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ORIGINAL ARTICLE

Clinical Trials and Investigations

Validation of energy expenditure and macronutrient oxidation measured by two new whole-room indirect calorimeters

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Abstract

Objective: The aim of this study was to validate two new whole-room indirfect calorimeters according to Room Indirect Calorimetry Operating and Reporting Standards (RICORS 1.0).

Methods: For technical validation, 16 propane combustion tests were performed to determine accuracy and precision of energy expenditure (EE) and ventilation rates of oxygen (VO_2), carbon dioxide (VCO_2), and respiratory exchange ratio (VCO_2/VO_2). For biological validation, eight participants (mean [SD], age 24.1 [2.5] years; BMI 24.3 [3.1] kg/m^2) underwent four 24-hour protocols under highly standardized conditions: (1) isocaloric sedentary, (2) fasting sedentary, (3) isocaloric active, and (4) fasting active. Reliability (coefficients of variation [CV]) and minimal detectable changes (MDC) were calculated for 24-hour EE, sleeping metabolic rate (SMR), physical activity energy expenditure (PAEE), thermic effect of food (TEF), and macronutrient oxidation rates.

Results: Technical validation showed high reliability and recovery rates for VO_2 (0.75% and 100.8%, respectively), VCO_2 (0.49% and 100.6%), and EE (0.54% and 98.2%). Biological validation revealed CV and MDC for active conditions of 1.4% and 4.3% for 24-hour EE, 1.7% and 5.9% for SMR, and 30.2% and 38.4% for TEF, as well as 5.8% and 10.5% for PAEE, respectively. Mean CV and MDC for macronutrient oxidation rates were 9.9% and 22.9%, respectively.

Conclusions: The precision of 24-hour EE and SMR was high, whereas it was lower for PAEE and poor for TEF.

INTRODUCTION

Modern respiratory exchange chambers (whole-room indirect calorimeter [WRIC]) provide high-resolution data for the assessment of circadian changes in energy expenditure (EE) [1] or the responses of macronutrient oxidation during meals, exercise, and sleep, including metabolic flexibility [2]. The technique is used in research on the impact of energy flux [3], time-restricted feeding [1, 4], or macronutrient composition of the diet [5, 6] on energy and macronutrient balance.

Studies involving WRICs provide a strictly controlled environment for human intervention trials, with standardization and close supervision of the daily routine, sleeping times, physical activity, and intake of food and beverages. Control for these confounders and high

precision of the metabolic measurement lead to the necessity for a lower number of participants required for a WRIC study when compared with interventions under free-living conditions that use doubly labeled water for measurement of EE and changes in body composition as estimates of energy and macronutrient balance (for reviews, see [7,8]). Because of the high expenditure of time, multicenter WRIC studies offer another means by which to reduce the number of participants needed at one study site. Because WRICs in many facilities are custom engineered and differ widely in the type of analyzers, software applications, room size, and structural installations, validation of each WRIC lab is mandatory before the fusion of data sets from different study centers [9]. The Room Indirect Calorimetry Operating and Reporting Standards version 1.0 (RICORS 1.0) guide validation to ensure reproducibility and facilitate comparisons of human WRIC studies across multiple centers [10,11].

In this study, two new WRICs built in 2019 at Kiel University (Germany) were validated according to RICORS 1.0. Limitations and applicability of physical activity energy expenditure (PAEE) and thermic effect of food (TEF) determined by "regression analysis" or "area under the curve method" were compared. Reproducibility of 24-hour EE, sleeping metabolic rate (SMR), respiratory exchange ratio (RER), PAEE, TEF, and macronutrient oxidation was assessed by repeated measurements of three different conditions: isocaloric sedentary (IsoSed), fasting sedentary (FastSed), and isocaloric active (IsoAct). Technical validation of gas exchange measurements was performed using the recovery rates of VO_2 (oxygen consumption) and VCO_2 (carbon dioxide production) by propane combustion. In addition, challenges to the validity of the outcome measures (e.g., habituation, excitement, tension) were analyzed to reveal biological determinants of bias.

Study Importance

What is already known?

- The Room Indirect Calorimetry Operating and Reporting Standards (RICORS) 1.0 for whole-room indirect calorimeter (WRIC) validation were developed to compare data of human energy metabolism from different study sites, because most of the WRICs are custom built.

What does this study add?

- RICORS 1.0 are feasible, and the application shows that the precision of Kiel WRICs in 24-hour energy expenditure (EE) and sleeping metabolic rate measurements was high, whereas it was lower for physical activity EE and macronutrient oxidation rates and poor for thermic effect of food.
- Situations with an influence on sympathetic nervous system activity like exercise under fasting conditions or excitement and tension due to an unaccustomed WRIC environment or birthday were identified to have an impact on the validity of components of EE.

How might these results change the direction of research or the focus of clinical practice?

- The high accuracy of EE and respiratory exchange ratio measurements facilitates data fusion for multicenter studies.
- Because of the high reproducibility for intraindividual comparison of EE as well as the time-consuming and elaborate study protocol in a WRIC, study designs based on intraindividual design have an advantage over interindividual comparisons except for objectives with a high effect size e.g., identification of thrifty and spendthrift metabolic phenotypes.

METHODS

The study was composed of a technical validation, using propane combustion and empty runs of the WRICs, and a biological validation, comprising healthy participants in a crossover intervention under isocaloric and fasting dietary conditions with sedentary and active protocols. Biological validation consisted of (1) reliability of measured components of EE and macronutrient oxidation rates, (2) a comparison of TEF and PAEE derived from regression analysis ($TEF_{regression}$, $PAEE_{regression}$, [12]) and area under the curve (AUC) subtraction method ($TEF_{subtraction}$ [13] and $PAEE_{subtraction}$), and (3) an analysis of the biological determinants of bias. Primary outcomes for the technical validation were ventilation rates of oxygen consumption (VO_2), carbon dioxide production (VCO_2), and respiratory exchange ratio (RER = VCO_2/VO_2, known at the cellular level as respiratory quotient [RQ]), along with simulated 10-hour EE. Accuracy was determined comparing the deviation of the measured value from the true value (i.e., the predicted ventilation rate of O_2 and CO_2 from propane combustion). The precision is given as means and standard deviation (SD) as well as intraclass correlation coefficients. In addition, we provide correlations between measured and predicted gas volumes in Supporting Information Figure S2. Outcome parameters for biological validation were 24-hour EE, SMR, RER, PAEE, TEF, and macronutrient oxidation rates.

