Room Indirect Calorimetry Operating and Reporting Standards (RICORS 1.0)

Obesity

Room Indirect Calorimetry Operating and Reporting Standards (RICORS 1.0): A Guide to Conducting and Reporting Human Whole-Room Calorimeter Studies

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Whole-room indirect calorimeters have been used to study human metabolism for more than a century. These studies have contributed substantial knowledge to the assessment of nutritional needs and the regulation of energy expenditure and substrate oxidation in humans. However, comparing results from studies conducted at different sites is challenging because of a lack of consistency in reporting technical performance, study design, and results. In May 2019, an expert panel was convened to consider minimal requirements for conducting and reporting the results of human whole-room indirect calorimeter studies. We propose Room Indirect Calorimetry Operating and Reporting Standards, version 1.0 (RICORS 1.0) to provide guidance to ensure consistency and facilitate meaningful comparisons of human energy metabolism studies across publications, laboratories, and clinical sites.

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Study Importance

What is already known?

- Studies of humans using whole-room indirect calorimetry have advanced our understanding of energy metabolism in humans.
- There are a limited number of operational calorimeters around the world, but several new calorimeters have opened in recent years.
- There are no agreed-upon standards for reporting the technical aspects or study results using whole-room indirect calorimeters.

What does this review add?

- Most calorimeters are custom built with variability in room dimensions, instrumentation, environmental controls, and data processing.
- Guidance is needed to improve the ability to compare data among studies, educate and guide new researchers to the field, and facilitate preparations and reviews of new publications
- To address these issues, an expert panel was convened to consider minimal requirements for conducting and reporting the results of human whole-room indirect calorimeter studies.

Introduction

The idea that the human body produces heat was a topic of fascination of the ancient Greeks, who believed that "vital heat" was produced by the heart, maintained by breathed air, and circulated through the blood. Aristotle postulated a link between energy intake, heat production, and breathing, nearly perfectly capturing the concepts of direct and indirect calorimetry (1). In the late 1700s, Antoine-Laurent Lavoisier made the first measurements of oxygen consumption in humans at rest, during exercise, and during exposure to different temperatures (2). Around the same time, Lavoisier and Laplace reasoned that the amount of ice melted by a hot object as it cooled down to 0 °C would be proportional to heat production. They subsequently demonstrated this using a guinea pig (3). In 1862, building on the progression of technology to measure expired gases, Pettenkofer constructed the first whole-room indirect calorimeter to measure respiratory gas exchange in humans

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(Figure 1) (4). In 1905, Atwater and Benedict built a calorimeter that measured both heat exchange (i.e., direct calorimetry) and respiratory gas exchange simultaneously, and they confirmed that both methods could accurately quantify human energy expenditure (EE) (5). With advances in technology, the use of whole-room indirect calorimeters expanded. Groups at Cambridge, UK (6,7), and in Lausanne, Switzerland (8-10), set the early standard for adapting new techniques to measurement of EE in humans and even extended its use in rural Gambia, Africa (8). The principles of room calorimetry have been extended to specialized calorimeters to measure metabolic rates in infants (11,12). Many other prominent groups have built on their expertise over the years, incorporating the latest technological advances into room calorimeter systems, which now employ indirect calorimetry almost exclusively.

How might these results change the direction of research?

- Recommending standards for performance testing and reporting will facilitate comparison of study results from different sites.
- This document can also serve as a guide to reviewers to ensure that appropriate information is reported to enhance the rigor and reproducibility of research using whole-room indirect calorimetry.
- The RICORS recommendations can guide further improvements in calorimetry technological developments and practical operations.

Whole-room indirect calorimetry allows participants to move freely, and the extended time of measurement (24 hours to several days) permits differentiation of the components of EE (e.g., sleeping, resting, physical activity, thermic effect of food). These systems are open-circuit, as the room is ventilated (diluted) with "fresh air," the expired gases by the study participant are mixed, and the excurrent air is sampled to determine oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) (Figure 2). Most calorimeter systems allow for a broad range of temperature settings, which can be useful to ensure thermal comfort or to study the effects of ambient temperature on energy metabolism. However, in most settings, temperature and relative humidity are kept relatively constant during a measurement period. Participants' physical activity is often monitored using wall- or ceiling-mounted Doppler-based motion sensing systems (13). Alternatively, wearable devices, such as accelerometers or heart rate sensors, and force platforms (14) can also quantify the intensity of participants' activity.

Because of the challenges of detecting small changes of gas concentrations in a large volume of air, early calorimeters had slow response times, with $\dot{V}O_2$ and $\dot{V}CO_2$ measured in intervals of 10 to 15 minutes (15,16). Improvements in technology and equipment, combined with the application of sophisticated signal processing techniques, have contributed to the development of systems with a response time on the order of one to several minutes (17-19). Most systems either push air into or pull air from the room, whereas some employ a "push-pull" that minimizes variability in CO_2 concentration in the calorimeter (17). Regardless of approach, leakage of air into or out of the room must be minimized. It is ideal to have a single air entry point to the room or at least confidence that all air entering is of the same composition. All systems also require complete mixing of air so the "sampled" air gas concentrations are accurate representations of the entire room. For comprehensive reviews on the history and technical advances in open-circuit respirometry, the reader is referred to the excellent textbooks by Paul Webb (16) and John Lighton (20), as well as a recent review by Schoffelen and Plasqui (21).

There are currently more than 40 research laboratories on four continents that house whole-room indirect calorimeters for human studies (Table 1). The limited availability is due to expense and the technical expertise required to support their operation and maintenance. Types of study include assessments of energy need for various populations, evaluation of wearable sensors, and interventions of diet, sleep, exercise, temperature, and pharmacological agents. However, there are currently no accepted standards for calibration and operation, quality control, protocol design, signal processing, or data reporting. As a result, comparing results between laboratories is often difficult, and the ability to perform multicenter trials is limited.

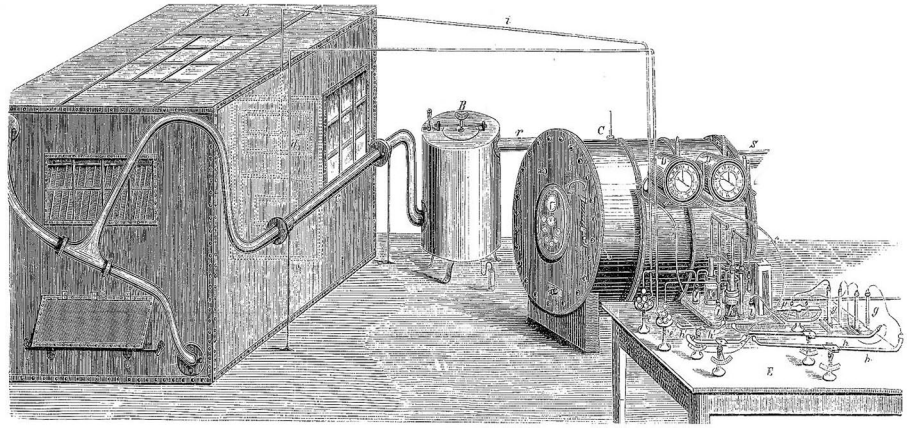

Figure 1 The first human open-circuit calorimeter built by Pettenkofer.

