Indirect calorimetry in humans: a postcalorimetric evaluation procedure for correction of metabolic monitor variability

Peter Schadewaldt, Bettina Nowotny, Klaus Straßburger, Jörg Kotzka, and Michael Roden

ABSTRACT
Background: Indirect calorimetry (IC) with metabolic monitors is widely used for noninvasive assessment of energy expenditure and macronutrient oxidation in health and disease.
Objective: To overcome deficiencies in validity and reliability of metabolic monitors, we established a procedure that allowed correction for monitor-specific deviations.
Design: Randomized comparative IC (canopy mode) with the Deltatrac MBM-100 (Datex) and Vmax Encore 29n (SensorMedix) were performed in postabsorptive (overnight fast >8 h) healthy subjects (n = 40). In vitro validation was performed by simulation of oxygen consumption (VO2) and carbon dioxide output (VCO2) rates by using mass-flow regulators and pure gases. A simulation-based postcalorimetric calibration of cart readouts [individual calibration control evaluation (ICcE)] was established in adults (n = 24).
Results: The comparison of carefully calibrated monitors showed marked differences in VCO2 and VO2 (P < 0.01) and derived metabolic variables [resting energy expenditure (REE), respiratory quotient (RQ), glucose/carbohydrate oxidation (Gox), and fat oxidation (Fox); P < 0.001]. Correlations appeared to be acceptable for breath gas rates and REE (R2 ~ 0.9) but were unacceptable for RQ (R2 = 0.3), Gox, and Fox (R2 = 0.2). In vitro simulation experiments showed monitor-dependent interferences for VCO2 and VO2 as follows: 1) within series, nonlinear and variable deviations of monitor readouts at different exchange rates; 2) between series, differences and unsteady variability; and 3) differences in individual monitor characteristics (eg, rate dependence, stability, imprecision). The introduction of the postcalorimetric recalibration by ICcE resulted in an adjustment of gas exchange rates and the derived metabolic variables with reasonable correlations (R2 > 0.9).
Conclusions: Differential, metabolic, monitor-specific deviations are the primary determinants for lack of accuracy, comparability, and transferability of results. This problem can be overcome by the present postcalorimetric ICcE procedure. Am J Clin Nutr 2013;97:763–73.

INTRODUCTION

Indirect calorimetry (IC) is a noninvasive method for the determination of energy expenditure by respiratory gas exchange analysis. The principles of IC were established in the 19th century (1–3). With the use of appropriate devices (metabolic monitor or cart), the rates of carbon dioxide output (VCO2) and oxygen consumption (VO2) are typically assessed under resting conditions. The data are used for calculation of resting energy expenditure (REE) and the respiratory quotient (RQ). The method also allows estimation of the differential contribution of macronutrient oxidation (ie, carbohydrate, fat, protein) to REE in vivo when protein oxidation is additionally evaluated. The procedure is well established and allows for more accurate estimates of REE than all of the available formula-based approaches (4–15).

An early clinical application was for diagnosis of thyroid dysfunction. Today, IC is of interest for evaluation of REE, eg, in intensive care units for assessment of caloric needs in severely ill patients (14, 16, 17). Experimental applications of IC in humans are widespread, eg, for studies on macronutrient oxidation, assessment of thermogenesis, measurement of energy expenditure during rest and muscular exercise, and exploration of pathogenesis of obesity and diabetes mellitus (5, 7, 18, 19).

Despite its broad use, IC has some limitations. In particular, commercially manufactured devices may differ in technical construction and analytic performance. For assessment of REE and RQ in humans, flow-through respirometry (ventilated hood/canopy mode) is the method of choice in the clinical and the experimental setting. Among canopy-based metabolic carts, the Deltatrac MBM-100 (Datex) is one of the most widespread used devices. The Deltatrac was supposed to exhibit the greatest accuracy and the least intercart variability of all such devices worldwide. Because this cart is no longer produced, there is a need for replacement by contemporary metabolic monitors (14, 20–25).

We here report on a comparative evaluation of the validity and reliability of 2 metabolic carts by applying simulation experiments in vitro. The results showed relevant deficiencies in cart performances, which led us to develop a specific postcalorimetric approach for correction of metabolic monitor variability.

calibration procedure allowing us to control for cart-dependent inaccuracy and variability in measurements with any canopy-based metabolic monitor.

SUBJECTS AND METHODS

Subjects

Inclusion criteria for participation in the IC studies were age >18 y and written informed consent. Exclusion criteria were the presence of an acute or chronic illness, pregnancy or breastfeeding in women, and contravention of the requirements of the study. Initial recruitment started in March 2008.

All subjects received detailed preparatory oral and printed information on the aims and prerequisites of the study. The following requirements for REE measurements were specifically stressed: 1) absence of vigorous physical activity or alcohol abuse for 20 h, 2) no intake of food or drinks other than water for at least 8 h, 3) abstention from nicotine for 3 h, 4) restriction of any physical activity in the morning of the study day to the absolute necessary minimum, and 5) arrival in the metabolic unit at 0800 (±0.5 h). Adherence to these guidelines was required for just before the measurements. Subsequent to the REE measurements, the participants underwent bioimpedance analysis (Body Impedance Analyzer Nutrigard-S; Data Inputy) for assessment of fat mass and lean body mass, and a generous breakfast was served after the tests.

The study protocol was examined and approved by the ethics committee of the University of Düsseldorf.

The following 3 cohorts participated in this study. The anthropometric data are summarized in Table 1.

Cohort 1 (cart comparison cohort): A total of 40 healthy adults participated in the metabolic monitor comparison study.

Cohort 2 (cart evaluation cohort): This group comprised 22 healthy adults. In this series, the mean rates of CO2 production and O2 consumption in vivo as read out by the metabolic carts were calibrated for each individual in a direct subsequent in vitro simulation experiment (mass-flow meter regulated infusion of pure gaseous CO2 and N2).

