not be distributed.

Participation

Participation in this research study is voluntary. You have the right to withdraw at any time or refuse to participate entirely.

Questions about the Research

If you have questions regarding this research study, you may contact Chelsea Irving, RD at (801) 913-9933, cirving18@gmail.com, or Susan Fullmer, PhD, RD, CD, S-277 ESC, Brigham Young University, Provo, UT, (801) 422-3349, susan_fullmer@byu.edu.

If you have questions regarding your rights as a participants in a research project, you may contact an IRB administrator at (801) 422-1461; A-285 ASB, Brigham Young University, Provo, UT 84602; irb@byu.edu.

Statement of Consent

I, ____, have read, understood, and received a copy of the above consent form and desire of my own free will to participate in this study.

____ ______________________

Participant Signature Date

Recruitment Script

I am conducting research on different criteria for measuring resting metabolic rate, or RMR, which is the rate at which your body burns fuel when completely at rest.

I will you need to you come to Brigham Young University for one visit which will last for about 2 hours. We will first explain the study procedures to you, and you will then signed an informed consent. Then we will measure your height and weight. Next, you will rest for 30 minutes, and then I will measure your RMR for 45 more minutes using an indirect calorimeter which is a canopy placed over your head and measures how much carbon dioxide you produce and how much oxygen you consume. You will need to fast for 12 hours before this and also not consume any caffeine or exercise for 24 hours before this.

This is completely voluntary and there is no pressure to participate. If you wish to participate, you can email me at cirving18@gmail.com. Thank you!

WANTED

Volunteers for a Resting Metabolic Rate Research Study

Have you ever wondered what your metabolic rate is?
If you are:

- between the ages of 18-65 years old
- are not currently pregnant or lactating

You could qualify for a study on campus!

There is no financial cost to you. Participation will include one visit to the Nutrition Assessment Lab, S-288 of the Eyring Science Center. Total time commitment will be about 2 hours. You will receive a print-out of your resting metabolic rate with the opportunity to discuss it and any other nutrition-related questions with a Registered Dietitian.

If interested, please contact Chelsea Irving, RD, CD at cirving18@gmail.com.

Department of Nutrition, Dietetics, & Food Science