Characteristics of the Kiel WRIC

The Institute of Human Nutrition at the University of Kiel has two identically constructed metabolic chambers. The respiratory exchange is measured by the Promethion (model GA-3m2/FG-250) integrated

WRIC (Sable Systems International; for details, see online Supporting Information).

The size of each WRIC is 9.8 m^2, and both are furnished for participant comfort (Figure 2C). The interior volume is 24,282 L, after correcting for that taken up by furnishings, toilet, sink, and cycle ergometer. Dual-level airtight air locks are used for the exchange of food, biological samples, and minor equipment.

RER is calculated from the gas exchange and is defined as the relationship of VCO_2 to VO_2. All metabolic parameters such as O_2, CO_2, water vapor pressure, excurrent flow rate, and barometric pressure are recorded every second and then amortized on a per minute basis prior to metabolic calculations. The 24-hour urinary nitrogen excretion, measured photometrically from 24-hour urine, is used to calculate macronutrient oxidation rates [14]. EE is calculated using the Weir equation [15].

Technical and biological validation

Propane combustion (99.2% propane, Scott Medical Products) was used for the technical validation process [16]. In each WRIC, sixteen 10-hour propane burns were conducted, monitoring the amount of burned propane with a digital scale every 30 minutes (OHAUS Explorer, OHAUS Europe GmbH). Accuracy and precision of the WRICs were determined through the recovery rates of simulated EE, VO_2, VCO_2, and RER. Additional information regarding formulas and propane combustion methodology is presented in detail elsewhere [16]. For quality assurance, monthly short propane burns were performed, each with a 5.5-hour duration [16]. In addition, empty runs of the WRIC were conducted, so VO_2 and VCO_2 were measured over several days to determine any abnormalities that may occur over the course of the measurement periods. If the WRICs are working correctly, there should be no measured VO_2, VCO_2, or EE detected during the empty test runs [10].

For the biological validation, healthy human participants followed a 5-week protocol, comprising two physical activity levels, sedentary and active, as shown in Figure 1. Metabolic measures composed of 24-hour EE, SMR, RER, TEF, PAEE, and macronutrient oxidation rates were conducted on two identical days. On these days, either isocaloric diets with sedentary (IsoSed) or active conditions (IsoAct) or inactivity and fasting (FastSed) were used, as shown in Figure 1. The 24-hour EE and RER were calculated for 24 hours from 6:30 AM to 6:30 AM SMR was calculated according to Schrauwen et al. [17] by taking the lowest mean EE during three consecutive hours between 11:30 PM and 6:30 AM The repeatability and plausibility of two different methods for assessing TEF and PAEE (regression vs. subtraction) were compared. Trapezoidal rule was used to estimate the AUC for EE, which enables the calculation of TEF [13] and PAEE as follows:

$$TEF_{subtraction} = 24h\ EE\ AUC\ isocaloric\ -\ 24h\ EE\ AUC\ fasting$$

$$PAEE_{subtraction} = 24h\ EE\ AUC\ active - 24h\ EE\ AUC\ inactive.$$

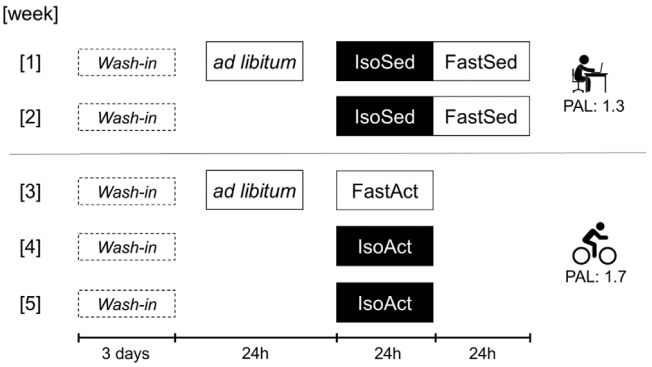

FIGURE 1 Outline of the study protocol with 24-hour interventions in the WRIC with four different conditions: isocaloric sedentary (IsoSed), fasting sedentary (FastSed), isocaloric active (IsoAct), and fasting active (FastAct). All boxes with solid line represent 24-hour stays in the WRIC. IsoSed and FastSed were conducted in a single stay over 48 hours. Isocaloric diets had a constant macronutrient ratio (52% carbohydrates, 35% fat, 13% protein). Different levels of physical activity were accomplished by cycling on an exercise bike with 50 W (female) or 75 W (male) and constant cadence (55–65 rpm) for defined time periods (3×20 minutes, three times a day; total of 3 h/d). A 3-day wash-in period with a controlled diet preceded each intervention phase. The figure displays the chronological sequence of the protocol. PAL, physical activity level

Regression analysis was applied for both parameters using the regression intercept between EE and acceleration volume per minute derived from accelerometry ($TEF_{regression}$, $PAEE_{regression}$, [12]), as shown in Figure 2B. However, the original regression method was not validated with accelerometry but with radar, which is a method that, in addition to lower limb activity, also detects chest and upper limb movements. Because the activity in our study on the exercise bike mainly involves the lower limbs, we used the acceleration volume derived from a triaxial accelerometer, which may have slightly underestimated the total movement. Spontaneous physical activity was determined according to Hall et al. [9], and resting metabolic rate (RMR) estimation was based on the assumption that SMR is 95% of RMR [18]. As an indication of sympathetic nervous system (SNS) activity dopamine excretion in 24-hour urine was measured by high-performance liquid chromatography.