Cohort 3 (intraindividual variability cohort): Each of 6 healthy adult subjects underwent multiple REE measurements on 4 different study days. The mean (±SD) total observation period was 21 ± 5 wk (median: 21 wk; range: 14–16 wk), and the mean (±SD) interval between measurements was 7 ± 5 wk (median: 6 wk; range: 4–16 wk).

Metabolic monitors and measurements

Carts

Two flow-through system metabolic carts allowing gas dilution–based canopy-mode measurements were comparatively evaluated: 1) the Deltatrac MBM-100 (Datex), equipped with an infrared CO2 sensor and a differential paramagnetic O2 sensor and fixed mass flow (40 L/min), and 2) the Vmax Encore 29n (SensorMedix; delivered by Cardinal Health Germany) with an infrared CO2 sensor and an electrochemical O2 sensor and equipped with a variable mass flow.

Maintenance was performed on the Deltatrac and the Vmax Encore before the study and the devices were checked extensively for adequate performance by the authorized technical service units at GE Health Care and Cardinal Health Germany, respectively. Before each use in the metabolic unit, the carts were prepared and calibrated according to the manufacturers’ instructions.

The Deltatrac and Vmax Encore provide VCO2 and VO2 in milliliters per minute and liters per minute, respectively, with the readout adjusted to standard temperature pressure dry conditions at temperature = 273 K and pressure = 1013 hPa.

Energy expenditure measurements

The subjects arrived at ~0800; they were allowed to void and were then asked to lie down in comfortable beds in a quiet special study room. Overall, study conditions were in accordance with

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cohort 1 (metabolic cart comparison)</th>
<th>Cohort 2 (metabolic cart evaluation)</th>
<th>Cohort 3 (intraindividual variability)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>28</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>Age (y)</td>
<td>38.8 ± 13.3</td>
<td>48.1 ± 10.1</td>
<td>40.2 ± 14.5</td>
</tr>
<tr>
<td>Median (range)</td>
<td>31.8 (20.8–62.6)</td>
<td>51.1 (27.9–60.0)</td>
<td>32.7 (25.8–63.9)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167 ± 8</td>
<td>181 ± 7</td>
<td>167 ± 8</td>
</tr>
<tr>
<td>Median (range)</td>
<td>167 (153–181)</td>
<td>184 (165–189)</td>
<td>170 (152–180)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66 ± 12</td>
<td>83 ± 18</td>
<td>68 ± 9</td>
</tr>
<tr>
<td>Median (range)</td>
<td>63 (49–93)</td>
<td>78 (68–135)</td>
<td>67 (49–84)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.6 ± 4.1</td>
<td>25.3 ± 4.9</td>
<td>24.5 ± 3.9</td>
</tr>
<tr>
<td>Median (range)</td>
<td>22.9 (18.9–36.7)</td>
<td>23.1 (21.6–37.8)</td>
<td>23.3 (18.4–33.4)</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>19 ± 7</td>
<td>17 ± 11</td>
<td>20 ± 6</td>
</tr>
<tr>
<td>Median (range)</td>
<td>18 (9–35)</td>
<td>16 (6–46)</td>
<td>20 (8–32)</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>47 ± 5</td>
<td>66 ± 9</td>
<td>48 ± 5</td>
</tr>
<tr>
<td>Median (range)</td>
<td>45 (40–58)</td>
<td>64 (56–83)</td>
<td>48 (41–59)</td>
</tr>
</tbody>
</table>

4Healthy subjects without stated acute or chronic illness entered the study in the postabsorptive state (overnight fast >8 h).

2Mean ± SD (all such values).
recommended best practice guidelines (16, 17). The room temperature was 20–22°C, and blankets were available when requested. After a resting period (10 min), the canopy of one of the metabolic carts was positioned over the participant’s head and IC measurement was started with an initial 10-min period to acclimatize the participant to the device and for equilibration. Subsequently, a 20-min recording period followed while the subject remained under strict resting conditions. Data readout (ie, VCO₂ and VO₂) was set at the highest available frequency (ie, Deltatrac: 1 readout/min; Vmax Encore, 3 readouts/min). After a second resting period (10 min), the subject received a second canopy measurement with the other metabolic cart with the use of the same time protocol as in the first measurement.

In general, 2 subjects were investigated in parallel with both carts, and the sequence of cart use was randomly assigned throughout the trials. Preparatory measures, calibration procedures, and IC measurements were performed or supervised by one researcher with special experience and expertise.

Gas-exchange simulation studies

In vitro, CO₂ production and O₂ consumption were simulated by infusion of gaseous CO₂ (purity: 4.5) and N₂ (purity: 5.0), respectively, by using a high-precision mass-flow regulator (series 358; range: 0–2 L/min; Analyt-MTC) calibrated to standard conditions (temperature = 273 K, pressure = 1013 hPa). On the basis of the known infused mass flow VinfusedCO₂ and VinfusedN₂, the expected readouts of the metabolic carts were then calculated as VcalcCO₂ and as VcalcO₂, respectively, as given below.

Initial experiments conducted in canopy mode with an artificial head model showed that steady state conditions were established within a time period comparable to the in vivo measurements. We then examined a direct mode. Artificial head and canopy were omitted and gaseous CO₂ and N₂ were directly introduced via the mass-flow regulator into the canopy hose of the metabolic cart (canopy removed; see Supplemental Scheme 1 under “Supplemental data” in the online issue). In the direct mode, steady state conditions were reached within a few minutes and the cart readouts were identical in both modes with either cart. The more rapid direct mode was therefore applied in the subsequent in vitro experiments.