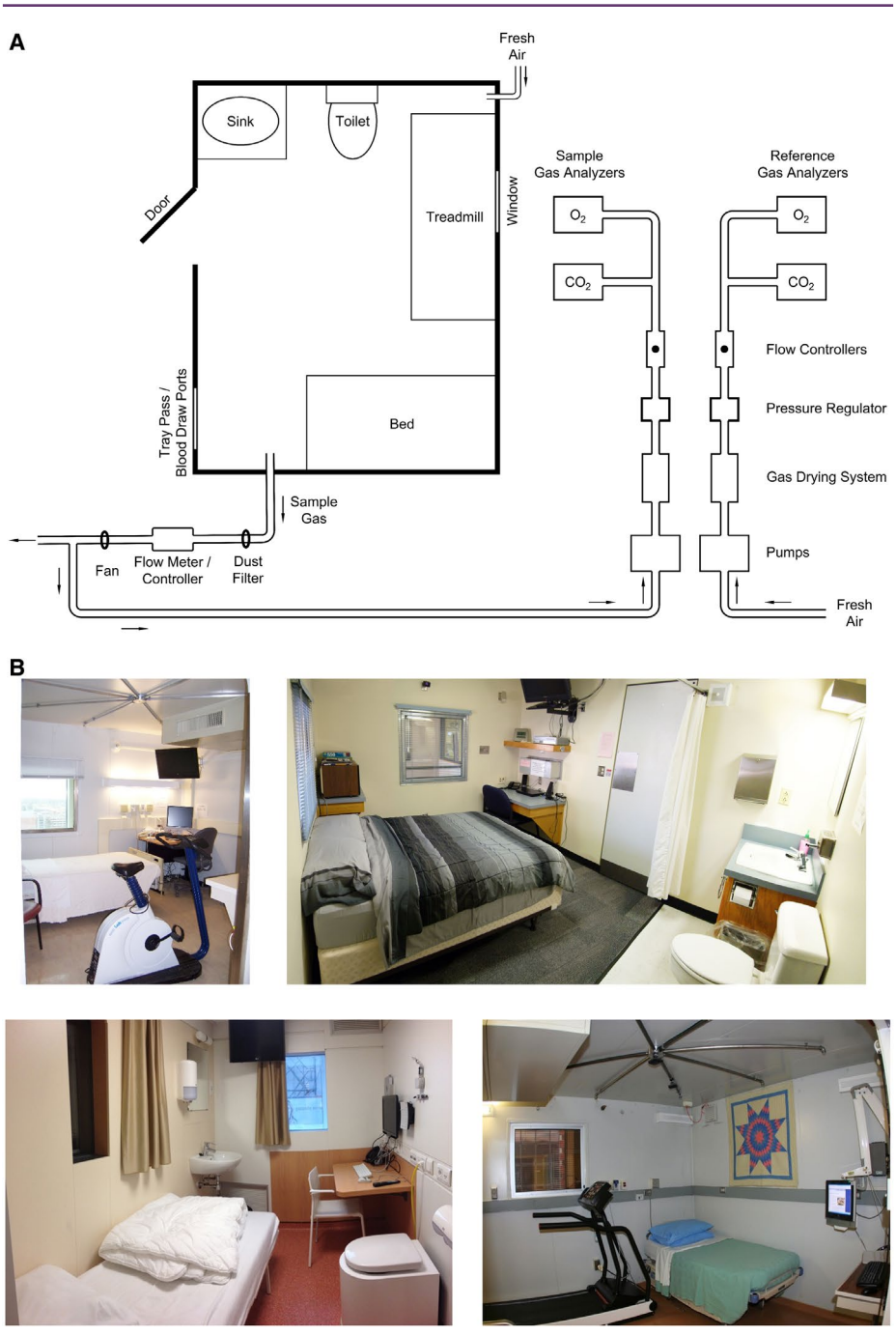

Figure 2 (**A**) Schematic layout of a room calorimeter. (**B**) Calorimeters located at the University of Colorado Anschutz Medical Campus (upper left), Pennington Biomedical Research Center (upper right), Maastricht University (lower left), and National Institutes of Health, Bethesda (lower right). [Color figure can be viewed at wileyonlinelibrary.com]

In May 2019, an international panel of calorimetry experts was convened at the Translational Research Institute for Metabolism and Diabetes in Orlando, Florida. The objective of this meeting was to establish minimum requirements for reporting (1) technical specifications; (2) data reduction and analytical approaches; (3) performance standards, including accuracy, precision, stability, and repeatability; (4) elements of study design, including the basis for power analyses, inclusion/exclusion criteria, and appropriate considerations of potential confounders (e.g., prestudy dietary intake and activity); and (5) outcome measures. The panel agreed this effort would improve the ability to compare data among studies, educate and guide new researchers to the field, and facilitate preparations and reviews of new publications. The result is the present

TABLE 1 Known research-based room calorimeters currently in operation

Center	Location	Number of rooms	Configuration
North America			
1. Baylor College of Medicine	Houston, Texas	4	Push-pull
2. Columbia University Medical Center	New York, New York	3	Pull
3. NIH/NIDDK Metabolic Clinical Research Unit	Bethesda, Maryland	3	Pull
4. NIH/NIDDK Phoenix Epidemiology and Clinical Research Branch	Phoenix, Arizona	2	Push
5. Pennington Biomedical Research Center	Baton Rouge, Louisiana	4	Pull
6. Mount Sinai St. Luke's Hospital	New York, New York	1	Pull
7. Translational Research Institute for Metabolism and Diabetes	Orlando, Florida	4	Push-pull
8. University of Alabama Birmingham	Birmingham, Alabama	1	Pull
9. University of Colorado Anschutz Medical Campus	Denver, Colorado	1	Pull
10. University of Massachusetts Amherst	Amherst, Massachusetts	2	Push-pull
11. University of North Carolina Kannapolis	Kannapolis, North Carolina	1	Pull
12. USDA Beltsville Human Nutrition Research Center	Beltsville, Maryland	2	Pull
13. USDA Western Human Nutrition Research Center	Davis, California	1	Pull
14. USDA Grand Forks Human Nutrition Research Center	Grand Forks, North Dakota	1	Push-pull
15. University of Wisconsin Madison	Madison, Wisconsin	1	Pull
16. Vanderbilt University Medical Center	Nashville, Tennessee	1	Pull
17. Virginia Commonwealth University	Richmond, Virginia	2	Push-pull
Europe			
18. NIHR Cambridge Clinical Research Facility	Cambridge, UK	2	Push
19. University of Warwick	Warwick, UK	2	Pull
20. Metabolic Research Unit Maastricht	Maastricht, The Netherlands	5	Pull
21. University Hospital of Pisa	Pisa, Italy	1	Push-pull
22. Christian-Albrechts University	Kiel, Germany	2	Pull
23. University of Hohenheim	Stuttgart, Germany	2	Pull
25. University of Copenhagen	Frederiksberg, Denmark	2	Pull
Asia			
25. National Institute of Health and Nutrition	Tokyo, Japan	2	Pull
26. University of Tsukuba	Tsukuba, Japan	4	Pull
27. KAO Corporation	Tokyo, Japan	2	Pull
28. Sendai University	Sendai, Japan	1	Pull
29. Doshisha University	Kyotanabe, Japan	1	Pull
30. Hirosaki University	Hirosaki, Japan	1	Pull
31. Fukuoka University Institute for Physical Activity	Fukuoka, Japan	1	Pull
32. Ritsumeikan University	Kusatsu City, Japan	1	Pull
33. Juntendo University	Tokyo and Inzai, Japan	2	Pull
34. Hosei University	Machida, Japan	1	Pull
35. Daikin Industries, Ltd.	Settsu, Japan	1	Pull
36. Singapore Institute for Clinical Sciences	Singapore, Singapore	2	Pull
37. BGI Group	Shenzhen, China	1	Pull
38. Shanghai University of Sport	Shanghai, China	1	Pull
39. Shanghai Jiao Tong University	Shanghai, China	2	Pull
40. Capital Medical University	Beijing, China	1	Pull
41. St. John's Medical College	Bangalore, India	1	Push
Australia			
42. University of Wollongong	Wollongong, Australia	2	Push

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Reporting Technical Specifications

The performance of a calorimeter system is defined by its accuracy and precision. Accuracy describes the proximity of measurements to traceable standards. Precision describes the variability in repeated

measures of the same quantity. Knowledge of these factors, along with biological variability, is necessary to appropriately power a study. This section focuses on determining and reporting the technical accuracy and precision of the calorimetry measurement systems. Minimizing confounding biological variabilities is a matter of protocol design, compliance, and supervision, which is addressed in later sections of this review.