Study protocol

Each participant went through seven interventions in the WRIC within 5 weeks. Participants either fasted or consumed isocaloric diets with a constant macronutrient ratio (52% carbohydrates, 35% fat, 13% protein). Physical activity level (PAL) was 1.3 on inactive days and 1.7 on active days. On inactive days, participants were allowed to move around freely but were instructed to spend their day mainly sitting without much activity (e.g., reading, computer work) and to avoid any athletic activity. On active days, physical activity was performed on a bicycle ergometer (opticare basic and ergoselect 4, ergoline GmbH)

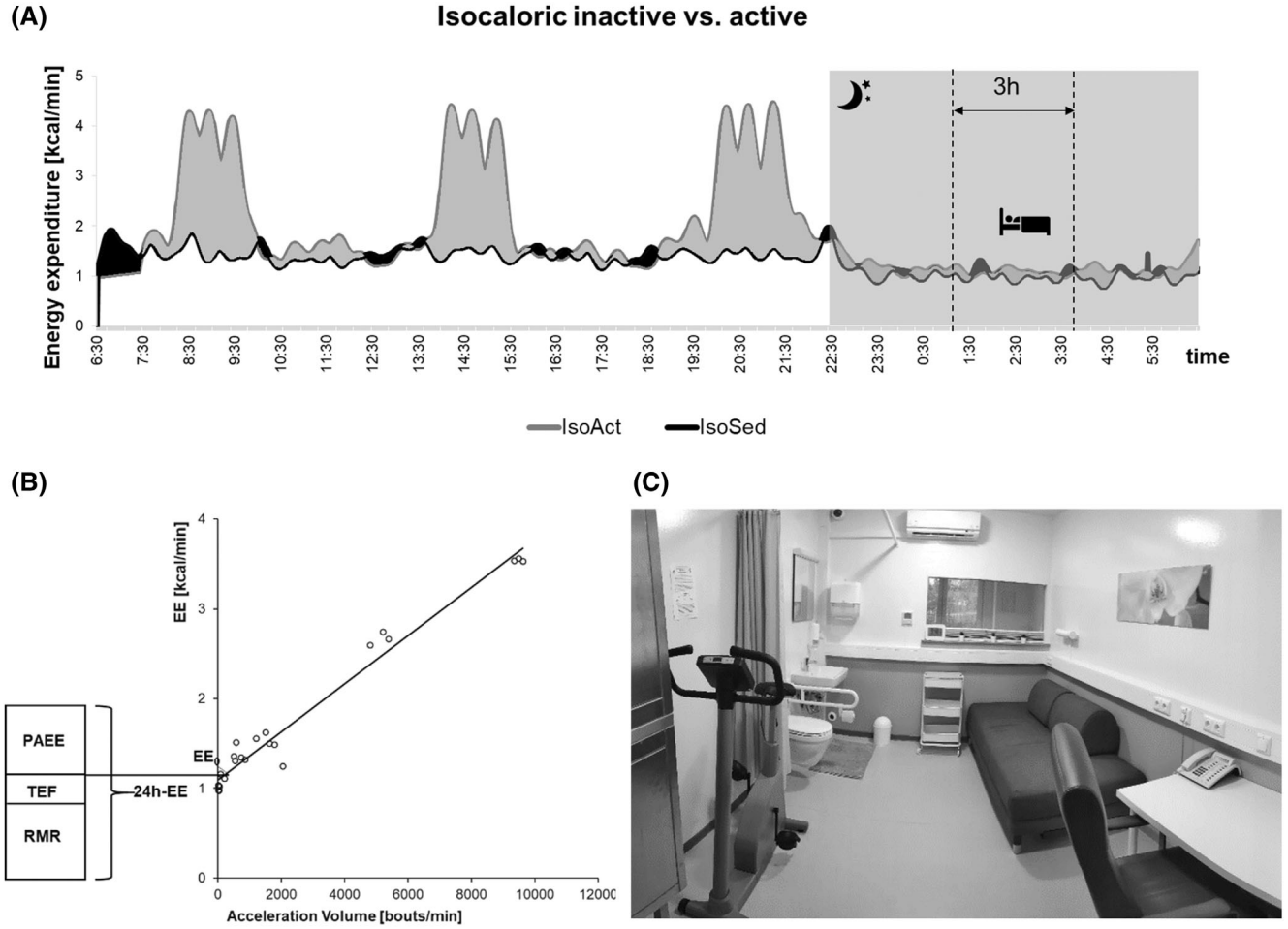

FIGURE 2 (A) Total energy expenditure (EE) over 24 hours, illustrated for one participant, comparing an inactive and an active day. Gray, area under the curve for EE on an active day being higher than on the inactive day. Black, area under the curve for EE on an inactive day being higher than on the active day. (B) Regression analysis, showing components of total daily energy expenditure (24-hour EE). Physical activity energy expenditure (PAEE) has a linear relationship with concurrent activity measured by triaxial accelerometer. Energy expenditure at zero activity (EE_0), i.e., the y-intercept, represents the sum of the resting metabolic rate (RMR) and thermic effect of food (TEF). RMR comprises sleeping EE and EE from arousal. Based on Schutz et al. [12] and Ravussin et al. [26]. (C) WRIC located at Kiel University, including a daybed, desk and chair, access to the internet, telephone, toilet, sink, and an exercise bike

for 3 × 20 minutes three times a day. Women were requested to cycle at 50 W and men at 75 W with a constant cadence (55–65 rpm). Physical activity was continuously monitored using step counts and acceleration volume per minute by a triaxial accelerometer (activPAL 4, Paltechnologies Ltd.). Each intervention week was initiated by a 3-day run-in period with a controlled diet and identical macronutrient composition to adapt macronutrient oxidation rates to macronutrient intake [19]. Participants were advised to maintain their habitual physical activity levels (< 1 h/d of exercise) and eat only the provided foods and noncaloric beverages without caffeine to ensure equal baseline conditions. Energy requirement under inactive and active conditions over 24 hours inside the WRIC was measured (preceding the IsoSed and fasting active [FastAct] days) with ad libitum energy intake (Figure 1). Participants entered the WRIC in the evening before each intervention period to adapt to the environment. A highly standardized diurnal rhythm (from 6:30 AM to 6:30 AM hours) was maintained throughout the study (wake-up at 6:30 AM; meals at 7:00 AM, 1:00 PM, 7:00 PM; and bedtime at 10:30 PM). Participants were asked to eat their meals within 30 minutes, without leftovers, on isocaloric intervention days. Individual diet composition was calculated using PRODI expert version 6.10 (Wissenschaftliche Verlagsgesellschaft Stuttgart; based on German Nutrient Data Base BLS 3.02). An outline of the study protocol is given in Figure 1, and a CONSORT (Consolidating Standards for Reporting Trials) is available in online Supporting Information.