In vitro, the experimental conditions required throughout were as follows: VCO₂ and VO₂ between 0.1 and 0.5 L/min, with VCO₂:VO₂ ratios in the range of 0.6–1.1 and, with the Vmax Encore, an additional expiratory CO₂ (FeCO₂) in the range of 0.6–0.9%. After a 5-min equilibration period, 10-min data recordings were performed under steady state conditions with maximal rates of data readout (see above). In these experiments, the mean within-series CVs of the Deltatrac readouts were 0.33 ± 0.25% (median: 0.28%; range: 0.00–1.82%) for VCO₂ and 0.56 ± 0.36% (0.46%; 0.00–1.82%) for VO₂. The respective mean CVs of the Vmax Encore were 0.56 ± 0.17% (0.53%; 0.26–0.17%) for VCO₂ and 0.88 ± 0.31% (0.82%; 0.27–1.89%) for VO₂. Of note, the CVs tended to be higher at lower gas-flow rates.

Case-related in vitro validation by individual calibration control evaluation

In a set of IC measurements in vitro, the metabolic cart readouts of VCO₂ and VO₂ were obtained as described above and then specifically validated by a subsequent in vitro simulation as follows. The means of the in vivo rates were calculated. Thereafter, gaseous CO₂ and N₂ were infused into the hose of the metabolic cart and the mass-flow meters adjusted until the mean rates as observed in an individual subject were exactly met. The resulting adjustment of the mass-flow regulator (in L/min, standard conditions; temperature = 273 K, pressure = 1013 hPa) was then taken to calculate the estimate for the true breath gas exchange rate for the subject (see below).

Data analysis and statistics

In vivo studies

The serial readouts of VCO₂ and VO₂ (in mL/min) were used to calculate the RQ

\[ RQ = V_{CO2} / V_{O2} \]  (1)

and the REE according to the abbreviated Weir equation (4)

\[ REE = (1.106 \times V_{CO2} + 3.941 \times V_{O2}) \times 1.44 \]  in kcal/d (2),

which is equivalent to

\[ REE = (1.106 \times V_{CO2} + 3.941 \times V_{O2}) \times 6.028 \]  in kJ/d (3)

The data presented are the within-series means of the 20 and 60 data points as obtained by the 20-min recordings with the Deltatrac and the Vmax Encore, respectively.

The proportional energy contribution of macronutrient oxidation [glucose/carbohydrate oxidation (Gox; %); fat oxidation (Fox; %); protein oxidation (Pox; %)] to REE was estimated on the basis of the assumption that Pox provided 15% of total REE (ie, Pox% = 15) in healthy subjects under postabsorptive conditions (overnight fast >8 h) (26) because urinary nitrogen excretion was not measured in these short-term experiments.

By using Atwater units of 16.74, 37.24, and 16.74 kJ/g of substrate (27, 28) as metabolic energy equivalents for carbohydrate, fat, and protein, respectively, Pox (in g/d) was estimated from REE (in kJ/d) as

\[ Pox = (0.15 \times REE) / 16.74 \]  (4)

By using established formulas (5, 9, 24–28), Gox and Fox (in g/d) were estimated from VCO₂ and VO₂ (mL/min) as

\[ Gox = [(4.55 \times V_{CO2} - 3.21 \times V_{O2}) \times 1.44] - 0.459 \times Pox \]  (5)

\[ Fox = [(1.67 \times V_{O2} - 1.67 \times V_{CO2}) \times 1.44] - 0.307 \times Pox \]  (6)

The relative energy contribution of carbohydrate and fat oxidation (ie, Gox% and Fox% in % REE) to total energy expenditure is given by
In vitro studies

Estimates for the expected VCO₂ and VO₂ in the simulation experiments in which defined infusion rates of gaseous CO₂ and N₂ (V_{infusedCO₂} and V_{infusedN₂} in standard liters per minute, normalized to a standard temperature of 0°C and pressure of 1013 hPa) were applied to mimic different rates of gas exchange rates considered the use of the Haldane transformation by the metabolic carts and were obtained as follows:

O₂ uptake (VO₂) and CO₂ exhalation (VCO₂) at a given rate of inspiration (V_i) and expiration (V_e) is calculated by

\[ V_{O2} = F_{iO2} \times V_i - F_{eO2} \times V_e \]  
\[ V_{CO2} = F_{eCO2} \times V_e - F_{iCO2} \times V_i \]

\( F_i \) and \( F_e \) represent the appropriate fractional amount of O₂ and CO₂ in the inspiratory and expiratory gas mixture, respectively.

\[ Gox(\%) = (Gox \times 16.74/REE) \times 100 \]  \( (7) \)
\[ Fox(\%) = (Fox \times 37.24/REE) \times 100 \]  \( (8) \)

\( V_e \) is known and adjusted by the metabolic cart (= V_{adj}), whereas \( V_i \) is unknown. The latter is derived by using the Haldane transformation. It is assumed that

\[ F_{iN2} \times V_i = F_{eN2} \times V_e \]

Then, \( V_i \) is calculated by

\[ V_i = F_{eN2} \times V_e \div F_{iN2}. \]  \( (11) \)

This is equivalent to

\[ V_i = [(I - F_{iO2} - F_{eCO2}) \div (I - F_{iO2} - F_{iCO2})] \times V_e \]  \( (12) \)

Thus, O₂ uptake and CO₂ output rates are calculated by the metabolic carts as

\[ V_{O2} = F_{iO2} \times [(I - F_{iO2} - F_{eCO2}) \div (I - F_{iO2} - F_{iCO2})] - F_{eO2} \times V_e \]  \( (13) \)

and

\[ V_{CO2} = F_{eCO2} \times V_e - F_{iCO2} \times [(I - F_{iO2} - F_{eCO2}) \div (I - F_{iO2} - F_{iCO2})] \times V_e. \]  \( (14) \)

