Original Article

A new indirect calorimeter is accurate and reliable for measuring basal energy expenditure, thermic effect of food and substrate oxidation in obese and healthy subjects☆

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A R T I C L E   I N F O

S U M M A R Y

Background & aims: The objectives of the study were to validate accuracy and reliability of the QUARK RMR, an indirect calorimeter *versus* the DELTATRAC II™, a well-established reference system which is no longer available, in resting and post-prandial conditions.
Methods: A crossover, randomized study was performed in 30 subjects for two consecutive days. Resting metabolic rate (RMR) was measured for three 45 min periods using alternating calorimeters. Means of RMR were then compared with Pearson's test and Bland and Altman plot. Thermic effect of food (TEF) and substrate oxidation were assessed for 3 h with each calorimeter, 15 min after meal ingestion, and were compared by longitudinal analysis.
Results: Means at rest of VO_2, VCO_2, RMR and substrate oxidation were not significantly different with both devices. The variability of VO_2, VCO_2 and RMR measurements, at rest, for each device, on two consecutive days, was similar to that measured with QUARK RMR and DELTATRAC II™ the same day, under standardized conditions. Longitudinal analysis of TEF and post-prandial substrate oxidation was equivalent with the two devices.
Conclusions: The QUARK RMR calorimeter seems to be a valid system to measure energy expenditure in resting and post-prandial conditions in obese and healthy subjects.
Clinical Trial Registration Number: NCT00848471.

1. Introduction

Measurement of energy expenditure (EE) has become a growing target in clinical practice to assess energy needs in patients, especially obese or malnourished ones, in determining optimal nutritional support.[1,2] EE can be measured by direct or indirect calorimetry or calculated with equations like the Harris–Benedict equation. These equations are not accurate, especially in pathological states, since values can be either under or over estimated in a range from 70 to 140% of actual resting metabolic rate (RMR).[3–5] Measurements by indirect calorimetry are widely used in clinical research to study variations in EE and substrate oxidation during nutritional intervention and to assess the mechanisms involved.[4,6] Unfortunately, indirect calorimetry is not a practical option in current clinical settings because of cost.[6,7] However, indirect calorimetry is an efficient tool for measuring EE with accuracy and precision under standardized conditions.[1,8]

Indirect calorimetry is based on measurement of gas exchange, oxygen consumption (VO_2) and carbon dioxide production (VCO_2), which reflect nutrient metabolism.[7] Open-circuit indirect calorimeter systems, using the so called "canopy dilution technique", are the most common devices for measuring EE.[9] The head of the patient is covered with a transparent plastic canopy hood, connected to a blower, generating a constant flow through the hood. Inhaled air enters from the surrounding environment (room air)

and the exhaled O_2 and CO_2 content is measured for calculation of O_2 consumption and CO_2 production.[10]

Indirect calorimetry should be performed as simply as possible with an easy-to-use indirect calorimeter assessing EE instantaneously at the bed-side. Most studies using indirect calorimetry have used the DELTATRAC II™ (Sensormedics, Yorba Linda, CA, USA) which became the reference tool validated for indirect calorimetry measurements.[5,11–16] However, this device is no longer on the market.[17] This causes a real problem for clinical research that needs a reliable and validated calorimeter to replace the DELTATRAC II™. Clinical research needs a reliable device which will become the reference to use in all clinical studies aiming to understand the mechanisms involved in metabolic pathways. Studies validating an indirect calorimeter to replace the DELTATRAC II™ are very important and of great relevance for guiding clinicians in their choice of an indirect calorimeter.

Different devices are now available, and we chose to test the accuracy and reliability of the QUARK RMR (Cosmed, Rome, Italy) compared to the DELTATRAC II™ because the two products are very similar. They are both metabolic carts used to measure EE. Both devices use the same analysis method namely the "canopy dilution technique". They both work in the same way: a canopy hood covers the patient's head and the patient breathes into it. Expired air is extracted by a pump to be analyzed by metabolic cart sensors. Measured O_2 consumption and CO_2 production are converted using the Weir equation in RMR. Both devices use the same kind of sensors to measure O_2 and CO_2. The slight difference is due to the blower flow which is constant with DELATRAC II™ and variable but continuously monitored with a turbine flowmeter with QUARK RMR.

The primary endpoint of this study was to assess the accuracy and reliability of the QUARK RMR in basal situations, in the whole population and in different body mass index (BMI) categories (normal weight, overweight and obese subjects) so as to have a wide range of metabolic situations. Accuracy and reliability were tested on RMR and substrate oxidations. The secondary endpoint was to test the QUARK RMR in post-prandial situations, in the same categories of subjects. Response to meal ingestion was tested by comparing the kinetics of substrate oxidation and thermic effect of food (TEF).

2. Methods

2.1. Subjects

Thirty four subjects were enrolled in the study, 3 subjects withdrew from the studies for personal reason and one for discomfort, headache and nausea under the canopy hood with the two calorimeters. So, 30 subjects divided into three BMI classes (normal weight ($18 < BMI \leq 25$ kg/m^2) – overweight ($25 < BMI \leq 30$ kg/m^2) and obese ($30 < BMI \leq 35$ kg/m^2)), with a sex ratio of one-half, completed the protocol. Voluntary subjects had low to moderate physical activity confirmed by Baecke's questionnaire. The study began on January 23, 2009 and ended on July 30, 2009. All subjects had stable weight with no weight variation during the 3 months preceding the study. We excluded volunteers performing intensive activity, smokers, claustrophobic subjects, pregnant or breast-feeding women, subjects with acute infections, chronic inflammatory disease or taking medication which could interfere with metabolic rate.

The written informed consent of all subjects was obtained. This study was approved by the Scientific Ethics Committee of Lyon Sud Est II and AFSSAPS (French health products safety agency) and complied with both the French, Huriet–Serusclat' law and the Second Declaration of Helsinki.

2.2. Material and technical specification of calorimeters

2.2.1. QUARK RMR (Cosmed, Rome, Italy)

The QUARK RMR is an open-circuit calorimeter for measurement of mechanically assisted patients and spontaneously breathing patients. The system can operate with "breath by breath" collection or the canopy dilution technique. This study refers to the canopy dilution mode only.