Study participants

Eight healthy adults (four women, four men) were recruited at Kiel University. Exclusion criteria were food allergies or intolerances, alternative eating habits, regular exercise (> 1 h/d), smoking, chronic

diseases or regular use of medications, claustrophobia, or > 5-kg weight change within 3 months before the study. Women were included only when using hormonal contraceptives continuously to avoid the influence of the menstrual cycle on EE [20]. The study protocol was approved by the Ethics Committee of the Medical Faculty of the University of Kiel, Germany, in accordance with the Declaration of Helsinki. Written, informed consent was obtained from all participants before participation.

Participants were invited to attend an in-person screening conducted within 2 weeks of the start of the interventions, before enrollment of the participants. Screening examinations took place after an overnight fast. Height was determined with a stadiometer (seca 274; seca GmbH & Co. KG). Body weight was measured on a calibrated scale, and fat mass was assessed using air-displacement plethysmography (BodPod, COSMED), both in underwear. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Fat-free mass index was calculated as fat-free mass divided by height squared (kg/m^2). Fat mass index was calculated as fat mass divided by height squared (kg/m^2). RMR was measured for 25 minutes via indirect calorimetry using two canopy hood devices (Q-NRG, COSMED). Both devices were calibrated according to the manufacturer's instructions prior to each measurement.

Statistical analysis

Normal distribution was checked via the Kolmogorov–Smirnov test. Paired *t* tests were used to examine differences between repeated measurements of 24-hour EE, SMR, PAEE, TEF, macronutrient oxidation rates, RER, VO_2, VCO_2, and technical EE (difference between measured and expected EE from propane combustion) as well as to compare results from propane combustion with stoichiometrically predicted values. Coefficient of variations (CV) given as a percentage was calculated as follows: CV (%) = (SD/mean) × 100. The minimal detectable change at 95% CI (MDC_{95}) was calculated from standard error of measurement (SEM) as $MDC_{95} = SEM \times 1.96 \times \sqrt{2}$, where 1.96 corresponds to the level of confidence adopted (95% in this case) and $\sqrt{2}$ represents the correction factor for measurement in duplicate.

Graphs were plotted using GraphPad Prism 9 for Windows (version 9.2). Data were analyzed using the SPSS Statistics software package version 27.0 (IBM Corp.). Furthermore, an appropriate statistical mixed model was applied [21] to calculate an applicable standard deviation using the statistical software R. The model included activity and energy intake, as well as their interaction term as fixed factors. The different participants and time effects were regarded as random factors. The residuals were assumed to be normally distributed and to be homoscedastic. These assumptions are based on a graphical residual analysis. Based on this model, a pseudo R^2 was calculated [22], and ANOVA was conducted, followed by multiple contrast tests [23] in order to compare the activity levels, as well as fasting and isocaloric conditions. Data are reported as means and SD unless otherwise specified. Significance was set at $p < 0.05$.

RESULTS

Technical validation

Results of repeated propane combustion tests are shown in Table 1. The average burning rate was 0.293 ± 0.033 g/min (range: 0.2311–0.3429 g/min). Measured gas volumes correlated well with values predicted by stoichiometry (VO_2, $r = 0.998$; VCO_2, $r = 0.999$; Supporting Information Figure S2), although measured values differed significantly from predicted values (VO_2, $\Delta = -24$ mL/g propane, $p < 0.001$; VCO_2, $\Delta = -12$ mL/g propane, $p < 0.001$), with errors of the expected gas volumes ranging from 0.06% to 1.62% for VO_2 and 0.13% to 1.15% for VCO_2. Recovery rates without correction for flow resistance were VO_2, 97.47% ± 1.40%; VCO_2, 94.91% ± 1.14%; and RER, 97.47% ± 0.69%. Errors for technical EE given as a percentage of expected EE ranged between 0.80% and 3.00%. Compared with the 10-hour propane burns, the shorter 5.5-hour tests ($n = 12$) performed for quality assurance showed similar recovery rates (VO_2, 101.81% ± 1.30%; VCO_2, 101.13% ± 1.47%; RER, 99.60% ± 0.73%; and EE, 100.9% ± 1.5%). The flow rate was stable during all propane combustions and the whole intervention study at 79.99 ± 0.01 L/min. The empty runs showed no abnormalities. Deviation from the default

TABLE 1 Reproducibility of 10-hour propane combustion ($n = 16$) and comparison of results (normalized for the amount of burned propane) against stoichiometry

	Mean	SD	CV (%)	SEM	MDC_{95}	MDC (%)	Recovery (%)
VO_2 (L)	2.567	0.019	0.75	0.005	0.013	0.52	100.84 ± 0.78
VCO_2 (L)	1.537	0.007	0.49	0.002	0.005	0.34	100.64 ± 0.51
RER	0.599	0.004	0.69	0.001	0.003	0.48	99.97 ± 0.55
EE (kcal)	3.42	0.38	0.54	0.001	0.004	0.40	98.17 ± 0.53

Note: Total amount of propane burned = 182.6 ± 20.7 g.

Abbreviations: CV, coefficient of variation; EE, energy expenditure; MDC, minimal detectable change; MDC_{95}, minimal detectable change at 95% confidence level; RER, respiratory exchange ratio; VCO_2, carbon dioxide production; VO_2, oxygen consumption.

TABLE 2 Baseline characteristics

	Women ($n = 4$)	Men ($n = 4$)	Total ($n = 8$)
Age (y)	22.4 ± 1.2	25.8 ± 2.4	24.1 ± 2.5
Height (m)	1.72 ± 0.0	1.78 ± 0.1	1.75 ± 0.1
Body weight (kg)	67.3 ± 8.9	81.6 ± 9.0	74.5 ± 11.5
BMI (kg/m^2)	22.9 ± 3.3	25.6 ± 2.1	24.3 ± 3.1
FMI (kg/m^2)	6.4 ± 2.9	5.3 ± 1.6	5.9 ± 2.4
FFMI (kg/m^2)	16.4 ± 0.4	20.3 ± 0.7	18.3 ± 2.0
RMR (canopy) (kcal/d)	1,613 ± 201	1,882 ± 138	1,748 ± 218

Note: Values are mean ± SD.
Abbreviations: FFMI, fat-free mass index; FMI, fat mass index; RMR, resting metabolic rate.

setting amounted to < 0.9% for VO_2, VCO_2, flow rate, and temperature of the gas analyzer.