System accuracy and variability

The panel recommends regular calibration of all calorimeter system components to traceable standards, including gas analyzers, flow meters, and environmental sensors. Once calibrated, system accuracy and precision can be determined using various validation tests. We recommend reporting gas exchange rates ($\dot{V}O_2$ and $\dot{V}CO_2$, in liters per minute, milliliters per minute, or liters per day) as primary outcomes for these validation tests because EE, macronutrient oxidation rates, and respiratory exchange ratio (RER, the ratio of $\dot{V}CO_2$ to $\dot{V}O_2$) are all derived from gas exchange rates. It should be noted that although RER reflects measurements made at the mouth (i.e., whole-body level) and respiratory quotient reflects gas exchange at the tissue or organ level, the two terms are often used synonymously.

To obtain accurate measurements of $\dot{V}O_2$ and $\dot{V}CO_2$, determining the room volume is vital. One approach is to use room dimensions to estimate volume, accounting for the volume of all impermeable objects in the room (e.g., furniture, sink, toilet, equipment) (18). Room volume can also be derived from the exponential change in gas concentrations in a closed room calorimeter after a "pulse" of gas is introduced into the room (17). This can be done using a gas infusion or combustion or even after a participant exits the room at the end of a study.

We recommend that performance of a calorimeter system be regularly verified using a combination of approaches. First, a "zero test" (i.e., recording of gas exchange rates in an empty, undisturbed room over a typical measurement period) will provide basic information about bias, drift, and inherent noise of the system (22). Second, system performance should be evaluated against traceable standards using the following two common approaches: (1) combustion of a flammable substance, such as ethanol, propane, or butane; or (2) infusion of dry gases (nitrogen and CO_2) to simulate $\dot{V}O_2$ and $\dot{V}CO_2$. With combustion, expected $\dot{V}O_2$ and $\dot{V}CO_2$ are calculated based on stoichiometry from the amount of fuel burned (e.g., measured by a calibrated scale). Combustion has the advantages of closely simulating fuel oxidation with heat and water vapor as products and is relatively easy to set up (Figure 3A-3B), and thus it has been widely practiced (18,19). Limitations of this approach include safety concerns with an open flame, assumed combustion efficiencies and purity (e.g., free of water and other contaminants), a resultant RER that is below the physiological range (0.667 for alcohols and 0.60 for alkanes), and the expense of high-grade fuels (e.g., a bottle of 99.2% propane is about $50, which lasts two 24-hour burns). Furthermore, even though the burning rate is manually set for a combustion test and typically not modifiable during the test (Figure 3A-B), progressive cooling of the liquefied gas as it evaporates gradually reduces the actual burning rate. As such, programmable devices may be used to set and control combustion rates more precisely. In contrast, gas infusion systems (Figure 3C) have the advantage of flexibility, allowing both independent and combined gas exchange simulations to mimic rises and falls in EE and RER. This is achieved using a pair of calibrated

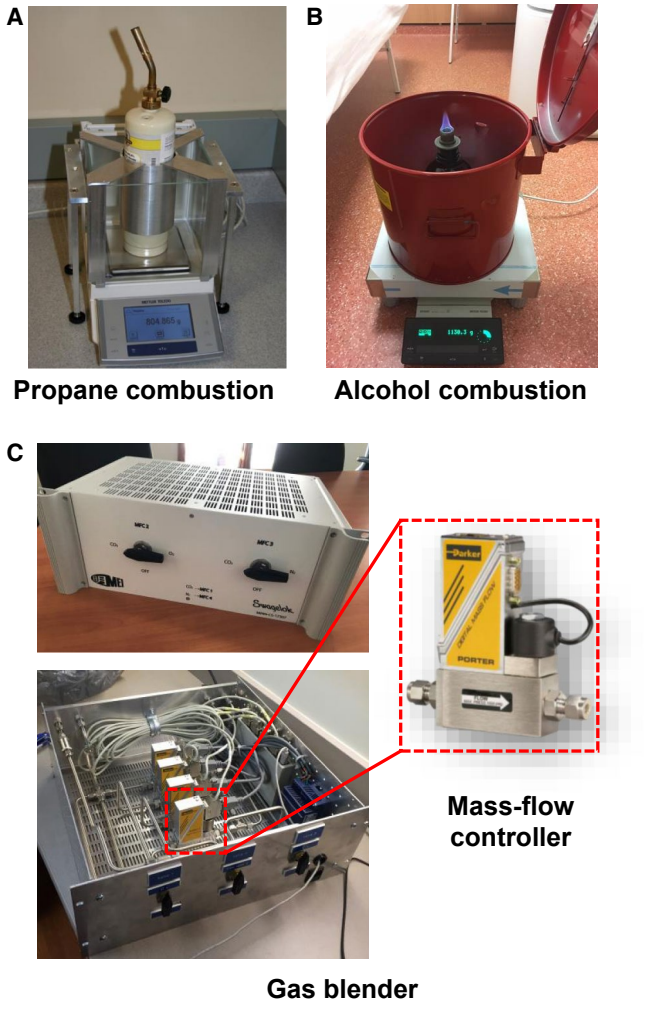

Figure 3 Room calorimeter validation methods. Combustion of (**A**) propane and (**B**) alcohol are well-established validation approaches that provide relatively constant rates of CO_2 production ($\dot{V}CO_2$) and O_2 consumption ($\dot{V}O_2$), verified by weight. Ratio of $\dot{V}CO_2/\dot{V}O_2$ is constant and stoichiometrically derived for each fuel source (0.6 for propane and 0.67 for alcohol). (**C**) Gas blenders infuse nitrogen and CO_2 at user-defined rates governed by mass-flow controllers to mimic variable, independent rates of $\dot{V}O_2$ (via oxygen dilution) and $\dot{V}CO_2$. Gas blender images provided by MEI Research Ltd. [Color figure can be viewed at wileyonlinelibrary.com]

mass-flow controllers to blend high-grade (>99%) nitrogen and CO_2 mixtures to simulate EE and RER profiles over the physiological range (17,23,24). Compared with the weighted combustion approach for validations of calorimetry systems, the technical requirements are more complex for gas infusions. For example, because mass-flow controllers are prone to drift, we recommend verification of their gas delivery rate during each infusion, independent of the stated manufacturer calibration. Gas infusions may also be done gravimetrically by observing the weight loss of a cylinder as gas flows from it or volumetrically using a drum, bellows, or wet-type gas meter. These meters offer a highly accurate (±0.2%) reference for volumetric gas flow measurements (15).