The QUARK RMR is equipped with a canopy hood for spontaneously breathing subjects. With this device, flow rate is directly measured with a digital turbine flowmeter. Ventilatory rate is regulated directly by the QUARK RMR. Calibration of the flowmeter is performed using a certified 3 L calibration syringe. Calibration of zero, span and delay alignment of the O_2 and CO_2 gas analyzers is performed before each test using a certified calibration gas. During each test, the readings are controlled and eventually compensated by means of periodic automatic room air calibrations.

Response times of O_2 and CO_2 sensors are less than 120 ms. The O_2 analyzer is a paramagnetic sensor which has a measuring range from 0 to 30% in the canopy mode. Accuracy of the O_2 sensor is 0.02%. The CO_2 analyzer is an infrared digital sensor which has a measuring range from 0 to 10%. Accuracy of the CO_2 sensor is 0.02%. These sensors are maintenance free.

The flowmeter which detects the ventilation rate of the canopy is a bidirectional turbine with an 18 mm diameter. The ventilation range is from 0 to 80 L/min. Accuracy of the flowmeter is 2%.

Software uses the Weir equation to assess EE at rest.

2.2.2. DELTATRAC II™ (Sensormedics, Yorba Linda, CA, USA)

The DELTATRAC II™ is an open-circuit calorimeter equipped with a canopy hood. It is used in intensive care, clinical nutrition and research for measurement of both mechanically ventilated and spontaneously breathing patients. The gas collection system is based on the air dilution technique by using a mixing chamber. Air dilution systems take the exhaled air, dilute it with room air, and then shunt the gases into a mixing chamber for analysis. In this mixing chamber, sensors sample the gas collection at factory-selected intervals.

Measurement ranges for O_2 consumption and CO_2 production are from 5 to 2000 mL/min. The O_2 analyzer is a paramagnetic oxygen sensor which offers a fast response time to measure oxygen changes within 150 ms; it provides excellent linearity and is maintenance free. The range of O_2 measurement is from 0 to 100%. Baseline drift is automatically compensated and gain drift is from 2%/24 h. The CO_2 analyzer is an infrared sensor which offers a fast response time to measure CO_2 change within 150 ms. The range of CO_2 measurement is from 0 to 10%. Baseline drift is automatically compensated and gain drift is from 2% of full scan/4 days.

The DELTATRAC II™ provides direct measurement of CO_2 production with room air dilution using a constant flow generator. Constant ventilation range is 80 L/min for obese adult patients, 40 L/min for adult patients, 12 L/min for children and 3 L/min for babies.

Software uses the Weir equation to assess energy expenditure at rest.

2.3. Standardized conditions followed

Food intake was calibrated after an interview with the dietician. Standardized evening meals were calibrated and an identical standard meal was served during the two consecutive test days. These meals were controlled by dietary record.

The same experienced nurse conducted the two consecutive days of test.

Environment conditions were also standardized: rooms were air-conditioned; temperatures and humidity were controlled regularly to maintain the same condition during the two consecutive test days.

Before each experiment, both calorimeters were warmed up following the manufacturer's instructions (30 min for DELTATRAC II™ and 10 min for QUARK RMR), calibrated with a gas of known and certified CO_2 and O_2 composition (CO_2 concentration of $5 \pm 0.03\%$ and O_2 concentration of $16 \pm 0.03\%$, Scott Gas, USA for QUARK RMR) – (CO_2 concentration of $5 \pm 0.03\%$ and O_2 concentration in balance, Sensormedics, USA for DELTATRAC II™) and with room air. Ethanol burning tests were performed on both units in order to control quality and stability of measurement before each consecutive test day.

2.4. Study design

This study was a crossover, randomized study performed for two consecutive days divided into two periods: basal and post-prandial after ingestion of a 687 kcal (2872 kJ) meal as shown in Fig. 1.

2.4.1. Basal period

After an overnight fast, fasting state was controlled on arrival at the centre by capillary glycemia and anthropometric measurements were performed. Subjects lay supine at complete physical rest, alone, and undisturbed in a quiet room during 30 min. Then, subjects were placed under the canopy and could read or watch movies. Nurses monitored subjects regularly to prevent them from falling asleep.

VO_2 and VCO_2 were measured with each calorimeter for three periods of 45 min. During these three periods, the order of calorimeters was inverted according to the sequence shown in Fig. 1 to assess inter and intra-variability of the two calorimeters on the same day or day-to-day. The order of passage of indirect calorimeters was randomized using software called "The Hat" (Version 2.3, Harmony Hollow). For each calorimeter, RMR and substrate oxidation were then calculated from VCO_2 and VO_2 data and compared.

2.4.2. Meal consumption

After the basal period, subjects consumed a 687 kcal (2872 kJ) standardized solid meal composed of 100 g minced steak (15% fat), 200 g cooked pasta, 1 yogurt (125 g), 100 g of cottage cheese (20% fat), 10 g sugar (44 g proteins (25.6%), 26.7 g lipids (34.9%), 67.8 g carbohydrates (39.5%)) to monitor substrate oxidation and TEF.

2.4.3. Post-prandial period

Measurements started 15 min after complete lunch consumption and continued for 3 h with the same calorimeter. The type of calorimeter was inverted on the second test day. During this period, post-prandial metabolic rate (PPMR), variation of substrate oxidation and magnitude of TEF were assessed with each calorimeter and compared. Measurement of urinary nitrogen excretion was performed during the 3 h of the post-prandial period using a chemioluminescence method (Antek 490, Alytech, Juvisy, France).[18]

2.5. Calculation

During basal and post-prandial periods, readings from the first 5 min were discarded to keep only values reflecting steady state. Software of both calorimeters was set for minute-by-minute reading report of VO_2 and VCO_2 measurement.