Biological validation

Four women and four men aged between 20 and 29 years and with BMI between 19.9 and 29.3 kg/m^2 participated in the study (Table 2). Three participants had overweight according to WHO criteria.

The CV for isocaloric repeated measurements of RER, 24-hour EE, and SMR ranged between 1.4% and 1.7% (Table 3). Moreover, all three conditions performed twice showed excellent test–retest reliability, assessed by concordance correlation coefficient, as shown in

TABLE 3 Reliability of components of energy expenditure and macronutrient oxidation rates on isocaloric days; comparison between inactive and active conditions

		Mean	SD	CV (%)	ICC	SEM	MDC_{95}	MDC (%)
(A) Reliability of components of energy expenditure								
RER	Inactive	0.853	0.013	1.7	0.897	0.004	0.012	1.4
	Active	0.825	0.010	1.8	0.814	0.004	0.012	1.4
24-hour EE (kcal/d)	Inactive	2,112	282	1.5	0.998		35.7	1.7
	Active	2,446	411	1.4	0.991	41.0	105.1	4.3
SMR (kcal/d)	Inactive	1,597	201	1.5	0.986	25.6	70.9	4.4
	Active	1,392	198	1.7	0.981	29.5	78.9	5.9
(B) Reliability of thermic effect of food; comparison of methods ($n = 6$)								
Subtraction method	Inactive	145	37	9.1	0.846	16.6	46.0	31.8
	Active	44	75	39.3	0.776	27.8	77.1	173.5
Regression method	Inactive	154	62	38.2	0.697	46.3	128.4	83.4
	Active	181	48	30.2	0.645	25.0	69.4	38.4
(C) Reliability of physical activity energy expenditure on active days; comparison of methods ($n = 6$), PAL and SPA								
Subtraction	Active	808	142	5.8	0.937	37.6	140.1	12.9
Regression	Active	992	208	5.8	0.977	37.6	104.3	10.5
PAL	Inactive	1.26	0.03	1.2	0.811	0.02	0.05	3.6
	Active	1.68	0.07	0.1	0.950	0.02	0.04	2.7
SPA	Inactive	44.53	17.28	49.5	0.113	26.18	72.57	163.0
	Active	118.30	35.13	16.3	0.910	11.46	31.77	26.9
(D) Reliability of macronutrient oxidation rates								
CHOOx (g)	Inactive	234.0	53.8	9.5	0.916	15.6	43.2	18.5
	Active	307.4	53.7	11.2	0.678	30.5	84.4	27.5
FatOx (g)	Inactive	98.0	35.8	12.1	0.943	8.6	23.7	24.2
	Active	150.9	40.0	8.8	0.912	11.9	32.9	21.8
ProtOx (g)	Inactive	67.6	9.0	10.7	0.283	21.1	21.1	31.2
	Active	73.9	13.2	6.2	0.915	10.7	10.7	14.5
Nitrogen excretion (g)	Inactive	5.8	1.9	17.7	0.695	1.1	2.9	50.3
	Active	6.5	2.3	9.9	0.948	0.5	1.5	22.5

Abbreviations: 24-hour EE, 24-hour energy expenditure; CHOOx, carbohydrate oxidation; CV, coefficient of variation; FatOx, fat oxidation; ICC, intraclass correlation coefficient; MDC, minimal detectable change; MDC_{95}, minimal detectable change on a 95% confidence level; PAL, physical activity level; ProtOx, protein oxidation; RER, respiratory exchange ratio; SMR, sleeping metabolic rate; SPA, spontaneous physical activity.

Supporting Information Figure S1. The reliability of the biological outcome parameters was tested between inactive (PAL = 1.23 ± 0.05) and active (PAL = 1.65 ± 0.05) conditions. Activity on the exercise bike led to a peak in EE up to 5.909 ± 0.989 kcal/min for a short period during the activity.

Comparison of mean values for TEF on active days between the regression and subtraction method is shown in Figure 3A (stratified into fasting and isocaloric conditions). Under isocaloric conditions, TEF was higher and more plausible using the regression method than the subtraction method (181 ± 48 kcal/d vs. 44 ± 75 kcal/d, $p < 0.05$). Under fasting conditions, the regression method resulted in a TEF of 75 ± 53 kcal/d (Figure 3A), which is explained by the model error of the regression analysis.

On active days, PAEE was significantly higher using the regression method when compared with the subtraction method. This was found during isocaloric as well as fasting conditions (Figure 3B, isocaloric condition: Δ −205 ± 101 kcal/d; $p < 0.01$; fasting: Δ −154 ± 107 kcal/d; $p < 0.05$).

The reliability of carbohydrate and fat oxidation, as well as protein oxidation along with nitrogen excretion under isocaloric treatment, is shown in Table 3. No differences in macronutrient oxidation rates were observed between repeated measurements.

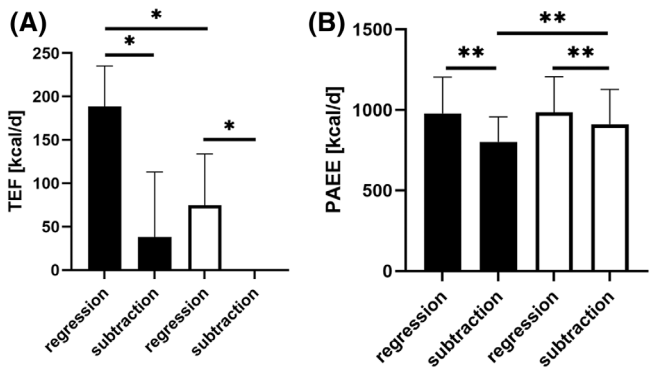

FIGURE 3 (A) Comparison of the thermic effect of food (TEF) and (B) physical activity energy expenditure (PAEE) between regression and subtraction method on active days stratified to isocaloric (black) and fasting (open bars) conditions. Fasting $TEF_{subtraction}$ is used as a reference and thus zero by definition. Values are mean ± SD. *$p < 0.05$; **$p < 0.01$ (paired t test)

No differences in carbohydrate or fat balances were found for repeated measurements on inactive days. On both inactive days, energy balance was positive (first IsoSed day: +75 ± 61 kcal, $p < 0.05$ vs. second IsoSed day: +133 ± 26 kcal, $p < 0.001$). On active days, there was a negative fat balance on the second day (Δ −20.5 ± 23.0 g, $p < 0.05$), showing that fat oxidation exceeded fat intake. By contrast, protein balance was significantly positive on active days (Δ +21.4 ± 9.1 g, $p < 0.001$) (Table 4).