In the simulation experiments, natural abundance in ambient air was assumed and fractional amounts of O₂ and CO₂ in inspired and expired air are given by the equations

<table>
<thead>
<tr>
<th>Metabolic cart</th>
<th>Vmax Encore 29n</th>
<th>Deltatrac MBM-100</th>
<th>Correlation ((R^2))</th>
<th>Difference ((P \text{ value}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCO₂ (mL/min)</td>
<td>170 ± 38⁴</td>
<td>181 ± 32</td>
<td>0.869</td>
<td>3.8 \times 10⁻⁵</td>
</tr>
<tr>
<td>VO₂ (mL/min)</td>
<td>162 (122–330)</td>
<td>173 (139–304)</td>
<td>0.865</td>
<td>1.4 \times 10⁻²</td>
</tr>
<tr>
<td>Median (range)</td>
<td>208 ± 41</td>
<td>211 ± 37</td>
<td>0.965</td>
<td>7.7 \times 10⁻⁸</td>
</tr>
<tr>
<td>RQ (ratio)</td>
<td>0.821 ± 0.052</td>
<td>0.856 ± 0.038</td>
<td>0.344</td>
<td>2.6 \times 10⁻⁴</td>
</tr>
<tr>
<td>Median (range)</td>
<td>0.851 (0.710–0.940)</td>
<td>0.860 (0.770–0.980)</td>
<td>0.966</td>
<td>6.1 \times 10⁻⁵</td>
</tr>
<tr>
<td>REE (MJ/d)</td>
<td>6.01 ± 1.21</td>
<td>6.16 ± 1.09</td>
<td>0.879</td>
<td>3.8 \times 10⁻⁵</td>
</tr>
<tr>
<td>Median (range)</td>
<td>5.79 (4.29–10.89)</td>
<td>5.79 (4.29–10.89)</td>
<td>0.966</td>
<td>6.1 \times 10⁻⁵</td>
</tr>
<tr>
<td>(kJ · h⁻¹ · kg⁻¹ LBM)</td>
<td>4.79 ± 0.52</td>
<td>4.93 ± 0.52</td>
<td>0.344</td>
<td>2.6 \times 10⁻⁴</td>
</tr>
<tr>
<td>Median (range)</td>
<td>4.74 (4.12–6.31)</td>
<td>4.93 (4.08–6.20)</td>
<td>0.966</td>
<td>6.1 \times 10⁻⁵</td>
</tr>
<tr>
<td>Substrate oxidation (%) of REE</td>
<td>0.141</td>
<td>0.141</td>
<td>0.141</td>
<td>6.1 \times 10⁻⁵</td>
</tr>
<tr>
<td>Gox</td>
<td>37 ± 20</td>
<td>50 ± 13</td>
<td>0.879</td>
<td>3.8 \times 10⁻⁵</td>
</tr>
<tr>
<td>Median (range)</td>
<td>40 (2–85)</td>
<td>50 (18–85)</td>
<td>0.966</td>
<td>6.1 \times 10⁻⁵</td>
</tr>
<tr>
<td>Fox</td>
<td>49 ± 20</td>
<td>35 ± 13</td>
<td>0.879</td>
<td>3.8 \times 10⁻⁵</td>
</tr>
<tr>
<td>Median (range)</td>
<td>45 (0–83)</td>
<td>35 (0–67)</td>
<td>0.966</td>
<td>6.1 \times 10⁻⁵</td>
</tr>
</tbody>
</table>

¹Forty subjects (cohort 1; see Table 1) underwent immediate subsequent indirect calorimetry measurements with both metabolic carts in random order. Fox, fat oxidation; Gox, glucose/carbohydrate oxidation; LBM, lean body mass; REE, resting energy expenditure related to lean body mass; RQ, respiratory quotient; VCO₂, carbon dioxide output rate; VO₂, oxygen consumption rate.

²Coefficient of determination as evaluated by linear regression analysis (least-squares method).

³As evaluated by Student’s t test.

⁴Mean ± SD (all such values).

⁵For estimation, protein oxidation was assumed to be 15% of total REE (see Subjects and Methods).
\[ F_{o2} = 0.2093 \] (15) 

\[ F_{eO2} = 0.2093 \times (V_e - V_{infusedN2} - V_{infusedCO2}) \times (1 + V_e) \] (16) 

and 

\[ F_{icO2} = 0.0004 \] (17) 

\[ F_{eCO2} = \left[ V_{infusedCO2} + 0.0004 \times (V_e - V_{infusedN2}) \right] \times (1 + V_e) \] (18) 

On the basis of these fractions, estimates of the rates of O\(_2\) consumption and CO\(_2\) output (\(V_{calc}\)) due to infusion of gaseous N\(_2\) and CO\(_2\) were calculated as 

\[ V_{calcO2} = 0.2646 \times V_{infusedN2} - 0.00016 \times V_{infusedCO2} \] (19) 

\[ V_{calcCO2} = V_{infusedCO2} - 0.00024 \times V_{infusedN2}. \] (20)

Of note, under the present test conditions, the correction for influence of infusion of CO\(_2\) on \(V_{calcO2}\) and of N\(_2\) on \(V_{calcCO2}\) is \(<0.1\%\) and can thus actually be neglected.

Results are the within-series means of the 10 and 30 data points as obtained by the 10-min recordings with the Deltatrac and the Vmax Encore, respectively, and RQ data were derived from the appropriate breath gas exchange variables as in the in vivo studies.