Gas infusions do not completely mimic human calorimeter studies because of a lack of heat and water vapor production. A participant will

add water content (upwards of 1 g/min) to the room air through respiration and perspiration. As a result, ingoing and outgoing air samples will have different water vapor contents. To measure $\dot{V}O_2$ and $\dot{V}CO_2$ accurately, the water vapor pressure must be accounted for, either by employing a system to remove water vapor from the samples of both the incurrent and excurrent or by precisely measuring water vapor pressure and mathematically removing the effects of water vapor on gas pressures (15,17,19,25,26). Inadequate drying is most clearly evident as an overestimate of $\dot{V}O_2$ (26). A variety of approaches have been employed to remove water vapor content in room calorimeter systems, including cooling systems that condense most of the water, chemical dryers, and counterflow devices that promote exchange of water vapor from the sample gasses using a continuous flow of dry gas around the liquid-permeable sample tubes. Depending on the drying approach, seasonal variations in ambient conditions may interfere with the performance (26). To more closely approximate human studies, gas infusion tests may be modified by introducing water vapor (e.g., evaporation on a boiling plate) and/or heat (e.g., electric heater with known wattage) into the calorimeter room. To identify issues caused by ineffective water vapor removal or temperature control, the results of infusion tests with these additional elements can then be compared with infusions with dry gas alone.

The panel recommends that the first manuscript following establishment or modification (major changes to hardware and/or software) of a room calorimeter include sufficient detail regarding instrumentation, system characteristics (e.g., room volume, analyzer and flow meter specifications, procedures for eliminating water vapor pressure effects on gas concentrations, temperature and humidity control), and results of validation tests (zero tests, combustion tests, infusion tests, and repeated measures on human participants). External validations against metabolic carts may also be informative, providing additional data that can be used to "fine tune" the performance of the system (26). The panel also recommends regular system validation using combustion as a minimum standard. The panel did not have consensus of what would constitute "regular," but it recommends combustion tests be performed at least quarterly. Other tests (infusion tests, zero tests, water evaporation tests) can be performed less frequently (e.g., after software/hardware modifications). The panel also recommends that the length of the validations be close to the length of the human studies (e.g., 24 hours) with rates of $\dot{V}O_2$ and $\dot{V}CO_2$ within the range expected when studying human participants. Accuracy should be reported as the mean (or median) difference of expected versus observed gas exchange rates. Precision (variability) should be reported as the SD of the expected versus observed differences for a single test or as the confidence limits of these differences for a group of tests performed over the duration of the protocol.

Signal processing

Early room calorimeters employed chemical or gravimetric approaches to measure $\dot{V}O_2$ and $\dot{V}CO_2$ (5,21). These methods were time and labor intensive, and, thus, studies were limited to steady states of metabolism or the aggregate of several metabolic states over a matter of hours. Gravimetric instrumentation and handwritten records have now been replaced with digital instrumentation and computers capable of measuring gas concentrations at rates less than 1 Hz (21). However, because the volume of most room calorimeters is approximately 50,000 to 100,000 times larger than the volume of $\dot{V}O_2$ and $\dot{V}CO_2$ each minute at rest, the practical limit to detect a change in gas concentration is on the order of 1 minute (20), and software-based algorithms are typically implemented to enhance the signal to noise ratio of room calorimeter systems.

The minimum detectable change in gas concentration for a room calorimeter is related to both instrumentations and physical dimensions. Modern gas analyzers (e.g., paramagnetic, infrared, fuel cell, mass spectrometry) differ in their physical properties that govern their relative sensitivity to different noise sources (e.g., vibration, water vapor content, electromagnetic interference, changes in flow and pressure), life-span, and propensity to drift, among other things (20). However, their response time (milliseconds to seconds) and resolution (<0.01%) to changes in gas concentration fall within a similar range (20), and thus the type of analyzer employed is not the major determinant of the response time, accuracy, and precision of a room calorimeter system.

The time between a sudden change in room gas concentration, such as the onset of exercise, and its detection by gas analyzers is defined by the calorimeter "time constant" (i.e., response time), which is calculated as the room volume divided by excurrent flow rate (15,20,21). The time constant is incorporated into the equations to determine $\dot{V}O_2$ and $\dot{V}CO_2$, which include a closed-system (first derivative) term, i.e., the change in room gas concentration over time multiplied by the room volume, either directly (15-18,25) or by using the Z-transformation method (20). The time constant can be reduced by decreasing room volume and/or increasing flow rate. However, because room volume is approximately 100 to 500 times larger than excurrent flow rate in a typical room calorimeter, it plays the greater role. Reducing room volumes can limit participant comfort and range of viable activities, whereas increasing flow rates can increase noise from the device used to control incurrent or excurrent flow.

While incorporating closed-system terms into equations for $\dot{V}O_2$ and $\dot{V}CO_2$ can analytically improve response time, it also amplifies noise inherent to O_2 and CO_2 measurements (20,25). Thus, some laboratories have developed software-based approaches to minimize this noise while retaining the improved response time using dynamic gas-infusion protocols as the validation criteria for their signal processing techniques. Early noise suppression efforts included averaging the gas concentration signals over time windows of various lengths prior to computing a discrete time derivative (17,19,25). Choosing the duration of the averaging window amounts to a tradeoff between a longer window with a less noisy steady state and slower response and a shorter window with a quicker response and noisier steady state (28). Others proposed fitting sliding windows of gas concentration data with two exponentials (27,29) or quadratic splines (25) prior to computing the derivative. These methods assume that changes in metabolic state will take a specific form and occur over a predetermined minimum duration. Though this approach yields fast responses and improves the signal to noise ratio when these assumptions are met (27,29), shorter-than-expected state changes may produce inaccurate calculations of $\dot{V}O_2$ and $\dot{V}CO_2$ (23). More complex signal processing techniques, such as Kalman filtering (30), deconvolution (30), wavelet denoising (23), and total variation denoising (28), have demonstrated promising improvements for noise suppression but they are more complex to implement and require independent validations on multiple room calorimeter systems before field-wide adoption. It should be noted that zero tests and validations (infusions and combustions) conducted without software filters can provide information about the statistical properties of noise and they are helpful in the design of signal processing algorithms.

The panel recommends troubleshooting and optimization of instrumentation and operational setup prior to development and implementation

of noise suppression methods, as software-based techniques should enhance system performance rather than correct for system inadequacies. Signal processing algorithms should be validated against traceable standards, not only to demonstrate improved short-term precision (e.g., reduced minute-to-minute error in $\dot{V}O_2$ and $\dot{V}CO_2$) but also to ensure that they do not compromise longer-term accuracy (e.g., unchanged total $\dot{V}O_2$ and $\dot{V}CO_2$ over hours and days). The panel also recommends that the first manuscript from a newly commissioned room calorimeter or following hardware/software modification to an existing system include sufficient signal processing details and performance comparisons to published approaches. Subsequent studies from the same system should then reference the original paper. This is particularly important if the reported calorimeter outcomes are of short durations or are dynamic in nature (such as $\dot{V}O_2$ during an exercise bout or slope of a postprandial RER); only reporting the validity of 24-hour performances using a static combustion test would not be sufficient under these circumstances.