2.5.1. Basal period

An average of VO_2 and VCO_2 values was taken from the subsequent 40 min steady state readings and was used to calculate RMR with the two calorimeters. RMR was determined using the Weir equation.[19] Respiratory quotient (RQ) was assessed as the VCO_2/VO_2 ratio. Substrate oxidation was assessed using Ferrannini's equations[20] for total carbohydrate and lipid oxidation; urinary nitrogen excretion was not measured and was estimated at 13 g/24 h which reflects nitrogen excretion of fasting subjects.[21–23]

2.5.2. Post-prandial period

An average was calculated from subsequent 15 min readings and was used to calculate PPMR with both calorimeters. PPMR was calculated using the modified Weir equation[19] taking into account total nitrogen excretion measured by a chemiluminescence method.[18] TEF i.e. the increment in EE above basal EE was calculated by substracting mean RMR obtained during the basal period from PPMR. The magnitude of TEF was calculated as the percentage of EE needed to assimilate the 687 kcal meal and compared between the two calorimeters.[24]

$$\%TEF = \left[\sum (PPMR - RMR) \times time \right] \Big/ ECM \qquad [1]$$

with TEF = thermic effect of food, PPMR: post-prandial metabolic rate (kcal/min), RMR: Resting Metabolic Rate (kcal/min), Time: time of measure (min), ECM: energy content of meal (kcal).

Substrate oxidation during the post-prandial period was assessed using Ferrannini's equations[20] for total carbohydrate and lipid oxidation. Variation of substrate oxidation was calculated by substracting mean substrate oxidation obtained during the basal period from substrate oxidation in the post-prandial period.

2.6. Statistical analysis

Sample size was defined by a power analysis of 2×2 crossover design to test equivalence, using maximal allowable differences that still result in equivalence and the standard deviation (SD) of the individual differences defined after previous measures of RMR with DELTATRACII™, for two consecutive days, under standardized
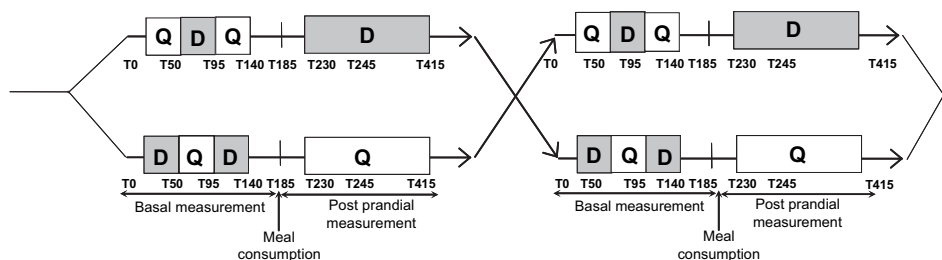

Fig. 1. Study design. This figure represents the study design. Seven days after selection, volunteers started their first metabolic test day. During this day, volunteers were first placed under resting conditions to measure RMR during three 45-min periods with each calorimeter (QUARK RMR, DELTATRAC II™ and QUARK RMR for the first randomization arm and DELTATRAC II™, QUARK RMR and DELTATRAC II™ for the second arm). After RMR measurement, subjects ingested a 687 kcal meal in 15 min. After this meal, PPMR was measured during three hours with the same calorimeter (DELTATRAC II™ for the first randomization arm and QUARK RMR for the second arm). On the second metabolic test day, subjects followed the same program but the order of calorimeter use was inverted.
RMR: Resting Metabolic Rate; PPMR: Post-Prandial Metabolic Rate; D: DELTATRAC IITM calorimeter; Q: QUARK RMR calorimeter.

conditions. With SD of 82 kcal/d and equivalence limits of −50 and +50 kcal/d, we needed a total sample size of 25 subjects to achieve 80% power at a 5% significance level and to show equivalence of QUARK RMR to DELTATRAC II™ for RMR measurement.

STATA 11 software (Statacorp LP, College Station, Texas, USA) was used for statistical analysis. All data are reported as means ± SD. Comparison of population characteristics on the two test days was performed using a Student's paired t test at $\alpha = 0.05$. Statistical reliability of mean VCO_2, VO_2, RMR, RQ and substrate oxidation at rest was determined by the correlation method using Pearson's correlation test. Accuracy and agreement of these parameters were determined by the Bland and Altman plot to assess the limits of agreement and were tested by a Pitman test.[24] Means of parameters obtained with the two calorimeters were compared by an ANOVA on repeated measures which took into account the type of calorimeter, sex, BMI class and randomization arm.

Longitudinal analysis of post-prandial metabolic rate variation between calorimeters was performed using a generalized estimating equations (GEE) model which took into account time, the type of calorimeters, sex, BMI class and randomization arm. TEF obtained with the two calorimeters was also compared using a GEE model as for substrate oxidation. Magnitude of TEF, measured with the two calorimeters, was compared using a paired t test for the whole population and with an ANOVA on repeated measures for BMI class.

For all statistical analysis the 0.05 level of significance was used.

3. Results

3.1. Subjects

Thirty subjects concluded the protocol: 10 normal weight, 10 overweight and 10 obese subjects with a sex ratio of one-half. Characteristics of the population at baseline are summarized in Table 1.

3.2. Ethanol burning test results

Reproducibility and repeatability of the QUARK RMR and the DELTATRAC II™ were assessed by using the currently accepted validation standard method, the ethanol burning test. Attempted result of RQ measured by the ethanol burning test was 0.67 ± 0.03. Reproducibility of both devices was assessed from 32 measurements made before each consecutive test day, under standardized conditions with calibrated devices in a ventilated room. Repeatability of both devices was assessed after 10 measures on the same day, under standardized conditions with calibrated devices in a ventilated room. Results of reproducibility and repeatability assessed with DELTATRAC II™ and QUARK RMR are summarized in Table 2.

Table 2
Reproducibility and repeatability of respiratory quotient measured with DELTATRAC II™ and QUARK RMR during an ethanol burning test.

Ethanol burning test				
	RQ with Deltatrac II™		RQ with Quark RMR	
	Mean ± Standard Deviation	Coefficient of Variation	Mean ± Standard Deviation	Coefficient of Variation
Reproducibility ($n = 32$)	0.66 ± 0.01	1.28%	0.69 ± 0.01	1.88%
Repeatability ($n = 10$)	0.66 ± 0.01	1.20%	0.66 ± 0.01	1.20%

RQ: Respiratory quotient.

3.3. Basal period

Table 3 summarizes data at rest obtained for DELTATRAC II™ and QUARK RMR for the whole population and each BMI class according to sex. Table 3 shows the averaged values of the two test days for VO_2, VCO_2, RQ, RMR and substrate oxidation with each calorimeter. No significant difference between the measurements obtained with DELTATRAC II™ and QUARK RMR was shown for the whole population or for BMI classes according to sex.