The 24-hour EE was higher during isocaloric condition than fasting on inactive days (2112 ± 248 kcal/d vs. 1883 ± 233 kcal/d, $p < 0.001$; Figure 4A). This difference disappeared on active days (IsoAct: 2914 ± 385 kcal/d vs. FastAct: 2875 ± 410 kcal/d, $p = 0.189$; Figure 4B). Following this discrepancy, 24-hour urinary dopamine excretion decreased with fasting on inactive days ($p < 0.001$; Figure 4C), whereas it tended to increase with fasting on active days ($p = 0.088$; Figure 4D). When comparing the two fasting interventions, activity resulted in higher dopamine excretion ($p < 0.05$; Figure 4).

One participant (male, 27 years) had his birthday on an inactive fasting day. On this occasion, his 24-hour EE was +13.5% (+323 kcal/d) higher than at the other inactive fasting day. This participant was therefore excluded from the analysis of inactive fasting days.

The impact of habituation to the WRIC was analyzed by comparing IsoSed measurements of EE from the second and fourth day. Mean 24-hour EE decreased (Δ −43 ± 28 kcal/d, $p < 0.01$), whereas SMR did not change with time (Δ +29 ± 42 kcal/d, $p = 0.131$). Repetition of the inactive fasting intervention (third vs. fifth day in the WRIC) showed a significant decrease in SMR (Δ −34 ± 34 kcal, $p < 0.001$) but not in 24-hour EE (Δ −39 ± 31 kcal, $p = 0.087$). Disregarding habituation effect, between-participant standard deviation for 24-hour EE was 352 kcal, whereas within-participant standard deviation was 101 kcal using the statistical mixed model analysis. Further analysis is shown in the online Supporting Information (Supporting Information Figure S1; Tables S1-S3).

DISCUSSION

Propane combustion tests have revealed a high validity (as evidenced by recovery rates of VO_2 and VCO_2) as well as a high precision

TABLE 4 Comparison of energy and macronutrient intake and balance between isocaloric sedentary (IsoSed) and isocaloric active (IsoAct) conditions

	Energy (kcal)	Carbohydrates (g)	Fat (g)	Protein (g)
IsoSed				
Intake	2,222 ± 259	234.0 ± 53.5	98.0 ± 10.4	64.8 ± 8.1
Balance	110 ± 42	37.6 ± 60.1	−7.1 ± 28.0	−2.9 ± 10.7
IsoAct				
Intake	2,872 ± 415	304.2 ± 56.9	149.8 ± 40.1	94.2 ± 16.8
Balance	−28 ± 82	34.0 ± 67.9	−23.7 ± 26.8	20.3 ± 8.7

Note: Values are mean ± SD.

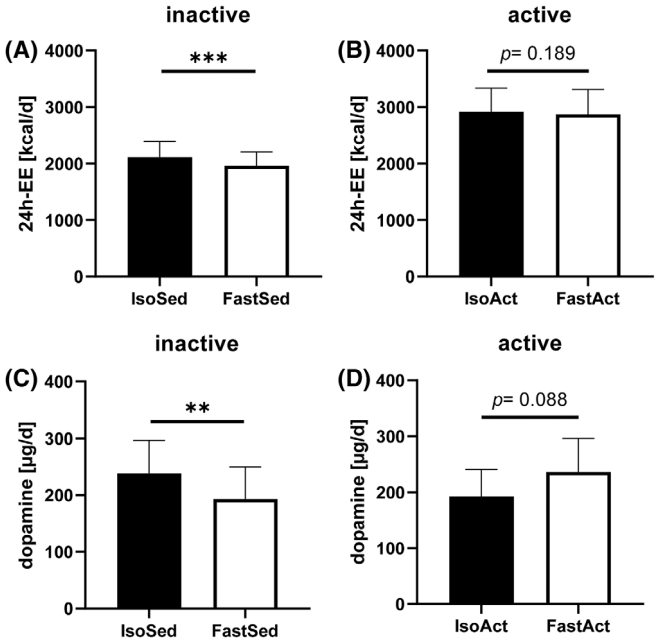

FIGURE 4 (A) 24-hour energy expenditure (EE) and (B) 24-hour dopamine urinary excretion are shown for inactivity and activity in comparison to isocaloric (black) and fasting (open bars) conditions. Values are mean ± SD. $^{**}p < 0.01$; $^{***}p < 0.001$ (paired *t* test)

(in repeated tests) of the WRIC at Kiel University. In the biological validation, the precision of 24-hour EE and SMR measurements was high, whereas it was lower for PAEE as well as macronutrient oxidation rates and poor for TEF. Situations with an influence on SNS activity were identified to have an impact on validity of components of EE.

Technical validation

The recovery rates for VO_2, VCO_2, RER, and technical EE for WRIC in Kiel are high. They are comparable with other validation studies, in which values ranged between −0.5% ± 1.6% for VO_2, −0.6% ± 0.9% for VCO_2, −0.5% ± 1.9% for RER, and 1.2% ± 1.5% for EE in 10-hour propane burns [16], or lay within 2% of the expected values [25, 26] despite differences in instrument construction and gas analyzers. Our 5.5-hour propane burns were found to be as precise as the 10-hour propane burns. So far, no recommendation has been made in RICORS 1.0 for the frequency of propane burns for quality check. During ongoing studies, we agree with the advice of Rising et al. to use monthly burns to check if parameters are out of range [16]. In addition, occasional empty runs can be recommended for troubleshooting.

According to RICORS, the rate and range of simulated EE from combustion should be similar to EE from humans [10, 16]. The 10-hour propane combustions led to an EE of 2,134 ± 247 kcal. This approximates the EE of one person on a whole intervention day. The technical error limits the detection of biological effects. Based on an EE of 2134 kcal, the technical error would be around 10 kcal (derived from MDC).