Reporting Data Analysis Approaches

While indirect calorimeters measure $\dot{V}O_2$ and $\dot{V}CO_2$, EE and/or substrate oxidation are the desired outcomes for clinical or physiological studies. Thus, it is important to report the analytical approaches used to determine the physiological determinants of energy metabolism. Components of 24-hour EE measured using room calorimetry include the following (31): (1) sleeping metabolic rate (SMR), (2) resting metabolic rate (RMR), (3) thermic effect of food (TEF), and (4) physical activity–related EE (PAEE) (Figure 4). With proper experimental design, it is also possible to measure additional components of 24-hour EE such as excess postexercise oxygen consumption (32). When comparing groups of disparate body size or composition, proper normalization procedures should be employed.

Components of 24-hour EE

The basal metabolic rate (BMR) is the rate of EE at complete rest; it represents the EE required to maintain vital functions, such as basic chemical reactions of the body, and is the primary contributor (60%-75%) of 24-hour EE measured in a room calorimeter (31). The BMR is typically measured using a ventilated hood. The strictest definition of BMR requires that an individual be in a postabsorptive state (10-12 hours without consumption of caloric food or beverages), physically undisturbed, awake, and in a thermally neutral environment (33). The measurement should be obtained in the morning following an overnight stay in a testing facility. The BMR can be measured in a room calorimeter if the conditions described above are met, although a longer measurement period is recommended (e.g., minimum of 30 minutes). If the resting measurements are obtained under less strict conditions, this is referred to as the RMR or resting EE.

In room calorimeter studies, SMR is sometimes used as an alternative measure of BMR/RMR. The SMR has the lowest intrasubject coefficient of variation and, consequently, is the most reproducible component of 24-hour EE (22,34). There are several challenges that must be considered when determining SMR. For example, EE during sleep decreases as the night progresses because TEF is sustained for at least 4 to 6 hours after consuming a meal (35). The RMR also varies by circadian phase; RMR is lowest late in the biological night at a time (~4-5 AM) that corresponds to the nadir in endogenous core body temperature (36). Thus, it is likely that SMR varies during the biological night, such that SMR measured during the early part of the biological night is higher than during later phases of the biological night. There is no clear consensus on how to define SMR. Some investigators have defined SMR as the lowest observed EE during the night over 3 consecutive hours (34), whereas others have defined SMR as the average EE during the three consecutive hours in the nighttime period with the lowest observed physical activity (body

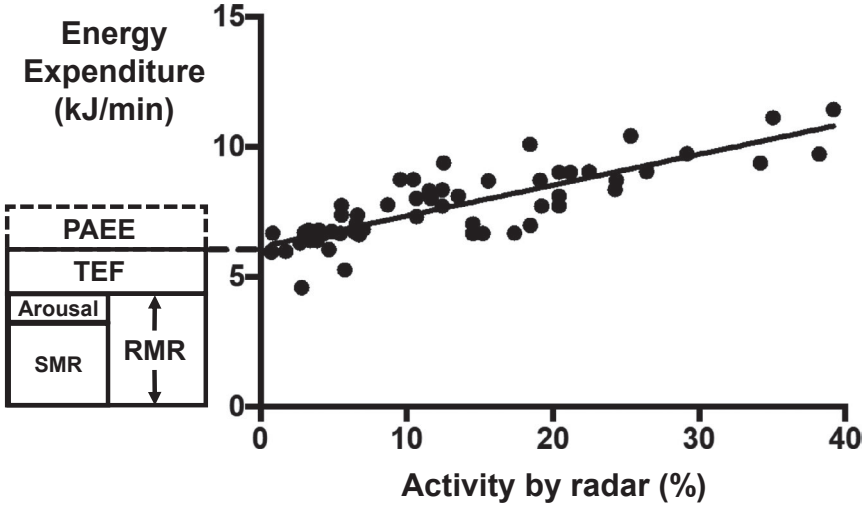

Figure 4 Components of total daily energy expenditure. Physical activity energy expenditure (PAEE) has a linear relationship with concurrent activity measured by radar. Energy expenditure at zero activity, i.e., the y-intercept, represents sum of the resting metabolic rate (RMR) and thermic effect of feeding (TEF). RMR comprises sleeping metabolic rate (SMR) and energy expenditure from arousal. Adapted from Ravussin et al. (18).

movement) (34,37,38). The latter approach requires a measure of body movement, such as accelerometry or in-room motion sensor system. Theoretically, the lowest body movement could be earlier in the night when TEF is still present. Thus, a more robust definition in this case would be the 3 consecutive hours with the lowest body movement that occur at least 6 hours after consumption of the last meal or the EE averaged from minutes of minimal activity over a prespecified post-TEF time period, such as 0100 to 0500 hours (39) or 0200 to 0500 hours (40). Finally, another factor that should be considered when determining SMR is the "first night" effect. It has long been known that when individuals sleep in an environment they are not accustomed to, sleep stages are disrupted such that the first night of "laboratory" sleep contains more awake periods, less rapid eye movement sleep, and delay in the onset of deep sleep stages. These disruptions in sleep quality rapidly dissipate during the second night of laboratory sleep (41). A first night effect on SMR has been shown in some calorimeter studies (42) but not others (34), which may be partly attributed to the method of SMR determination. Similarly, some studies have shown EE to differ between sleep stages (43,44), whereas others have not (45). Nonetheless, the panel recommends that the first night effect should be considered when measuring SMR and may be particularly important for crossover design studies.

The TEF is the increase in EE above BMR or RMR caused by food intake. The TEF is dependent on both the size and macronutrient composition of the meal (46). When healthy participants are in approximate energy balance (EE ≈ energy intake) and consuming a mixed diet, TEF is ~10% of 24-hour EE. In a room calorimeter study, it is difficult to independently attribute increases in EE above BMR or RMR to TEF and PAEE, unless one of these components is minimized by design. For example, with a participant studied awake and under complete bed rest, the increase in EE above RMR represents TEF. Conversely, in a participant studied under fasted conditions, the increase in EE above RMR represents PAEE. Some rigorous studies of TEF take advantage of the day-long measurement capability of room calorimeters by fasting participants for one day and feeding on another day while minimizing activity each day (47-49). Because this protocol requires 2 separate days and burdens participants with a prolonged fast, alternative methods to estimate TEF using a measurement of physical activity have been proposed (47,48,50,51). With this approach, physical activity is regressed against EE per unit of time (Figure 4). The *y*-interecept is the EE in the inactive state, and the difference between this value and RMR is considered to represent the cumulative TEF. However, TEF computed by this method had poor reproducibility when participants were studied on more than one occasion (48). Thus, methodology to measure TEF using whole-room calorimeters is an area ripe for development.

Macronutrient oxidation rates

Stoichiometric equations of different substrates reacting with oxygen to produce carbon dioxide can be used to estimate macronutrient oxidation. The earliest equations published by Zuntz et al. (52) assumed the amount of protein oxidized was negligible. Because room calorimetry measures are typically carried out over many hours, the contribution of protein oxidation cannot be ignored, and thus more current calculations of macronutrient oxidation also include protein oxidation determined by the quantification of nitrogen excretion in the urine. Many sites use Weir's (53) equations to derive macronutrient oxidation, which account for the fraction of protein oxidation (assumed or measured), although alternative equations have been presented (54-56). Depending on the choice of equation, fat oxidation can vary by ~3%, whereas carbohydrate oxidation can vary by up to 6% (57). Thus, the panel recommends that the equations used to calculate macronutrient oxidation be specified, and they strongly recommend that protein oxidation be determined from measured urinary nitrogen excretion rather than by using an assumed value (53).