Table 4 shows accuracy and agreement of QUARK RMR and DELTATRAC II™ at rest under standardized conditions for the whole population. A significant correlation between values was obtained with DELTATRAC II™ and QUARK RMR. Values of correlation are summarized in Table 4 and represented in Fig. 2. To test the accuracy of the two calorimeters, a Bland and Altman plot was calculated. It represents for each parameter (VO_2, VCO_2, RQ, RMR and substrate oxidation) the mean of the two values measured for each subject with DELTATRAC II™ and QUARK RMR versus the difference of these two values (Table 4 and Fig. 2). A Pitman test performed on the Bland and Altman plot determined the significance of this test, i.e. it looked for a significant correlation between the difference and the means obtained for parameters measured with the two devices. Limits of agreement of the Bland and Altman plot were determined as the bias of mean difference +/− two standard deviations of the results with both devices. Accuracy and agreement were good with both calorimeters according to the Pitman test although limits of agreement seemed to be high. However, as seen in Table 4, similar limits were obtained with the same calorimeter on two consecutive days for VO_2, VCO_2, RMR, RQ and substrate oxidation values for the whole population. For example, day-to-day intra-variability of RMR for each calorimeter for the whole population was estimated at 26 ± 93 kcal/d (CI 95%: [−166; 213]) for DELTATRAC II™ and −20 ± 86 kcal/d (CI 95%: [−191; 152]) for QUARK RMR and was comparable to day-to-day inter-variability calculated between

Table 1
Subjects characteristics at baseline.

	Unit	Normal weight 18 < BMI < 25		Overweight 25 < BMI < 30		Obese 30 < BMI < 35	
		Men ($n = 5$)	Women ($n = 5$)	Men ($n = 5$)	Women ($n = 5$)	Men ($n = 5$)	Women ($n = 5$)
Age	y	26.8 ± 4.9	37.4 ± 13.4	29.2 ± 10.1	30.4 ± 9.9	39.4 ± 5.5	35.0 ± 4.9
Weight	kg	68.8 ± 9.8	59.3 ± 5.7	94.9 ± 10.4	73.0 ± 7.2	95.9 ± 3.8	82.3 ± 11.6
Height	m	1.76 ± 0.04	1.62 ± 0.04	1.82 ± 0.08	1.62 ± 0.06	1.76 ± 0.03	1.59 ± 0.09
BMI	kg/m^2	22.1 ± 2.5	22.8 ± 2.3	28.6 ± 1.0	27.8 ± 1.7	31.1 ± 0.6	32.6 ± 1.5
Fat mass	kg	13.6 ± 3.1	18.1 ± 3.1	28.2 ± 6.5	25.6 ± 2.4	30.7 ± 6.0	31.0 ± 5.6
Fat free mass	kg	55.0 ± 6.4	41.2 ± 4.2	65.5 ± 10.5	46.8 ± 4.9	64.8 ± 5.8	50.5 ± 5.6
Glycemia	mM	4.7 ± 0.4	4.4 ± 0.2	5.3 ± 0.4	4.6 ± 0.5	5.3 ± 0.5	5.0 ± 0.5
CRP	mg/L	1.7 ± 1.5	1.5 ± 0.9	1.4 ± 0.8	4.0 ± NC	3.1 ± 2.5	4.6 ± 2.1
Creatinemia	μM	83 ± 15	73 ± 7	95 ± 16	73 ± 10	87 ± 18	79 ± 10
TSH	mUI/L	1.9 ± 0.8	1.8 ± 0.7	2.2 ± 1.2	2.2 ± 1.7	1.8 ± 1.0	1.6 ± 0.8
Baecke's score		14.1 ± 1.7	11.3 ± 3.0	11.7 ± 2.6	9.8 ± 3.3	12.9 ± 1.9	12.4 ± 1.7

Values are mean ± SD.

Table 3
Statistical comparison of means obtained at rest with QUARK RMR and DELTATRAC II™ for VCO$_2$, VO$_2$, respiratory quotient (RQ), resting metabolic rate (RMR), carbohydrate and fat oxidation in the whole population and in normal weight, overweight and obese women and men.

Paired t test for mean +/−SD

Measure at rest		VCO$_2$ (mL/min)		VO$_2$ (mL/min)		RQ		P value
		Q	D	Q	D	Q	D	
The Whole population ($n = 30$)		195 ± 39	193 ± 36	239 ± 49	235 ± 43	0.82 ± 0.03	0.82 ± 0.03	NS
Men ($n = 15$)	Normal weight ($n = 5$)	198 ± 21	204 ± 18	238 ± 23	246 ± 20	0.83 ± 0.04	0.83 ± 0.05	NS
	Overweight ($n = 5$)	229 ± 14	220 ± 20	281 ± 14	271 ± 22	0.81 ± 0.02	0.81 ± 0.03	NS
	Obese ($n = 5$)	246 ± 36	239 ± 34	306 ± 47	289 ± 40	0.81 ± 0.04	0.83 ± 0.03	NS
Women ($n = 15$)	Normal weight ($n = 5$)	152 ± 12	155 ± 11	184 ± 12	188 ± 10	0.83 ± 0.03	0.83 ± 0.03	NS
	Overweight ($n = 5$)	171 ± 17	169 ± 12	209 ± 19	207 ± 14	0.81 ± 0.03	0.82 ± 0.03	NS
	Obese ($n = 5$)	176 ± 11	169 ± 10	217 ± 15	209 ± 11	0.81 ± 0.02	0.81 ± 0.02	NS

Measure at rest		RMR (kcal/d)		CHO oxidation (mg/kg min^{-1})		Fat oxidation (mg/kg min^{-1})		P value
		Q	D	Q	D	Q	D	
The Whole population ($n = 30$)		1670 ± 337	1641 ± 299	95 ± 44	96 ± 53	56 ± 23	54 ± 23	NS
Men ($n = 15$)	Normal weight ($n = 5$)	1668 ± 160	1721 ± 140	110 ± 51	112 ± 63	50 ± 19	53 ± 26	NS
	Overweight ($n = 5$)	1964 ± 102	1892 ± 158	113 ± 39	103 ± 52	70 ± 13	69 ± 22	NS
	Obese ($n = 5$)	2127 ± 324	2024 ± 281	111 ± 58	133 ± 66	83 ± 31	67 ± 29	NS
Women ($n = 15$)	Normal weight ($n = 5$)	1290 ± 87	1314 ± 73	75 ± 27	74 ± 34	36 ± 10	38 ± 13	NS
	Overweight ($n = 5$)	1461 ± 132	1443 ± 99	79 ± 33	80 ± 34	47 ± 12	45 ± 15	NS
	Obese ($n = 5$)	1512 ± 101	1455 ± 78	80 ± 34	73 ± 31	50 ± 12	49 ± 12	NS

All values are means ± standard deviation compared with a paired t test ($\alpha = 0.05$) Q corresponds to QUARK RMR, D to DELTATRAC II™ NS: no significant.