Biological validation

All three conditions performed twice (IsoSed, FastSed, and IsoAct) showed excellent test–retest reliability for 24-hour EE (Supporting Information Figure S1). Our results are, however, limited by a homogeneous, healthy, and young study population. Precision was comparable with values reported by other groups for repeated measurements of 1% to 5% for 24-hour EE and 1% to 4% for SMR [25-29]. Precision is decisive for determining differences in intervention studies, in which each participant serves as his/her control. On the contrary, high accuracy is crucial for combining data sets from multicenter studies in order to minimize the required sample size (for review, see [30]). Variances in 24-hour EE within individuals (791 kcal) and among individuals (79,758 kcal, after adjusting for FFM) were comparable with variances found by others (within individual, 1843 kcal/d; among individuals, 80,420 kcal/d [27]). Using intraindividual comparisons, we were able to detect a difference of 43 kcal/d in 24-hour EE between inactive isocaloric and fasting conditions (Figure 4A). This result was accompanied by a decrease in dopamine excretion and thus, lower SNS activity with fasting (Figure 4C). Interestingly, under active conditions, 24-hour EE did not decrease with fasting (Figure 4B). This discrepancy may be due to a slightly higher dopamine excretion (Figure 4D). This presumption is supported by others who found elevated plasma epinephrine and norepinephrine concentrations in fasting compared with fed participants during exercise varying in intensity and volume [31].

Besides higher SNS activity with exercise on fasting days, excitement and tension due to unaccustomed WRIC environment or having a birthday might contributed to a systematic bias of the 24-hour EE measurement (see *Results*). Therefore, standardization of measurement conditions (i.e., avoidance of emotional or mental stress) is important in order not to override small biological effects. Our findings highlight that complete adaptation to the chamber environment takes at least 3 days in the WRIC. However, our results need to be confirmed using intraindividual measurements over several days under exactly the same conditions of diet and activities.

In contrast to 24-hour EE, the reproducibility of the TEF was much lower (Table 3). Similar to our results, others found CV for TEF between 15% and 43% using WRIC [30, 33, 26]. There may be several factors with an impact on the reproducibility of TEF. First, a higher chamber volume may increase the error due to dilution. This is why several room calorimeter labs use a smaller room for RMR and TEF measurements [34]. Second, physical activity may impact the choice of the method for TEF determination. Under active conditions, the subtraction method led to implausibly low values for TEF. For the regression method, the determination of the *y*-intercept revealed some weaknesses because a spurious TEF was found under fasting conditions (Figure 3A and Table 3). A positive TEF with fasting might result from an overestimation of the

y-intercept due to not recorded, nonexercise activity thermogenesis by accelerometry (for review, see [35]).

The MDC_{95} for PAEE was 104 to 140 kcal (Table 3). Because habitual physical activity in a WRIC is artificially low, intervention protocols should use a treadmill or exercise bike to simulate free-living conditions in order to avoid confounding adverse effects of inactivity on metabolism [35]. Regarding the accuracy of PAEE measurements, the subtraction method systematically underestimates PAEE (Figure 3B) because spontaneous physical activity is included in 24-hour EE under inactive conditions and thus disregarded when subtracted from 24-hour EE with activity. Therefore, results from both methods cannot be used interchangeably because they are based on a different concept. Spontaneous physical activity is a behavioral component that might impair reliability. In fact, spontaneous physical activity showed a high variance between participants. Absolute differences in kilocalories per day were, however, low (Table 3). The difference between $PAEE_{regression}$ and $PAEE_{subtraction}$ was 216 ± 93 kcal. This difference could be explained by spontaneous physical activity and associated nonexercise activity thermogenesis. Levine et al. described nonexercise activity thermogenesis as 15% of 24-hour EE in very sedentary individuals [36].

CONCLUSION

In conclusion, our results show an excellent technical and biological validity of 24-hour EE using Kiel WRICs. The findings contribute to evaluating whether biological effects in components of EE can be detected using WRIC and facilitate the choice of study design (intraindividual vs. interindividual comparison) and sample size calculation.O

AUTHOR CONTRIBUTIONS

Anja Bosy-Westphal designed research; Anja Bosy-Westphal, Rebecca Dörner, Franziska A. Hägele, Jana Koop conducted research; Rebecca Dörner, Franziska A. Hägele, Russell Rising, Thomas Foerster, Mario Hasler and Jana Koop analyzed data; Rebecca Dörner and Anja Bosy-Westphal wrote the paper and had primary responsibility for final content; Anja Bosy-Westphal, Rebecca Dörner, Franziska A. Hägele, Jana Koop, Russell Rising, Thomas Foerster, Thomas Olsen and Manfred J. Müller discussed the data. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

TF is an employee of Sable Systems International, North Las Vegas, Nevada, and RR is the owner of the company D&S Consulting Services Inc., New York, New York. All authors declare no conflict of interest related to the study.