As eloquently detailed by Livesey and Elia (58), there is more uncertainty when using indirect calorimetry to determine macronutrient oxidation than in the uncertainty of using indirect calorimetry to measure EE. There are several sources of potential error in calculating macronutrient oxidation from gas exchange rates and nitrogen excretion. First, small errors in the measurement of $\dot{V}O_2$ and/or $\dot{V}CO_2$ can lead to large errors in carbohydrate and fat oxidation (58). Second, estimates of protein oxidation from nitrogen excretion can vary substantially (58). Part of this is due to variability in nitrogen assay performance. Thus, the accuracy of the methods used to determine urinary nitrogen concentrations should be reported. There are a number of assays available for performing urine nitrogen analyses, including colorimetric and chemiluminescence assays (59). Although not commonly employed, urinary urea nitrogen is ~80% to 100% of total urinary nitrogen and thus can be used as a means of verifying total urine nitrogen. It is essential that all urine be collected over the 24-hour period, including a void obtained at the end of the calorimeter study.

RER

It is common practice to report RER as an index of relative macronutrient oxidation, either over 24 hours or during discrete periods (e.g., during exercise or sleep). Although not discouraging this practice, the panel recommends that care be taken in determining the RER values, particularly over short periods of time. RER is a dimensionless ratio defined as $\dot{V}CO_2/\dot{V}O_2$. Noise inherent to the measurement of CO_2 and O_2 results in minute-to-minute variation in $\dot{V}CO_2$ and $\dot{V}O_2$. Because noised-based variations in $\dot{V}CO_2$ and $\dot{V}O_2$ are not covariant, this can lead to spuriously high or low values when RER is computed over short durations (i.e., minutes). Assuming noise is normally distributed and biological state is constant, using total $\dot{V}CO_2$ and $\dot{V}O_2$ over longer durations (i.e., hours) to calculate RER will minimize the effects of this noise, whereas calculating RER on a minute-to-minute basis results in a greater likelihood of incorporating values near zero or infinity into the average.

Normalization procedures

The major determinant of BMR/RMR and 24-hour EE in humans is fat-free mass (FFM) (18), although fat mass contributes to a small but perhaps important proportion (60). Thus, EE data must be adjusted or "normalized" to compare values between individuals of different body size or composition. Dividing EE by body weight or FFM may lead to misinterpretation because of a "non-zero intercept artifact" (31,61), as illustrated in Figure 5. In this example, RMR is plotted as a function of FFM for both men (open circles) and women (closed circles). Men and women fall on a shared regression line with a nonzero intercept, indicating the relationship between RMR and FFM is independent of sex. However, dividing RMR by FFM results in a significant difference between women and men (0.143 [0.012] and 0.128 [0.080] MJ/kg for women and men, respectively, $P < 0.0001$) (62). Regression-based methods are considered to be the preferred approach when comparing EE data from groups of different body composition (63) or when assessing changes in EE resulting from interventions that alter body composition.

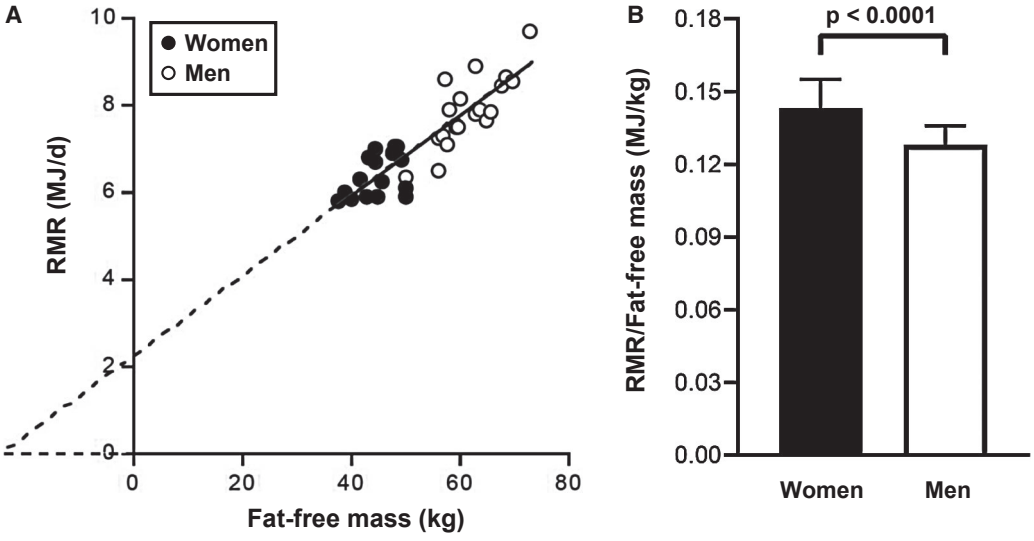

Figure 5 Body composition adjustments for energy expenditure. (**A**) Plotting resting metabolic rate (RMR) as a function of fat-free mass for 17 women (closed symbols) and 20 men (open symbols) results in a shared linear regression (RMR [MJ/d] = 2.27 + 0.091 fat-free mass [kg]; $r^2 = 0.78$), suggesting a sex-independent relationship between RMR and fat-free mass. (**B**) Dividing RMR by fat-free mass, however, yields a greater "normalized" RMR for women because of the nonzero intercept in panel A. Adapted from Westerterp (62).

For reporting data analysis approaches, the panel recommends that the methods used to determine the components of EE be reported. If BMR or RMR is measured in the calorimeter, procedures to ensure that the measure was obtained under standard conditions should be reported, including whether participants were monitored to be sure that they remained awake and motionless during the period of measurement. Physical activity may be measured using a variety of methods, but recent advances have made using wearable monitors more feasible. This also has the advantage that the same wearable device can be worn outside of the calorimeter (e.g., during preceding study days), which permits a comparison of the physical activity level in the calorimeter to habitual physical activity levels. Although TEF is not commonly measured, the panel suggests that this is an area for further methodological development and investigation. If SMR is measured, the period of time and additional criteria used to determine sleep versus wakefulness should be reported. If macronutrient oxidation rates or RER are measured over discrete periods of time (e.g., during a bout of activity), data should be presented or referenced to demonstrate the ability of the system to accurately and reliably measure these outcomes over such short intervals. Finally, when normalizing data to permit comparisons of groups of disparate size/body composition, the panel recommends regression-based approaches that consider both FFM and fat mass as covariates.

Reporting Elements of Study Design and Conduct

Appropriate study design for room calorimeter studies requires consideration of confounding factors that may affect EE and/or substrate oxidation (e.g., preceding diet, physical activity, sleep, weight stability, menstrual cycle phase, and state of energy balance). There are generally three types of studies performed in room calorimeters: observational, longitudinal, and interventional. (64). Observational studies involve measuring participants under one or more conditions (e.g., high-fat vs. low-fat diet; exercise vs. no exercise) within a short period of time. Longitudinal studies measure changes over time, with measurements performed at two or more time points. Interventional studies (e.g., diet, exercise training, weight loss, overfeeding) require measurements pre-, post-, and sometimes during the intervention. Almost all published room calorimeter studies to date are based on studies performed at a single site. To our knowledge, only one multisite study has been published (64). Multisite studies must take careful consideration to remove as many site-specific variables as possible to align the entire data set.