**Statistical analyses**

Results are presented as means ± SD (median and range in parentheses). Linear regression (least-squares method) was applied for correlation analysis. Differences between paired or unpaired data sets were assessed by using 2-tailed Student’s \(t\) test as appropriate. Significant differences required \(P < 0.05\). Inter- and intraindividual CVs (CV\(_i\)) were calculated from the total CV (CV\(_t\)) and analytic CV (CV\(_a\)) as

**FIGURE 1.** Comparison of the Vmax Encore 29n (SensorMedix) and Deltatrac MBM-100 (Datex) breath gas-exchange measurements in healthy subjects. Postabsorptive adults (\(n = 40\)) underwent indirect calorimetry measurements with either cart in random order as detailed in Subjects and Methods (see also Table 1). Bland-Altman plots of \(V_{CO2}\) (A, C) and \(V_{O2}\) (B, D) data are shown. Mean differences between carts and ranges for ±1.96 SD and 95% CIs are indicated. Abs., absolute; CI\(_{lower}\), lower CI; CI\(_{upper}\), upper CI; DT, Deltatrac MBM-100; Rel., relative; \(V_{CO2}\), carbon dioxide output rate; Vmax, Vmax Encore 29n; \(V_{O2}\), oxygen consumption rate.
\[
CV_i = \sqrt{CV_i^2 - CV_a^2}
\]

(21)

Method comparison was visualized by using Bland-Altman plots. The 95% limits of agreement (CL) were calculated as

\[
CL = mean \pm 1.96 \times SD
\]

(22)

The limits of the 95% CI were calculated as

\[
CI = mean \pm (1.96 \times SD + 1.71 \times t(95\%,n-1) \times SD \div \sqrt{n})
\]

(23)

and are shown as recommended (29–32).

RESULTS

Comparative in vivo assessment of the metabolic carts

The metabolic carts were compared by performing parallel IC measurements in 40 fasted subjects under strictly controlled conditions. A summary of results is presented in Table 2. Significant differences between carts were found with all variables (\(P < 0.02\)). Correlation between carts was only moderate for the primary variables \(\text{VCO}_2\) and \(\text{VO}_2\) and REE (\(R^2 > 0.86\)), poor for RQ (\(R^2 < 0.35\)), and unacceptable for the estimates for macronutrient oxidation (Gox and Fox, \(R^2 < 0.15\)).

Variations were essentially due to a distinct variability in \(\text{VCO}_2\) and \(\text{VO}_2\) measurement (see Figure 1). In relation to the mean value of both methods, the relative differences of both devices (ie, Vmax Encore minus Deltatrac) in \(\text{VCO}_2\) and \(\text{VO}_2\) were \(-7 \pm 6\%\) (range: \(-22\%\) to +8\%) and \(-2 \pm 4\%\) (\(-14\%\) to +5\%), respectively.

These differences propagated differentially into derived metabolic estimates. With REE, the mean relative difference was small and amounted to \(-3 \pm 4\%\) (\(-13\%\) to +6\%). With RQ, the mean difference was 0.044 \(\pm\) 0.042 (\(-0.15\) to +0.04) units (Figure 2). Macronutrient oxidation estimates were strongly affected. The mean relative differences were \(-47 \pm 59\%\) (\(-182\%\) to +122\%) with Gox and 23 \(\pm 57\%\) (\(-200\%\) to +200\%) with Fox, and correlation between methods was practically abolished (see Figure 3).

In vitro evaluation of cart performance characteristics

Toward an explanation of the differences between monitors, in vitro simulation experiments were performed in parallel with both carts by using calibrated gas-flow regulators and a pure mixture of 21\% \(\text{CO}_2\) in \(\text{N}_2\) to imitate \(\text{VCO}_2\) and \(\text{VO}_2\) at a fixed RQ = 1.00.
Accuracy of VCO₂ and VO₂ readouts

In simulation experiments (n = 9) comprising 20 different flow rates in series (range: >0.1 to <0.5 L/min), linear-regression estimates of slope and intercept between cart readouts and mass-flow regulator-adjusted flow were 1.0 and within ±0.018 L/min, respectively, and overall \( R^2 \) was >0.995 with either cart (see Supplementary Figure 1 under “Supplemental data” in the online issue). A closer inspection of data showed distinct differences.

Between devices, VCO₂ and VO₂ were significantly different (\( P < 0.05 \)) in a nonlinear fashion over the whole measuring range and at a flow >0.27 L/min, respectively (see Supplementary Figure 2 under “Supplemental data” in the online issue).

The deviation of readouts from adjusted values is shown in Figure 4. With the Deltatrac, differences were significant (\( P < 0.05 \)) over the whole range and with the Vmax Encore at flows <0.22 L/min. Deviations in Deltatrac readouts of VCO₂ and VO₂ were approximately linear and inverse linear correlated to the mass-flow regulator-adjusted flow, respectively. A complex nonlinear deviation pattern was found with the Vmax Encore. Of note, interassay variability in readouts was high, leading to a considerable broadening of confidence limits with both carts.

Variability and long-term stability

In simulation experiments, the CVs of VCO₂ and VO₂ readouts were <1% within series and <3% between series with both monitors. With the Vmax Encore, within- and between-series CVs tended to be higher than with the Deltatrac (Table 3).

Long-term stability (10-h period) was excellent with both carts, although some significant but very low drifts were observed at higher flow rates (Table 4).

Fractional mean estimates for the impact of analytic variability on metabolic cart measurements of VCO₂ and VO₂ in humans were as follows—within series: <0.02; between series: <0.04 and <0.20 for interindividual and intraindividual assays, respectively (Table 5).

Exploitation of RQ readouts

The RQ in simulation experiments was 1.0045 units throughout. The Deltatrac readouts yielded significantly elevated RQ values.
over the whole flow range, with a mean increment of +0.090 ± 0.026 units (range: 0.025–0.176 units) \((n = 180)\). With the Vmax Encore, the mean RQ was not significantly different from the assigned value and amounted to 0.002 ± 0.033 units (range: 0.048 to 0.102 units; see Supplementary Figure 3 under “Supplemental data” in the online issue).