QUARK RMR and DELTATRAC II™ (−29 ± 110 kcal/d (CI 95%: [−248; 190])).

The same results were obtained for men and women analyzed separately. For men, day-to-day inter-variability was estimated at −40 ± 255 kcal/d (CI 95%: [−295; 214]) with a significant correlation ($r = 0.899$; $p < 0.0001$). Day-to-day intra-variability was estimated at 27 ± 213 kcal/d (CI 95%: [−186; 241]) for DELTATRAC II™ and 3 ± 195 kcal/d (CI 95%: [−192; 198]) for QUARK RMR and was comparable to day-to-day inter-variability calculated between QUARK RMR and DELTATRAC II™. For women, day-to-day inter-variability was estimated at −17 ± 276 kcal/d (CI 95%: [−193; 159]) with a significant correlation ($r = 0.790$; $p < 0.0001$). Day-to-day intra-variability was estimated at 26 ± 163 kcal/d (CI 95%: [−137; 189]) for DELTATRAC II™ and −43 ± 135 kcal/d (CI 95%: [−177; 92]) for QUARK RMR and was comparable to day-to-day inter-variability calculated between QUARK RMR and DELTATRAC II™.

3.4. Post-prandial period

Figure 3 summarizes mean ± SD of VO$_2$, VCO$_2$ (these values are cumulative data measured for a 25 min period taking account of the 10 min interval after each 50 min period of post-prandial measurement) obtained with the two calorimeters for the whole population during the 3 h following ingestion of the test meal. Longitudinal analysis showed no significant difference between results obtained with DELTATRAC II™ and QUARK RMR. The same results were obtained with women and men analyzed separately.

Table 4
Inter and intra-variability of QUARK RMR and DELTATRAC II™ to assess the accuracy and availability of QUARK RMR measurements recorded at rest in the whole population and validated by a Pearson's test, a Bland and Altman plot and the Pitman test.

Pearson's correlation	Inter-variability between QUARK RMR and DELTATRAC II™		Intra-variability of QUARK RMR		Intra-variability of DELTATRAC II™	
	r	p value	r	p value	r	p value
VCO$_2$ (mL/min)	0.929	$p < 0.05$	0.944	$p < 0.05$	0.953	$p < 0.05$
VO$_2$ (mL/min)	0.947	$p < 0.05$	0.974	$p < 0.05$	0.949	$p < 0.05$
RQ	0.296	$p < 0.05$	0.360	$p < 0.05$	0.558	$p < 0.05$
RMR (kcal/day)	0.947	$p < 0.05$	0.972	$p < 0.05$	0.953	$p < 0.05$
Carbohydrate oxidation (mg/kg min^{-1})	0.482	$p < 0.05$	0.446	$p < 0.05$	0.730	$p < 0.05$
Fat oxidation (mg/kg min^{-1})	0.426	$p < 0.05$	0.740	$p < 0.05$	0.651	$p < 0.05$
Bland and Altman representation	Mean of difference ± 2SD	Limits of agreement	Mean of difference ± 2SD	Limits of agreement	Mean of difference ± 2SD	Limits of agreement
VCO$_2$ (mL/min)	−3 ± 14	[−31; 26]	−3 ± 14	[−30; 25]	5 ± 12	[−18; 28]
VO$_2$ (mL/min)	−4 ± 16	[−36; 28]	−3 ± 12	[−27; 21]	3 ± 14	[−24; 31]
RQ	0.00 ± 0.04	[−0.07; 0.08]	0.00 ± 0.03	[−0.07; 0.07]	0.01 ± 0.03	[−0.06; 0.07]
RMR (kcal/day)	−29 ± 110	[−248; 190]	−20 ± 85	[−191; 152]	26 ± 93	[−160; 213]
Carbohydrate oxidation (mg/kg min^{-1})	1 ± 41	[−80; 83]	−4 ± 42	[−88; 80]	10 ± 33	[−56; 76]
Fat oxidation (mg/kg min^{-1})	−2 ± 17	[−37; 32]	1 ± 16	[−31; 32]	−2 ± 15	[−31; 27]
Pitman Test	r	p value	r	p value	r	p value
VCO$_2$ (mL/min)	0.187	$p < 0.05$	0.297	NS	−0.183	NS
VO$_2$ (mL/min)	0.368	$p < 0.05$	0.372	$p < 0.05$	0.059	NS
RQ	−0.074	NS	0.095	NS	0.332	NS
RMR (kcal/day)	0.398	$p < 0.05$	0.369	$p < 0.05$	0.081	NS
Carbohydrate oxidation (mg/kg min^{-1})	0.111	NS	0.149	NS	0.313	NS
Fat oxidation (mg/kg min^{-1})	−0.276	$p < 0.05$	0.118	NS	0.08	NS