In a well-performing system, biological variability should comprise the largest source of total variability. To estimate the number of study participants required, the variability in repeated measurements (intraindividual variability) should be determined (Figure 6). In many studies, EE is the primary study outcome. The coefficient variability of 24-hour EE measured by indirect room calorimeters have been reported to be 1% to 5% (18,22,54,65-67), whereas the coefficient variability of macronutrient oxidation are reported to be 5% to 25% (66,67). In our review of published studies, in many cases, the primary study outcomes were not clearly stated. The panel strongly recommends that calorimeter studies clearly define the primary outcome and that information to justify the sample size be presented.

A checklist for reporting participant characteristics, prestudy controls, and participant preparation is presented in Table 2. Where appropriate, these are listed according to type of study. The minimum information to report includes participant age, sex, ethnicity, weight, height, and body composition (including method of determination). Additional inclusion and exclusion criteria, such as weight stability, medication, alcohol, and nicotine use,

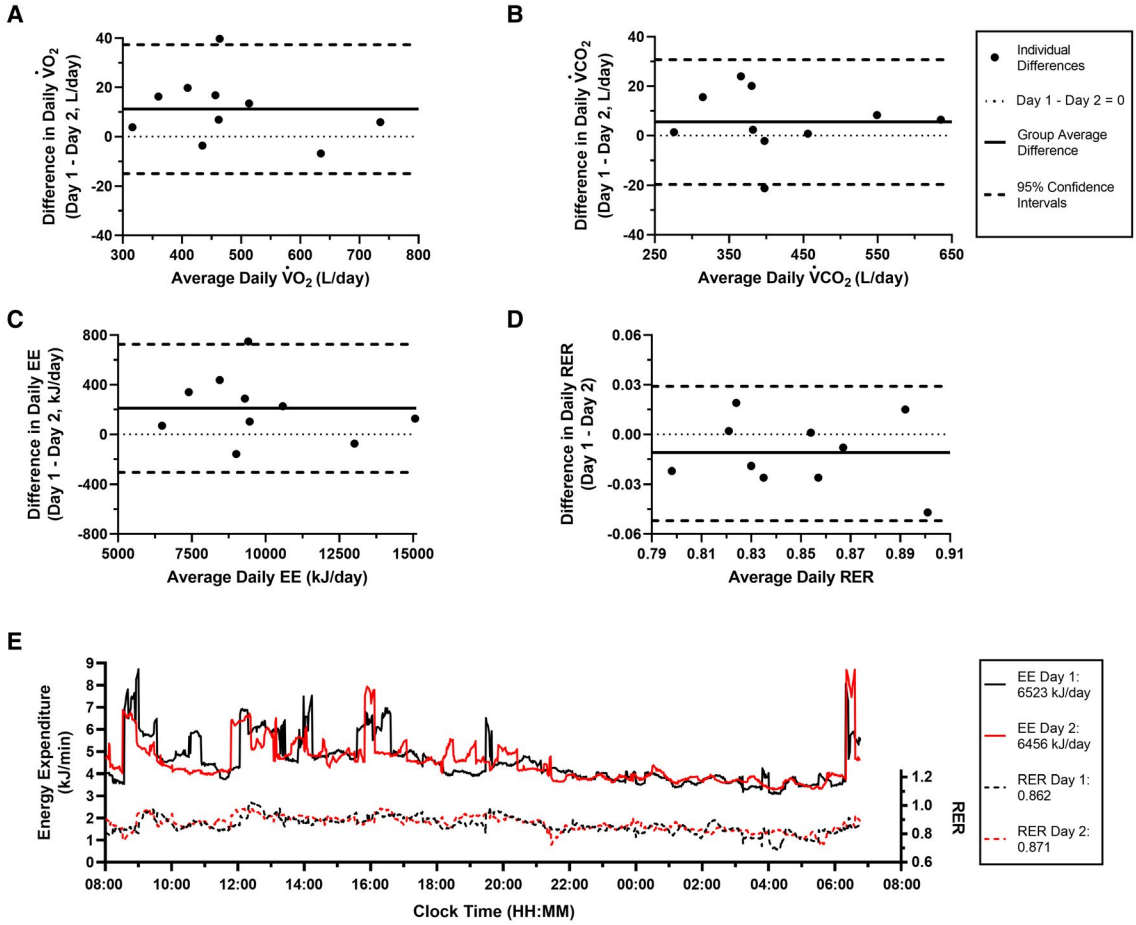

Figure 6 Repeated 23-hour participant measurements to assess biological intraindividual variability. Bland-Altman plots of the difference between day 1 and day 2 versus their average for (**A**) oxygen consumption ($\dot{V}O_2$), (**B**) carbon dioxide production ($\dot{V}CO_2$), (**C**) energy expenditure (EE), and (**D**) respiratory exchange ratio (RER) for 10 participants fed a eucaloric, energy balanced diet and instructed to approximate their same daily routine both days. Average differences for the group are as follows: $\dot{V}O_2$, 11.2 ± 13.3 L/d (2.6% ± 2.9%); $\dot{V}CO_2$, 5.6 ± 12.8 L/d (1.5% ± 3.4%); EE, 211.1 ± 262.4 kJ/d (2.4% ± 2.9%); and RQ, −0.01 ± 0.02 (−1.3% ± 2.4%). (**E**) Minute-by-minute tracings over 23 hours for EE (solid lines, left y-axis) and RER (broken lines, right y-axis) on day 1 (black) and day 2 (red) for the participant with the lowest daily EE and activity. Data provided by KY Chen (unpublished). [Color figure can be viewed at wileyonlinelibrary.com]

should be reported. Studies of female participants should report menstrual status because ovarian hormones impact EE (68) and perhaps physical activity (69). When premenopausal women are studied, whether studies were performed in a specific menstrual cycle phase (luteal vs. follicular) should be reported, along with the methods used to determine cycle phase (70). Scheduling all premenopausal female participants in the same phase of the cycle presents many logistical challenges. Thus, when this cannot be achieved, the panel recommends measuring ovarian hormone concentrations (e.g., estrogen, progesterone) and using this as covariates in the statistical models. Whether premenopausal women using hormonal contraceptives have been included or excluded should also be reported. When postmenopausal women are studied, the methods used to determine menopausal status should be reported (e.g., self-report based on cessation of menstrual cycle or confirmation by ovarian hormone) as well as whether women using hormonal replacement therapy were included or excluded.

In many calorimeter studies, a "run-in" period is included in which exercise and energy intake are prescribed or controlled (71). Although not commonly practiced, a consistent sleep schedule may also be prescribed prior to calorimeter studies (72). The purpose of this run-in phase is to minimize the impact of variations in these parameters on energy stores, EE, and substrate oxidation (73). Any prestudy restrictions (e.g., diet, physical activity, sleep, etc.) should be reported, along with the prescribed duration of these restrictions and how they were verified. In some studies, energy and macronutrient intake is strictly controlled by providing food to participants. In these cases, formulae used to estimate energy and macronutrient requirements and methods to track compliance should be reported. If participants were instructed to complete an overnight fast prior to the study day, the length of this fast should also be reported.