Together, these variabilities point at the physiologically significant impact of cart readout errors on derived secondary metabolic variables.

**Impact of cart series**

Due to the inherent variability of metabolic monitors, cart differences might also be suspected in instruments of the same type. In fact, when series of simulation assays were performed using 2 Vmax Encore devices of identical construction (ie, Vmax Encore 29n) in parallel, considerable variability in simultaneously obtained VCO2 and VO2 readouts was found with but minor effects on the overall mean values (Supplementary Figure 4 under “Supplemental data” in the online issue).

**Accomplishment of accurate IC measurements in humans by individual calibration control evaluation**

To reduce interferences of metabolic cart deviations and variability in human studies, the postcalorimetric individual calibration control evaluation (ICcE) procedure was introduced. The procedure evaluates cart performance for each individual IC measurement by an appropriate in vitro simulation measurement.

**Value of case-related in vitro validation**

The relevance of ICcE was evaluated in vivo by performing comparative IC measurements with Deltatrac and Vmax Encore devices in postabsorptive subjects \((n = 22)\). A summary of data is presented in Table 6. Distribution of and variation in VCO2 and VO2 readouts and in derived metabolic variables were comparable to the findings in the first in vivo study, and cart-dependent differences were confirmed.

By means of ICcE, distinct errors of the individual VCO2 and VO2 readouts were detected, which were comparable to the findings in the in vitro simulation studies (see Supplementary Figure 5 under “Supplemental data” in the online issue). Accordingly, considerable error was found in the derived estimates for REE, RQ, Gox, and Fox (see Supplementary Figure 6 under “Supplemental data” in the online issue).

Adjustment of the individual cart readout for the deviations detected by the ICcE procedure resulted in a considerably better agreement of results. Although some minor differences remained, in particular with the VCO2 values, the correlation between methods improved significantly. In this data set, the coefficients of determination \(R^2\) for the primary variables, VCO2 and VO2, were 0.936 and 0.953, respectively, and for the estimates of the derived variables REE, RQ, Gox, and Fox were 0.922, 0.929, 0.939, and 0.927, respectively.

**DISCUSSION**

Among metabolic monitors, the Deltatrac was supposed to provide the greatest accuracy and least intercart variability of all such devices worldwide (ie, a “gold standard” for canopy-based IC), but it is no longer produced (14, 21, 25). In the present study in healthy volunteers, we compared the Deltatrac with the Vmax Encore metabolic monitor. The data were similar to results reported by Cooper et al (25). The primary data, ie, breath gas exchange rates, as well as the derived secondary metabolic results differed significantly between devices, although carefully designed preparatory measures were imposed on the subjects under study and strictly controlled experimental conditions were applied for the measurements. Specifically, mean VCO2 was found...
steady state conditions by using the maximal rate of data readout (ie, Vmax).

Ten-minute recordings were performed under calibrated mass-flow regulators; and variable gas-flow rates (between 0.1 and 0.5 L/min adjusted by means of

rate; VO2, oxygen consumption rate.

Between-series CV

Gox, and Fox. On the whole, differences in cart readouts led marked differences were observed with the estimates for RQ, Gox, and Fox. Accordingly, REE estimates,
to be substantially higher with the Deltatrac, with more subtle differences in VO2 measurements. Accordingly, REE estimates, to be substantially higher with the Deltatrac, with more subtle differences in accuracy.

In studies in which different carts devices are applied. In the latter case, data may additionally be confounded by device-specific differences in accuracy.

The foregoing considerations point toward the need for rigorous control of intra- and interdevice variability as a necessary

to substantially higher with the Deltatrac, with more subtle differences in VO2 measurements. Accordingly, REE estimates, which depend primarily on VO2, differed less markedly, but marked differences were observed with the estimates for RQ, Gox, and Fox. On the whole, differences in cart readouts led to a physiologically relevant variability between methods with a generally poor correlation of data and an essentially un-acceptable correlation of the estimates of RQ, Gox, and Fox.

However, because no uniform procedure is available to ensure accuracy and precision of metabolic carts, virtually no final preference can be assigned to either cart. Thus, the causes for the differences remain obscure. We therefore conducted an analysis of performance with either cart by in vitro simulation experiments with the use of pure gases and calibrated gas-flow regulators. The outcome, however, was somewhat disillusioning. The Deltatrac, the presumed reference cart, exhibited physiologically significant variation in measurements within series with respect to a rate-dependent deficiency of accuracy as well as between series with respect to a variable imprecision in repeatability. Similar problems with accuracy and repeatability were encountered with the Vmax Encore. Interestingly, however, the performance characteristics showed distinct differences, eg, with a nearly linear rate dependence of accuracy with the Deltatrac compared with a nonlinear dependence with the Vmax Encore. Of note, the effect of device-inherent variability on differences in the measurements was also shown when parallel examinations were performed with 2 identical Vmax Encore carts.

Clearly, an inevitable within- and between-series variability in metabolic carts exists. Thus, deviation of the cart readout from the true value cannot be predicted reliably. Any given individual measurement is therefore uncertain to an unknown extent. In principle, confidence limits for a cart readout can be estimated from the present experimental data, but these variables are inappropriate for evaluation of an individual measurement or repeat measurements in one subject at different occasions. Variability within carts of the same series may finally level out in population-based studies with large numbers of participants but will remain in studies in which different carts devices are applied. In the latter case, data may additionally be confounded by device-specific differences in accuracy.