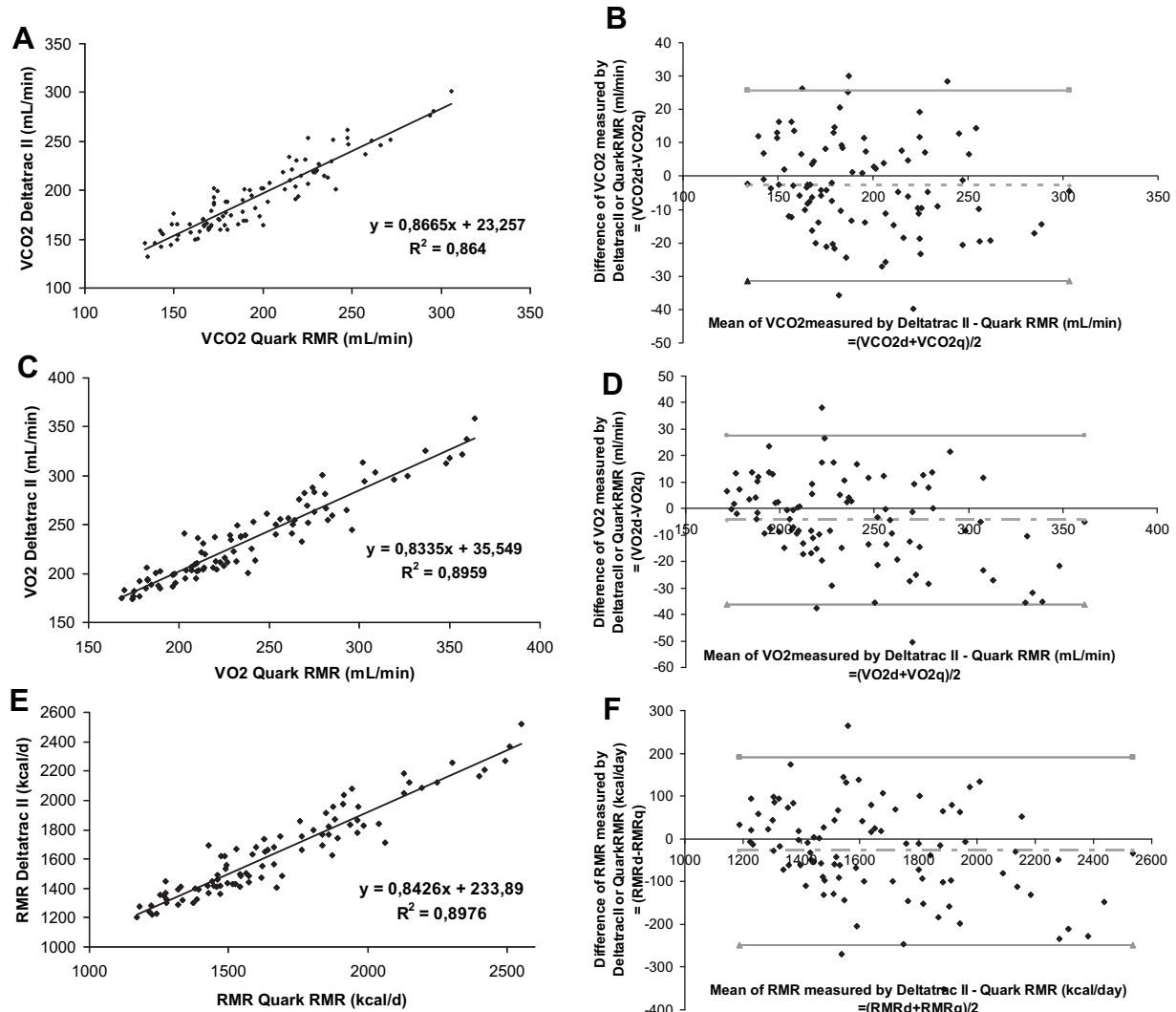RQ: respiratory quotient − SD: Standard Deviation − RMR: Resting Metabolic Rate − NS : No Significant.

Fig. 2. Correlation and Bland and Altman representation plot of VCO_2, VO_2 and RMR measured by DELTATRAC II™ and QUARK RMR. A and B represent VCO_2 correlation and the Bland and Altman plot respectively. C and D represent VO_2 correlation and the Bland and Altman plot respectively. E and F represent RMR correlation and the Bland and Altman plot respectively. All figures represent pooled data from all 6 groups of subjects (Normal, Overweight, Obese men and women (n = 30)).
—— Mean of differences ±2 Standard Deviations between DELTATRAC II™ and QUARK RMR.
− - − - Mean of differences between DELTATRAC II™ and QUARK RMR.

Figure 4 represents TEF and substrate oxidation during the 3 h following the test meal in the whole population. No significant difference between QUARK RMR and DELTATRAC II™ measurements was observed. The GEE model did not show any difference between calorimeters whatever the BMI class, sex or randomization arm.

Magnitude of TEF was about 4.9 ± 1.9% with DELTATRAC II™ versus 4.9 ± 2.0% with QUARK RMR when considering the whole population (NS). Considering BMI class, magnitudes of TEF were respectively about 5.4 ± 1.4% versus 5.0 ± 1.1% for normal weight, 4.5 ± 1.5% versus 4.5 ± 2.7% for overweight and 5.0 ± 2.5 versus 5.1 ± 2.0% for obese subjects (NS). Considering men and women categories, magnitudes of TEF were respectively about 5.6 ± 2.1% versus 5.1 ± 2.0% and 4.3 ± 1.3% versus 4.6 ± 2.0% (NS).

4. Discussion

We were able to show the equivalence between QUARK RMR and DELTATRAC II™ for measurement of RMR, basal substrate oxidation,

TEF and post-prandial substrate oxidation in normal weight, overweight and obese subjects. To our knowledge, this crossover study conducted in three BMI categories is the first to compare the QUARK RMR calorimeter to the DELTATRAC II™, known to be the reference tool for measurement of these parameters under standardized conditions.[1,8]

The primary endpoint of this study was to compare reliability and accuracy of the two devices in the whole population and then in different BMI classes in order to test a wide range of VO_2 and VCO_2 in resting conditions.

Data for repeatability and reproducibility of both devices obtained with the ethanol burning test permits, first, to conclude to the ability of both devices to assess values of 0.67 ± 0.03 for RQ with coefficients of variation (CV) below 5% (Table 2).

