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A simplified equation for total energy expenditure in mechanically ventilated critically ill patients

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Abstract

Introduction: **"tight calorie control" concept arose to avoid over- and under-feeding of patients.**

Objective: **to describe and validate a simplified predictive equation of total energy expenditure (TEE) in mechanically ventilated critically ill patients.**

Methods: **this was a secondary analysis of measurements of TEE by indirect calorimetry in critically ill patients. Patients were allocated in a 2:1 form by a computer package to develop the new predictive equation TEE (prediction cohort) and the validation cohort. Indirect calorimetry was performed with three different calorimeters: the Douglas-bag, a metabolic computer and the Calorimet®. We developed a new TEE predictive equation using measured TEE (in kcal/kg/d) as dependent variable and as independent variables different factors known to influence energy expenditure: age, gender, body mass index (BMI) and type of injury.**

Results: **prediction cohort: 179 patients. Validation cohort: 91 patients. The equation was: TEEPE (kcal/Kg/d) = 33 - (3 x A) - (3 x BMI) - (1 x G). Where: A (age in years): ≤ 50 = 0; > 50 = 1. BMI (Kg/m²): 18.5 – 24.9 = 0; 25 – 29.9 = 1; 30 – 34.9 = 2; 35 – 39.9 = 3. G (gender): male = 0; female = 1.**

The bias (95% CI) was -0.1 (-1.0 – 0.7) kcal/kg/d and the limits of agreement (± 2SD) were -8.0 to 7.8 kcal/kg/d. Predicted TEE was accurate (within 85% to 115%) in 73.6% of patients.

Conclusion: **the new predictive equation was acceptable to predict TEE in clinical practice for most mechanically ventilated critically ill patients.**

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Key words: *Total energy expenditure. Resting energy expenditure. Critically ill patients. Mechanical ventilation.*

ECUACIÓN SIMPLIFICADA PARA EL CÁLCULO DEL GASTO ENERGÉTICO TOTAL EN PACIENTES CRÍTICOS CON VENTILACIÓN MECÁNICA

Resumen

Introducción: **el concepto de "control calórico estricto" surgió para evitar la excesiva y la deficiente nutrición de los pacientes.**

Objetivo: **describir y validar una ecuación simplificada para el cálculo del gasto energético total (GET) en pacientes críticos con ventilación mecánica.**

Métodos: **análisis secundario de las mediciones de GET por calorimetría indirecta en pacientes críticos. Los pacientes fueron asignados de forma 2:1 por un paquete estadístico; el primer grupo se empleó para desarrollar la nueva ecuación predictiva del GET (grupo predictivo) y el segundo para validarla (grupo validación). La calorimetría indirecta se realizó con tres calorímetros diferentes: la bolsa de Douglas, un computador metabólico y el equipo Calorimet®. Hemos desarrollado la nueva ecuación predictiva del GET utilizando el GET medido (en kcal/kg/d), como variable dependiente, y como variables independientes los diferentes factores que influyen en el gasto energético: edad, género, índice de masa corporal (IMC) y tipo de lesión.**

Resultados: **el grupo de predicción incluyó 179 pacientes y el de validación 91 pacientes. La ecuación predictiva fue: GETEP = 33 - (3 x E) - (3 x IMC) - (1 x G). Donde: E (edad en años): ≤ 50 = 0; > 50 = 1. IMC (kg / m2): 18,5-24,9 = 0; 25-29,9 = 1; 30-34,9 = 2; 35-39,9 = 3. G (género): hombre = 0; mujer = 1.**

El sesgo (IC del 95%) entre el GET medido y el predicho fue de -0,1 (-1,0 a 0,7) kcal/ kg/día y los límites de acuerdo (± 2SD) fueron -8,0 a 7,8 kcal/kg/d. El GET por la ecuación predictiva fue preciso (entre el 85% y el 115%) en el 73,6% de los pacientes.