The foregoing considerations point toward the need for rigorous control of intra- and interdevice variability as a necessary

to be substantially higher with the Deltatrac, with more subtle differences in VO2 measurements. Accordingly, REE estimates, which depend primarily on VO2, differed less markedly, but marked differences were observed with the estimates for RQ, Gox, and Fox. On the whole, differences in cart readouts led to a physiologically relevant variability between methods with

| TABLE 3 | Variability of metabolic cart measurements in vitro |
| Metabolic cart | | |
| Variable | Vmax Encore 29n | Deltatrac MBM-100 |
| Within-series CV | | |
| VCO2 | 0.56 ± 0.17 | 0.33 ± 0.25 |
| VO2 | 0.88 ± 0.31 | 0.56 ± 0.37 |
| Between-series CV | | |
| VCO2 | 3.26 ± 0.85 | 1.03 ± 0.22 |
| VO2 | 2.91 ± 0.89 | 1.77 ± 0.45 |

1 Data are percentages as evaluated in analyses with a fixed VCO2:VO2 ratio (adjusted by infusion of a calibrated gas mixture of 21% CO2 in N2) and variable gas-flow rates (between 0.1 and 0.5 L/min adjusted by means of calibrated mass-flow regulators; see Supplemental Figure 1 under “Supplemental data” in the online issue). Ten-minute recordings were performed under steady state conditions by using the maximal rate of data readout (ie, Vmax Encore, 3 readouts/min; Deltatrac, 1 readout/min). VCO2, carbon dioxide output rate; VO2, oxygen consumption rate.

2 SensorMedix.

3 Datex.

4 Values are based on successive serial measurements of VCO2 and VO2 at 20 different rates, respectively (see Supplemental Figure 1 under “Supplemental data” in the online issue), with a total of 9 repeats performed on different working days. Of note, CVs were essentially independent from the flow rate.

5 Mean ± SD (all such values).

6 | TABLE 4 | Long-term stability of metabolic cart measurements in vitro |
| Metabolic cart | | |
| Variable | Vmax Encore 29n | Deltatrac MBM-100 |
| VCO2 (mL/h) | -0.12 ± 0.13V (-0.04 ± 0.03) | 0.06 ± 0.21HIIV (0.009 ± 0.063) |
| VO2 (mL/h) | 0.32 ± 0.12IIIIV (0.13 ± 0.05) | 0.02 ± 0.02IIIIV (0.0003 ± 0.0837) |
| RQ (units) | -0.0018 ± 0.0004IVIIIIV | 0.0008 ± 0.0017 |
| REE (kJ/h) | 0.28 ± 0.10IIIIV (0.10 ± 0.04) | 0.14 ± 0.40IV (0.0005 ± 0.0012) |

1 Values are mean ± SD changes with time of the 4 slope estimates obtained and were evaluated in parallel with both carts by using a calibrated gas mixture (21% CO2 in N2) at 4 different flow rates [ie, levels I, II, III, and IV at 0.65, 0.95, 1.25, and 1.80 L/min, respectively, which is equivalent to VCO2:VO2 ratios of 141:140, 206:205, 271:270, and 391:389, respectively]. At each level, time-dependent changes (ie, slope estimates) over a total observation period of 10 h were evaluated by linear regression analyses on the basis of data from five 20-min recordings. These measurements were equally distributed over time and were performed under steady state conditions. Time-dependent changes at the different gas flow rates were in a comparable range. For convenience, means ± SDs of the relative changes observed at the 4 flow levels (given in %/h) are shown in parentheses. IVIIIIV Significant changes with time (linear regression analysis, least squares) at P < 0.01 at gas-flow rates for levels I, II, III, or IV, respectively. REE, resting energy expenditure (as estimated by the abbreviated Weir equation; see Subjects and Methods); RQ, respiratory quotient; VCO2, carbon dioxide output rate; VO2, oxygen consumption rate.

2 SensorMedix.

3 Datex.
requirement for a reasonable comparability of data from canopy-based IC studies in humans. The possible effect of device-specific variability on the primary readout of metabolic monitors and the impact on derived metabolic variables have not been appropriately reviewed in the literature (8, 9, 13–17, 20–25).

To overcome these problems, we have now developed a new postcalorimetric procedure, ie, the ICcE, for quantitative evaluation of each individual measurement. To avoid any change in performance or setup of the instrument, this evaluation procedure has to be performed in series directly after an IC assessment. The

Table 5

Impact of analytic variability on metabolic cart measurements in humans

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total variation</th>
<th>Vmax Encore 29n&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DeltaTrac MBM-100&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV in %</td>
<td>fraction of total mean CV/upper 95% CL</td>
<td></td>
</tr>
<tr>
<td>Within-series CV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCO₂</td>
<td>6.2 ± 2.4</td>
<td>&lt;0.01/0.01</td>
<td>&lt;0.01/0.01</td>
</tr>
<tr>
<td>VO₂</td>
<td>4.9 ± 1.9</td>
<td>&lt;0.02/0.06</td>
<td>&lt;0.01/0.05</td>
</tr>
<tr>
<td>Between-series CV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interindividual assays</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCO₂</td>
<td>12.5</td>
<td>&lt;0.04/0.12</td>
<td>&lt;0.01/0.01</td>
</tr>
<tr>
<td>VO₂</td>
<td>10.8</td>
<td>&lt;0.04/0.10</td>
<td>&lt;0.02/0.04</td>
</tr>
<tr>
<td>Intraindividual assays</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCO₂</td>
<td>7.2 ± 2.9</td>
<td>0.11/0.27</td>
<td>0.01/0.03</td>
</tr>
<tr>
<td>VO₂</td>
<td>4.9 ± 1.9</td>
<td>0.20/0.68</td>
<td>0.07/0.16</td>
</tr>
</tbody>
</table>

<sup>a</sup>As evaluated in 20-min standard indirect calorimetry recordings in postabsorptive healthy subjects (cohort 1; see Table 1 and footnote 1 of Table 2). For comparative purposes, metabolic data were normalized to unit weight of lean body mass, CL, confidence limit; VCO₂, carbon dioxide output rate; VO₂, oxygen consumption rate.