Secondly, during the basal state, the Bland and Altman plot showed agreement and reliability of both calorimeters. The mean difference observed in the whole population was similar to that observed in other studies which compared metabolic carts or
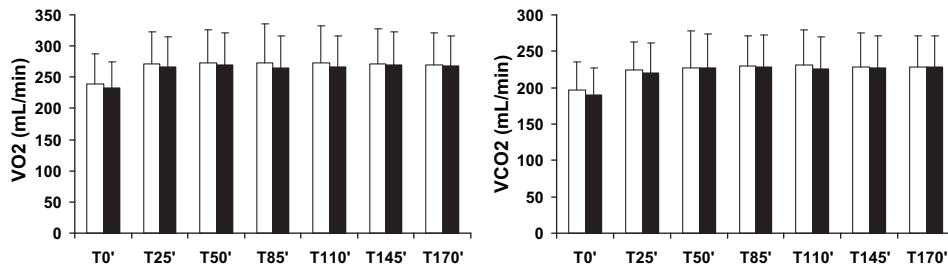
Fig. 3. VO_2 and VCO_2 variation measured in post-prandial phase with the QUARK RMR or the DELTATRAC II™ during the 3 h following test meal ingestion in the whole population (white bars : data from QUARK RMR and black bars : data from DELTATRAC II™).

portable devices to DELTATRAC II™ (-2.8 ± 14.2 mL/min for VCO_2; -4.3 ± 16 mL/min for VO_2; -28.9 ± 109.5 kcal/day for RMR when we compared QUARK RMR to DELTATRAC II™). Wahrlich et al.[11] who compared the VO2000® calorimeter (a portable metabolic system) to the DELTATRAC MB100™ in 33 healthy patients (normal weight, overweight or obese subjects) and who validated the VO2000™ calorimeter (Medgraphics, USA) for RMR measurement, found a difference for VO_2 of -4.44 ± 12.44 mL/min, for VCO_2 of -5.52 ± 14.12 mL/min, for RQ of -0.01 ± 0.01 and for RMR of -145 ± 341 kJ/day (-34.6 ± 81.4 kcal/day). Cooper et al., reported a mean difference for RMR of about -26 ± 155 kcal/day when they compared the VmaxEncore29 (Viasys Healthcare, Sensor Medics Corp, USA) system to the DELTATRAC II™ calorimeter and a mean difference for RMR of about -6 ± 131 kcal/day when they compared the True One® 2400 (Parvo Medics, USA) to the DELTATRAC II™ in 18 patients.[17] Stewart et al., showed, in 50 healthy patients, a mean difference for oxygen consumption of 0.58 ± 15.33 mL/min and a mean difference for RMR of 4.66 ± 113.39 kcal/day when they compared the MedGem RMR® (Microlife, USA), a portable device and the DELTATRAC II™.[5]

It is important to specify that the indirect calorimeters described above were developed to measure EE linked to physical activity and are often portable devices which only use an oxygen sensor to measure EE and approximate VCO_2. The QUARK RMR was developed specially to measure basal metabolic rate at rest which differentiates it from other calorimeters currently marketed, allowing for more sensitive VO_2 and VCO_2 measurement.

The limits of agreement obtained with the Bland and Altman plot were high ([-31; 26] mL/min for VCO_2; [-36; 28] mL/min for VO_2; [-248; 190] kcal/day for RMR) when we compared QUARK RMR to DELTATRAC II™. However, we found the same limits of agreement in other studies such as that performed by Walrich et al.[11] (-826 to 537 kJ/day or -197 to 128 kcal/day for RMR which compared VO2000™ to DELTATRAC II™). This variability is due to study design. In fact, it is impossible to compare the two calorimeters simultaneously for a given subject at the same period. Some day-to-day variability appeared due to biological changes in subjects and to instrument variability. This variability can reach $2-10\%$ under standardized conditions.[2] In our study, the day-to-day variability of the same calorimeter confirmed these data (RMR for DELTATRAC II™, 26 ± 93 kcal/d (CI 95%: [-166; 213]) and for QUARK RMR, -20 ± 86 kcal/d (CI 95%: [-191; 152])) and was comparable to the day-to-day inter-variability calculated between QUARK RMR and DELTATRAC II™.

Our principal limitation was not being able to use QUARK RMR and DELTATRAC II™ simultaneously. One study tried to measure RMR simultaneously with a Cosmed K4b^2 (Cosmed, Italy), a portable calorimeter, and a DELTATRAC II™ placing the Cosmed K4b^2 under the canopy of the DELTATRAC II™ to reduce confounding factors. Unfortunately, this generated errors in RMR results, due to gas leakage resulting in poor reproducibility.[25] It is impossible to perform
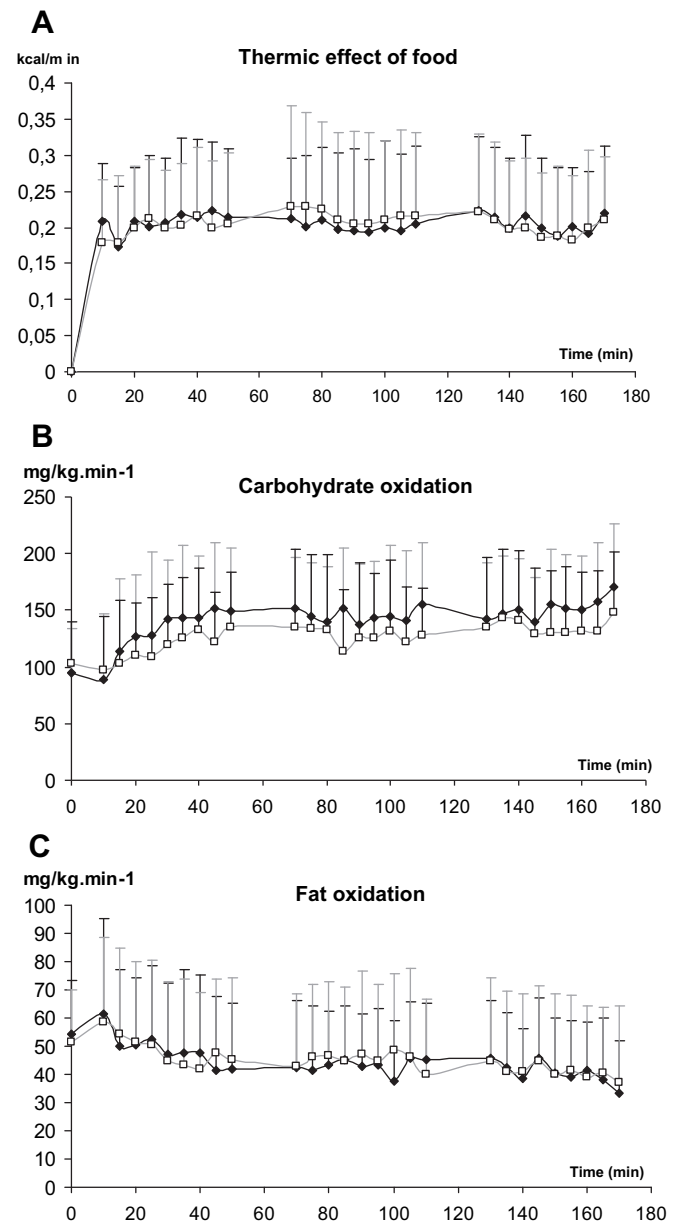