Prior to the start of a study, consideration should be given to orienting the participant to the room calorimeter and study protocol. The panel recommends that a scripted orientation be provided to each study participant, including a review of the room layout and facilities, such as access to food ports, toilets, safety (e.g., emergency call buttons), and privacy features (e.g., video monitors and windows that view into the room and whether the participant has any control over these). A written script of

TABLE 2 Summary of recommendations

	Recommendation
Participant characteristics	
Age, sex, ethnicity, race, weight, (height), BMI	• Report
Body composition	• Report (including method of determination) • Report fat mass and fat-free mass (kg)
Prescription and over-the-counter medications	• Report if inclusion/exclusion criteria
Weight stability	• Report if inclusion/exclusion criteria
Tobacco/nicotine use	• Report if inclusion/exclusion criteria
Female participants	• Report if oral contraceptives and/or hormonal replacement therapy used • Report if menstrual cycle phase was determined and if visits were performed in a particular phase
Technical specifications	
Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$)	• Report as primary outcomes • Report/reference accuracy of $\dot{V}O_2$ and $\dot{V}CO_2$ against traceable standard and method of determination (e.g., combustion, infusion) • When reporting over specific time intervals (e.g., during exercise, during the night) report as mL/min, L/min, or L/h; when reporting 24-hour values, report as L/d • Combustion results are considered the minimum standard for establishing accuracy; additional measurements (e.g., infusions, evaporation test, zero tests) are recommended • Rate and range of simulated energy expenditure from combustion or infusion should be similar to range of human studies • Validation studies should be close to the length of human studies (24 hours) • For dynamic performance measures (e.g., during and after bouts of exercise, post meal, drug intervention), report signal processing details, and variable-rate infusion validations are recommended
Signal processing	• Details and performance comparison to established methods should be reported in the first manuscript after room commissioning or following substantial hardware/software modifications; subsequent studies from the same system should reference the original paper
Data analysis	
24-hour expenditure	• Because SI unit for energy is joules (J), reporting in kJ/min or MJ/d is preferred rather than kcal/min or kcal/d • When measured over a shorter time interval than 24 hours (e.g., 23 hours, 23.5 hours), indicate how extrapolation to 24 hours was done
Components of total daily energy expenditure	• Report approaches used to determine activity energy expenditure, thermic effect of food, and basal, resting, or sleeping metabolic rate • For basal/resting metabolism, report procedures to ensure measure was obtained under standard conditions • For sleeping metabolic rate, state period of time and criteria used to determine sleep vs. wakefulness
Physical activity	• If measured, report methods used to measure spontaneous physical activity
Respiratory exchange ratio	• Use respiratory exchange ratio rather than respiratory quotient; the former is more appropriate for gas exchange measured at the whole-body level • If measured over discrete periods of time, report/reference accuracy
Macronutrient oxidation	• Reference equations used to determine macronutrient oxidation • Report methods used to determine protein oxidation
Normalization of energy expenditure	• Preferred method is to use fat mass and fat-free mass as covariates
Study design	
Sample size and power	• Report how sample size was determined and statistical power to detect differences in outcomes reported
Prestudy control	• Report any prestudy dietary and physical activity prescription or controls
Procedures for orienting participants to study design and safety procedures	• Report
Meals during calorimeter study	• Report time of meals and whether meals were required to be consumed within a certain amount of time • At a minimum, report energy and macronutrient composition of total diet • Report how energy needs were determined
Physical activity monitoring	• If monitored, report methods used
Safety procedures/participant monitoring	• Report
Bedtime/lights out time/napping	• Report if controlled/prescribed

the study protocol should be provided, including mealtimes, sample collection procedures (e.g., urine and/or blood) and schedule, and any prescribed exercise, sleep, and wake times. Participants should also be given instructions if meals are to be consumed within a prescribed period of time (e.g., 30 minutes), whether they should consume all food provided, and what activities they are permitted or required to perform during the study. These practices should minimize potential sources of biological variability, enhancing the ability to detect significant differences. The panel recommends that regardless of study design, physical activity during the calorimeter study be monitored and reported. Finally, participants should be instructed whether the use of cellular phones and other personal electronic devices is permitted in the calorimeter and the time limits if they are permitted. Personal device use after bedtimes should be discouraged, as this may interfere with sleep.

As we were finalizing this manuscript for submission, the coronavirus disease pandemic has become a serious health issue in many countries. Considering the seriousness of its impact, we believed it prudent to provide recommendations to reduce the risk of accidental transmission of viruses in calorimetry-related clinical research. It is recommended that users of calorimeters implement rigorous cleaning procedures prior to and following each participant study. Some calorimeters are located in hospital facilities or affiliated with medical centers, and procedures are often performed according to hospital standards. However, some calorimeters are not affiliated with hospital or medical centers, and these locations should adopt appropriate procedures. The panel recommends that before and after each human study, all surfaces inside the calorimeter chamber (sinks, toilets, tables) as well as devices such as computer keyboards and televisions be thoroughly wiped with a disinfectant. Floors should be mopped with a disinfecting solution. Cleaning should be performed using nitrile gloves. Trash cans should be lined with plastic disposable bags that are changed after each study. Any linens provided should be changed and cleaned. In many calorimeters, a portion of the excurrent air is vented into the anterior chamber areas, which could promote the transmission of viruses into areas outside of the calorimeter. In these settings, the panel recommends that a suitable filter be placed in-line of the venting system.

Another consideration for reducing the risk of accidental transmission is screening individuals for recent illnesses. The need to protect spread of viruses during the process of metabolic measurements is an often-overlooked area. In most situations, participants will perform the consent process and any health screening days or weeks prior to their scheduled study visits. Thus, the panel recommends that sites employ procedures to screen individuals immediately prior to the study visit to inquire about recent illnesses. Participants should be screened about recent (e.g., previous 2-3 weeks) incidents of fever, cough, shortness of breath, or any other illness or symptom, and these cases should be reviewed by appropriate medical personnel to determine whether the study visit should be rescheduled.

Summary and Future Directions

The future of room calorimetry is bright; compared with 20 years ago, technological advancements are making instruments more affordable, and the acquisition, processing, and analysis of data are more streamlined and efficient. New room calorimeters are being constructed around the world, particularly in Asia and North America. Most calorimeters are custom built with variability in room dimensions, instrumentation, environmental controls, and data processing. Thus, standardizing performance testing and reporting can facilitate comparison of different sites.

Because of the specialized nature of building, operating, and maintaining room calorimeters, there are currently limited opportunities for comprehensive training. Furthermore, unlike metabolic carts, there are few companies that specialize in the design and construction of room calorimeters. We can offer several recommendations for those that wish to pursue learning opportunities in this field. First, Table 1 lists the sites known to maintain operating room calorimeters. Second, although not exclusively focused on room calorimetry, the triannual conference entitled "Recent Advances and Controversies in Measuring Energy Expenditure" (www.RACMEM.org) devotes numerous sessions on technical and operational components that are covered in this document.

We propose RICORS 1.0 criteria (Table 2) as the minimum guidance for performing, analyzing, and reporting human whole-room calorimetry studies. There are many decisions and assumptions made during study design and conduct, data acquisition, and data analysis that should be considered and reported when performing such studies. The RICORS recommendations are intended to provide consistency in reporting to permit proper evaluation and interpretation of results, as well as to provide consistency so that results from different studies can be compared. Moreover, the RICORS recommendations can guide further improvements in calorimetry technological developments and practical operations, such as developing new algorithms to increase sensitivity and reduce response time and measuring TEF, excess postexercise oxygen consumption, and sedentary EE. The RICORS criteria were developed with the consultation of many room calorimetry experts, some of whom have performed studies in this area for several decades. As the first consensus panel report, RICORS should be evaluated by operators in the field and updated as needed.

The authors welcome suggestions from the research community to refine these recommendations (please send suggestions to the corresponding author). Finally, we envision that this document will serve as a template for other fields where standardization in reporting is lacking (e.g., animal calorimetry studies).O

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