<sup>b</sup>Means ± SDs of series with 40 different participants (see footnote 1 of Table 2).

<sup>c</sup>Estimates were based on the analytic imprecision data compiled in Table 4; the maximum is indicated as the 95% CL.

<sup>d</sup>SensorMedix.

<sup>e</sup>Datex.

<sup>f</sup>Variability of measurements in 40 different subjects (see above).

<sup>g</sup>Means ± SDs from 6 healthy subjects undergoing a series of 4 different intraindividual measurements within a mean period of 3 mo (see Subjects and Methods).

Table 6

Accuracy of indirect calorimetry measurements and derived metabolic variables in healthy subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vmax Encore 29n&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DeltaTrac MBM-100&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Readout</td>
<td>Calibrated&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>VCO₂ (mL/min)</td>
<td>154 ± 21</td>
<td>170 ± 22&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>VO₂ (mL/min)</td>
<td>189 ± 26</td>
<td>202 ± 26&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Median (range)</td>
<td>184 (144–257)</td>
<td>200 (159–270)</td>
</tr>
<tr>
<td>RQ</td>
<td>0.815 ± 0.049</td>
<td>0.841 ± 0.046&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Median (range)</td>
<td>0.808 (0.726–0.903)</td>
<td>0.832 (0.765–0.937)</td>
</tr>
<tr>
<td>REE (MJ/d)</td>
<td>5.52 ± 0.74</td>
<td>5.92 ± 0.75&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Median (range)</td>
<td>5.45 (4.21–7.40)</td>
<td>5.82 (4.70–7.83)</td>
</tr>
<tr>
<td>Gox (g/h)</td>
<td>4.6 ± 2.5</td>
<td>6.4 ± 2.6&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Median (range)</td>
<td>3.8 (0.1–10.3)</td>
<td>5.8 (2.2–13.0)</td>
</tr>
<tr>
<td>Fox (g/h)</td>
<td>2.9 ± 1.1</td>
<td>2.6 ± 1.1&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Median (range)</td>
<td>3.0 (1.1–5.4)</td>
<td>2.7 (0.6–4.9)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Twenty-two postabsorptive subjects without acute or chronic illness received immediate subsequent indirect calorimetry measurements with both metabolic carts in random order. Results are presented as means ± SDs; median and range in parentheses).<sup>1</sup>–<sup>III</sup>Significant differences (Student’s t test)—I: vs appropriate cart readout, P < 0.05; II: between carts, P < 0.005; III: between carts, P < 0.005. Fox, fat oxidation (protein oxidation was assumed to account for 15% of total REE; see Subjects and Methods for details); Gox, glucose/carbohydrate oxidation; NA, not applicable; NS, no difference between carts; REE, resting energy expenditure; RQ, respiratory quotient; VCO₂, carbon dioxide output rate; VO₂, oxygen consumption rate.

<sup>b</sup>SensorMedix.

<sup>c</sup>Datex.

<sup>d</sup>Cart readouts were calibrated for each individual in a subsequent simulation experiment (ie, mass-flow regulator infusion of pure gaseous CO₂ and N₂ as detailed in Subjects and Methods).

<sup>e</sup>Means ± SDs of deviation of readouts as related to calibrated estimates (with the minimal/maximal deviation in parentheses).
determination of the appropriate deviations in the cart readout from the true in vivo values is achieved by mimicking the readout of the in vivo measurement. This is done in vitro by simulating exactly the in vivo rate readout by means of mass-flow regulator-controlled infusion of pure gaseous CO₂ and N₂ via appropriate tubes into the hose of the flow-through system of the metabolic cart. In detail, the flow of gaseous CO₂ is upregulated via the regulator until the cart readout exactly matches the mean VCO₂ that was observed in the previous in vivo measurement. The best estimate for the true value for VCO₂ in the patient is now shown directly on the CO₂-flow regulator. Similarly, the N₂ flow (VN₂) is upregulated to yield a cart readout that matches the mean VO₂ in vivo. The best estimate for O₂ consumption rate in vivo is calculated from VN₂ as VO₂ = VN₂ × 0.2646. The factor accounts for 1) the fact that N₂ infused in the system replaced not only oxygen but whole room air and 2) the numeric contribution of the Haldane transformation on the metabolic cart readout.

According to our experience, the mean in vivo values can be reproduced in vitro on the metabolic cart with an agreement better than ±2 mL/min in ~15 min. Of note, for reliable and accurate performance of the ICcE, it is mandatory that the gas-flow regulators are carefully maintained and (re)calibrated according to the manufacturers’ recommendations and that the standard temperature pressure dry conditions applied for regulator calibration are identical to those that are used in the metabolic cart under evaluation.

The applicability and usefulness of the ICcE procedure were verified in another Deltatrac compared with Vmax Encore IC study in vivo. In this human study, the agreement of the metabolic variable estimates for REE, RQ, and the macronutrient oxidation rates (Gox and Fox) increased significantly, and only minor differences between the 2 carts remained.

In conclusion, the ICcE procedure established in this study appears to be suitable to control for variability in devices based on flow-through respiratory measurements in canopy mode, independent of device, time, and place.

We gratefully acknowledge the skillful help of Waldemar Merck with construction and setup of the homemade ICcE unit.

The authors’ responsibilities were as follows—PS: design and supervision of experiments and primary responsibility for the final content; PS, BN, and JK: collection and evaluation of data; PS, KS, and JK: data analysis and interpretation; and PS, BS, KS, JK, and MR: writing of the manuscript. All authors contributed substantially to the concept, data collection and analysis, or preparation of manuscript and read and approved the final manuscript. None of the authors had a conflict of interest to declare.

REFERENCES