Fig. 4. Representation of thermic effect of food, carbohydrate and fat oxidation measured by DELTATRAC II™ and QUARK RMR during the 3 h following test meal ingestion in the whole population. Figure A represents thermic effect of food in the whole population during the 3 h after test meal ingestion. Figure B represents carbohydrate oxidation in the whole population during the 3 h after test meal ingestion. Figure C represents fat oxidation in the whole population during the 3 h after test meal ingestion. All figures represent pooled data from all 6 groups of subjects (Normal, Overweight, Obese men and women (n = 30))
—◆— DELTATRAC II™.
- -□- -QUARK RMR.

simultaneous measurements of RMR using two metabolic carts because of gas leakage.

An important point to take into account in a crossover design to test equivalence between two devices is to use an adequate number of subjects defined by a statistical power analysis for crossover design. For that, we considered that a difference of RMR of 50 kcal/day with a SD of 82 kcal/day was acceptable to detect non equivalence between the two calorimeters. This tolerated difference of 100 kcal [−50; +50 kcal/day] generally represents, for a healthy subject with a RMR of 1500 kcal/day, about 3% error in estimating RMR measurement which is clinically quite acceptable. For this tolerated difference and SD, 25 subjects were required to achieve 80% power at a 5% significance level. We examined 30 subjects with a large range of BMI as described in the methods section of this paper to test the equivalence of RMR, post-prandial metabolic rate and substrate oxidation measurement made indifferently with the QUARK RMR and DELTATRAC II™ in the whole population. However, we could have perhaps used more subjects in the different BMI and sex categories to have more power to test accuracy and availability, i.e. the equivalence of QUARK RMR *vs* DELTATRAC II™ in these subgroups of categories.

Some calorimeters are reliable for measuring EE but are not available for measuring post-prandial metabolic rate after a test meal. The DELTATRAC II™ was used for many years to assess both EE and substrate oxidation at rest and to provide appropriate nutritional support in critical situations. It was also used to study variations in these parameters during nutritional interventions in clinical research and to search for the mechanisms involved. This is why it seemed important to test the capacity of the QUARK RMR to track modification of EE and substrate oxidation after a test meal. Our secondary endpoint was to validate QUARK RMR performance *versus* DELTATRAC II™ performance and assess the kinetics of TEF and substrate oxidation after a test meal of 687 kcal.

To our knowledge, no publications have compared metabolic carts to the DELTATRAC II™ reference tool during the post-prandial period.

Beyond the interest of assessing the magnitude of TEF in clinical research, it is important to appreciate variation in carbohydrate and fat oxidation rate after meal consumption. Following ingestion of a mixed meal, we observed a similar increase in carbohydrate oxidation and a decrease in fat oxidation, whatever calorimeter was used. Data on substrate oxidation kinetics obtained with DELTATRAC II™ and QUARK RMR were similar and clinical results could be superimposed.

If we consider the magnitude of TEF, we showed that QUARK RMR and DELTATRAC II™ measurements did not differ. The magnitude of TEF was about $4.9 \pm 1.9\%$ with DELTATRAC II™ *versus* $4.9 \pm 2.0\%$ with QUARK RMR when considering the whole population (NS). Considering BMI class, the magnitudes of TEF were respectively about $5.4 \pm 1.4\%$ *versus* $5.0 \pm 1.1\%$ for normal weight, $4.5 \pm 1.5\%$ *versus* $4.5 \pm 2.7\%$ for overweight and $5.0 \pm 2.5\%$ *versus* $5.1 \pm 2.0\%$ for obese subjects (NS). In the literature, the theoretical magnitude of TEF is estimated at 7–9% of EE after meals of 400–1200 kcal/day.[1] Assessment of complete TEF depends on the composition, consistence and calorie content of meals and the time to measurement of post-prandial EE.[26] We chose to give a high protein and low fat diet (44 g proteins (26%), 27 g lipids (35%), and 68 g carbohydrates (39%)) to assess 60–70% TEF after 3 h of post-prandial measurement.[27] Whatever the calorimeter used, DELTATRAC II™ or QUARK RMR, we assessed complete TEF as 54% with our 687 kcal meal after 3 h of measurement. Riggs et al., using a Medgem (HealthTech, Golden, CO) portable calorimeter, compared the effect of high protein-high fat diet *versus* high protein-low fat diet in normal and overweight women. They observed that a 440 kcal meal with 30.8 g protein (28%) and 11.8 g fat (24%) induced a magnitude of TEF of $5.9 \pm 1.1\%$ in normal weight women after a 210 min measurement.[28]

5. Conclusion

Our results highlight the validity of the QUARK RMR calorimeter for measuring basal and post-prandial EE and substrate oxidation in normal and overweight subjects.

The QUARK RMR appears to be a useful alternative to the DELTATRAC II™ for measurement of EE in normal and overweight patients. Given we did not test its ability in critical care patients; we now await research in this area.

Conflict of interest

The authors who contributed to this article have disclosed their relationships with industry. Martine Laville, PhD, MD has received grant support from Cosmed Sarl, Rome, Italy corresponding to provision of the indirect calorimeter (QUARK RMR) but she has no affiliation with Cosmed Sarl, Rome, Italy or Sensormedics, Yorba Linda, CA, USA manufacturers.

Other authors have no financial arrangement or affiliation with Cosmed, Rome Italy or Sensormedics, Yorba Linda, CA, USA.

Statement of authorship

EB participated in elaboration of design experiment, in collection and in analysis of data, in writing of the manuscript. CM participated in elaboration of design experiment, in collection and in analysis of data, in provision of significant advice. SN participated in revision of manuscript and in provision of significant advice. MS participated in conception of standardized meal and in analysis of food record. HR participated in statistical analysis of data. JG participated in laboratory data analysis and interpretation and in revision of manuscript. ML participated in elaboration of design experiment, in revision of manuscript and in provision of significant advice.

All authors read and approved the final manuscript.

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