CLINICAL TRIAL REGISTRATION

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REFERENCES

1. Ravussin E, Beyl RA, Poggiogalle E, Hsia DS, Peterson CM. Early time-restricted feeding reduces appetite and increases fat oxidation but does not affect energy expenditure in humans. *Obesity (Silver Spring)*. 2019;27:1244-1254.
2. Carnero EA, Bock CP, Distefano G, et al. Twenty-four hour assessments of substrate oxidation reveal differences in metabolic flexibility in type 2 diabetes that are improved with aerobic training. *Diabetologia*. 2021;64:2322-2333.
3. Nas A, Büsing F, Hägele FA, Hasler M, Müller MJ, Bosy-Westphal A. Impact of energy turnover on fat balance in healthy young men during energy balance, energetic restriction and overfeeding. *Br J Nutr*. 2020;123:30-40.
4. Nas A, Mirza N, Hägele F, et al. Impact of breakfast skipping compared with dinner skipping on regulation of energy balance and metabolic risk. *Am J Clin Nutr*. 2017;105:1351-1361.
5. Bush NC, Resuehr HES, Goree LL, et al. A high-fat compared with a high-carbohydrate breakfast enhances 24-hour fat oxidation in older adults. *J Nutr*. 2018;148:220-226.
6. Oliveira CLP, Boulé NG, Sharma AM, et al. A high-protein total diet replacement increases energy expenditure and leads to negative fat balance in healthy, normal-weight adults. *Am J Clin Nutr*. 2021;113:476-487.
7. Müller MJ, Bosy-Westphal A, Lagerpusch M, Heymsfield SB. Use of balance methods for assessment of short-term changes in body composition. *Obesity (Silver Spring)*. 2012;20:701-707.
8. Schutz Y. Respiration chamber calorimetry and doubly labeled water: two complementary aspects of energy expenditure? *Eur J Clin Nutr*. 2018;72:1310-1313.
9. Hall KD, Chen KY, Guo J, et al. Energy expenditure and body composition changes after an isocaloric ketogenic diet in overweight and obese men. *Am J Clin Nutr*. 2016;104:324-333.
10. Chen KY, Smith S, Ravussin E, et al. Room Indirect Calorimetry Operating and Reporting Standards (RICORS 1.0): a guide to conducting and reporting human whole-room calorimeter studies. *Obesity (Silver Spring)*. 2020;28:1613-1625.
11. Montaurier C, Richard R, Boirie Y. Two functional calorimetric chambers in France complete the room indirect calorimetry operating and reporting standards (RICORS) 1.0 guide list. *Obesity (Silver Spring)*. 2021;29:631. doi:10.1002/oby.23138
12. Schutz Y, Bessard T, Jéquier E. Diet-induced thermogenesis measured over a whole day in obese and nonobese women. *Am J Clin Nutr*. 1984;40:542-552.
13. Tataranni PA, Larson DE, Snitker S, Ravussin E. Thermic effect of food in humans: methods and results from use of a respiratory chamber. *Am J Clin Nutr*. 1995;61:1013-1019.
14. Jéquier E, Acheson K, Schutz Y. Assessment of energy expenditure and fuel utilization in man. *Annu Rev Nutr*. 1987;7:187-208.
15. de V. Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol*. 1949;109:1-9.
16. Rising R, Foerster T, Arad AD, Albu J, Pi-Sunyer X. Validation of whole room indirect calorimeters: refinement of current methodologies. *Physiol Rep*. 2017;5:e13521. doi:10.14814/phy2.13521

17. Schrauwen P, van Marken Lichtenbelt WD, Westerterp KR. Energy balance in a respiration chamber: individual adjustment of energy intake to energy expenditure. *Int J Obes Relat Metab Disord*. 1997;21:769-774.
18. Nas A. *Metabolic chamber studies on energy- and macronutrient metabolism: Impact of meal skipping and energy flux*. Dissertation. University of Hohenheim, Institute of Nutritional Medicine; 2019. http://opus.uni-hohenheim.de/volltexte/2019/1571/
19. Hill JO, Peters JC, Reed GW, Schlundt DG, Sharp T, Greene HL. Nutrient balance in humans: effects of diet composition. *Am J Clin Nutr*. 1991;54:10-17.
20. Zhang S, Osumi H, Uchizawa A, et al. Changes in sleeping energy metabolism and thermoregulation during menstrual cycle. *Physiol Rep*. 2020;8:e14353. doi:10.14814/phy2.14353
21. Pinheiro JC, Bates DM. *Mixed-Effects Models in S and S-PLUS*. Springer; 2000.
22. Nakagawa S, Schielzeth H. A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods Ecol Evol*. 2013;4:133-142.
23. Bretz F, Hothorn T, Westfall PH. Applications. In: *Multiple Comparisons Using R*. Chapman & Hall/CRC Press; 2011:69-126.
24. Melanson EL, Ingebrigtsen JP, Bergouignan A, Ohkawara K, Kohrt WM, Lighton JRB. A new approach for flow-through respirometry measurements in humans. *Am J Physiol Regul Integr Comp Physiol*. 2010;298:R1571-R1579.
25. Ravussin E, Lillioja S, Anderson TE, Christin L, Bogardus C. Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. *J Clin Invest*. 1986;78:1568-1578.
26. Astrup A, Thorbek G, Lind J, Isaksson B. Prediction of 24-h energy expenditure and its components from physical characteristics and body composition in normal-weight humans. *Am J Clin Nutr*. 1990; 52:777-783.
27. Allerton TD, Carnero EA, Bock C, et al. Reliability of measurements of energy expenditure and substrate oxidation using whole-room indirect calorimetry. *Obesity (Silver Spring)*. 2021;29:1508-1515.
28. de Boer JO, van Es AJ, van Raaij JM, Hautvast JG. Energy requirements and energy expenditure of lean and overweight women, measured by indirect calorimetry. *Am J Clin Nutr*. 1987;46:13-21.
29. Usui C, Ando T, Ohkawara K, et al. Validity and reproducibility of a novel method for time-course evaluation of diet-induced thermogenesis in a respiratory chamber. *Physiol Rep*. 2015;3:e12410. doi:10.14814/phy2.12410
30. Dulloo AG, Jacquet J, Montani J-P, Schutz Y. Adaptive thermogenesis in human body weight regulation: more of a concept than a measurable entity? *Obesity Rev*. 2012;13:105-121.
31. Pequignot JM, Peyrin L, Pérès G. Catecholamine-fuel interrelationships during exercise in fasting men. *J Appl Physiol Respir Environ Exerc Physiol*. 1980;48:109-113.
32. Ogata H, Kobayashi F, Hibi M, Tanaka S, Tokuyama K. A novel approach to calculating the thermic effect of food in a metabolic chamber. *Physiol Rep*. 2016;4:e12717. doi:10.14814/phy2.12717
33. Rising R, Whyte K, Albu J, Pi-Sunyer X. Evaluation of a new whole room indirect calorimeter specific for measurement of resting metabolic rate. *Nutr Metab*. 2015;12:46. doi:10.1186/s12986-015-0043-0
34. Donahoo WT, Levine JA, Melanson EL. Variability in energy expenditure and its components. *Curr Opin Clin Nutr Metab Care*. 2004;7:599-605.
35. Stephens BR, Granados K, Zderic TW, Hamilton MT, Braun B. Effects of 1 day of inactivity on insulin action in healthy men and women: interaction with energy intake. *Metabolism*. 2011;60:941-949.
36. Levine JA. Measurement of energy expenditure. *Public Health Nutr*. 2007;8:1123-1132.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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