Conclusiones: **La nueva ecuación predictiva fue aceptable para predecir el GET de la mayoría de pacientes críticos con ventilación mecánica en la práctica clínica.**

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Palabras clave: *Gasto energético total. Gasto energético en reposo. Pacientes críticos. Ventilación mecánica.*

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Introduction

Optimal energy requirements in critically ill patients remain to be a controversial topic[1]. Different recent studies showed an association between negative cumulative energy balance and increased nosocomial infection and ICU mortality in critically ill patients with prolonged mechanical ventilation[2-7], highlighting the need for a close monitoring of caloric and protein intake[6,8]. In this setting, the concept of "tight calorie control"[5] arose to avoid over- and under-feeding of these patients.

In spite of these findings, many patients are underfeeded[9]. Causes for underfeeding usually include, but are not limited to, a prescribed energy intake lower than total energy expenditure (TEE), since TEE is not measured by the difficult in performing indirect calorimetry and is calculated by predictive equations too arduous for clinical practice. Additionally, patients are usually given energy intake lower than that prescribed due to the interruption of nutritional support by different causes[1,4].

Then, the only way to achieve "the tight calorie control" is measuring TEE by using a calorimeter for 24 hours. However, it is not feasible in clinical practice. A more feasible alternative is measuring resting energy expenditure (REE) by indirect calorimetry in short periods of time and adding estimated energy expenditure by daily activity, obtaining the TEE. Finally, as previously noted, TEE can be calculated from predictive equations which have also been questioned because they can lead to over- and under-prediction of TEE[10-13].

Our objective was to describe and validate a new simplified equation to predict TEE in critically ill patients undergoing mechanical ventilation.

Methods

Patients

This study was a retrospective analysis of indirect calorimetry measurements performed in mechanically ventilated critically ill patients included in previous studies[14-17]. For the purpose of this secondary analysis we excluded patients who were aged < 18 years-old, with a body mass index (BMI) < 18.5 Kg/m^2 or ≥ 40 Kg/m^2, and body temperature <36 ºC or > 38 ºC.

Patients were allocated in a 2:1 form with a computer package. Approximately 66% of patients (prediction cohort) were used to describe a new TEE predictive equation and the rest to validate the predictive equation (validation cohort).

Patients were studied in the morning at rest, in the semirecumbent position, hemodynamically stable with or without vasoactive drugs, and after two or more days of mechanical ventilation. All patients were mechanically ventilated in volume control mode and most of them received continuous infusions of sedatives and analgesics. Measurements of indirect calorimetry were performed during the continuous administration of enteral or parenteral feeding with a maximal caloric intake of 30 kcal/Kg body weight. Tracheal suctioning, physiotherapy, postural changes, radiologic studies or body washings were not carried out during the 30 minutes prior to the measurement.

The study was approved by the research committee of the hospital. The need of informed consent was waived since this was a retrospective secondary analysis.

Indirect calorimetry: apparatus and techniques

Indirect calorimetry was performed from measured oxygen consumption (VO_2) and carbon dioxide excretion (VCO_2) for short periods of time which were used to calculate the REE using the abbreviated Weir's equation[18]:

$$REE = 1.44\ (3.9\ VO_2 + 1.1\ VCO_2)$$

Where: REE was measured in kcal/d and VO_2 and VCO_2 in mL/min; the factor 1.44 is derived by multiplying 1440 min/day by kcal/1000 mL.

We considered as measured TEE the REE measured by indirect calorimetry increased by 10% corresponding to the estimated daily patient activity[19].

VO_2 and VCO_2 measurements were performed with three different calorimeters used in four different studies: the Douglas-bag[14,17], the metabolic computer[16] and the Calorimet ® (Caloric Measurement Unit, ICOR, Bromma, Sweden)[15]. VO_2 and VCO_2 measurements were corrected for standard temperature and pressure dry gas (STPD) conditions. The reproducibility of the measurement of VO_2 and VCO_2 was close to10%[20]. We briefly describe the three indirect calorimeter methods used:

– Douglas-bag: Based in open-circuit method, the inspired and expired gases were collected in the Douglas bag for a period of 4-5 minutes and analyzed for volume and gas fractions. The volume of expired gas was measured with a spirometer. The analysis of oxygen and CO_2 fractions was done with a polarographic electrode of blood gas analyzer. Measurements of VO_2 and VCO_2 were made twice, and the mean of the two measurements was used.
– Metabolic computer: VO_2 was measured with a metabolic computer (Gambro Engström, Bromma, Sweden) and VCO_2 was measured with an external infrared CO_2 analyzer (Eliza, Gambro Engström, Sweden) connected to the metabolic computer. We recorded the mean VO2 and VCO_2 during a 60-minute period.
– Calorimet®. This device measures VO_2 by continuously monitoring the change in the volume of the gas in a closed breathing circuit[20]. VO_2 measurements were performed in duplicate, in periods

of 100 breaths for each measure, and the mean of the two measurements was recorded. This method allows VO_2 measurement in patients requiring oxygen concentrations from 21 to 100%. The limitation of the device was that it did not measure VCO_2 and the REE calculation (in kcal/day) was made with the Weir's equation[18] only using the VO_2 measurement (in ml/min). The VCO_2 was estimated using a respiratory quotient of 0.85: $VCO_2 = VO_2 \times 0.88$.

Predictive equation

We developed a new TEE predictive equation in the predictive cohort of patients using measured TEE (in kcal/Kg/d) as dependent variable and as independent variables different factors known to influence TEE: age, gender, weight, height and type of injury. The continuous independent variables were categorized to simplify the predictive equation. The female gender and age over 50 years-old have the value 1 in multiple regression equation. Current body weight (at the day when indirect calorimetry was performed) and height were replaced by the BMI. The patients were stratified into BMI categories according to World Health Organization criteria, similarly to study of Zauner et al[21]. BMI categories have the following values in linear regression: normal weight (BMI 18.5 – 24.9) value of 0, pre-obese (BMI 25 – 29.9) value of 1, obese class I (BMI 30 – 34.9) value of 2 and obese class II (BMI 35 – 39.9) value of 3. The type of injury had a value of 0 for trauma patients, a value of 1 for medical patients and a value of 2 for surgical patients in the linear regression analysis.

Validation of predictive equations

Our new TEE predictive equation and other common predictive equations (detailed below) were evaluated in the validation cohort patients against measured TEE by taking the difference (predicted – measured) as a percentage of measured TEE. Appropriate predicting was defined when predicted TEE was within 90-110% of measured TEE and acceptable predicting within 85-115%[22]. Under- and over-prediction was considered when TEE predicted by equations were above and below of the respective limits of TEE measured[22].

The common predictive equations evaluated were:

– *Harris-Benedict equation (TEEHB)*
  Men: 66.5 + (13.8 x weight) + (5.0 x height) – (6.8 x age)
  Women: 655 + (9.6 x weight) + (1.8 x height) – (4.7 x age)
  REE predicted by Harris-Benedict equation was increased by 30% for physical activity (10%)[19] and injury factor (20%)[23] to predict TEE.

– *Ireton-Jones 1992 (TEEIJ92)*[24] *and 1997 (TEEIJ97)*[25] *equations*
  TEEIJ92 = 1925 – (10 x age) + (5 x weight) + (281 if male) + (292 if trauma present) + (851 if burns present)
  TEEIJ97 = 1784 + (5 x weight) – (11 x age) + (244 if male) + (239 if trauma present) + (840 if burns present)
  REE predicted by Ireton-Jones equations were increased by 10% for physical activity[19] to predict TEE.

Data collection

The following baseline information was collected for each patient included: demographic data (age, gender, height, weight and body mass index (BMI); Simplified Acute Physiology Score (SAPS II) at ICU admission; type of injury (medical, surgical or trauma). During indirect calorimetry we recorded the measured value of REE, body temperature and the caloric and nitrogen intake per day.

Statistical analysis

Data are expressed as mean ± standard deviation (SD) or as median and interquartile range (IQR) as appropriate. Independent samples t-test was used to compare continuous variables, and chi-square or Fisher exact tests were used to compare proportions. The new TEE predictive equation was developed using forward stepwise linear regression using measured TEE as dependent variable. The Bland-Altman analysis[26] was used to assess the bias and limits of agreement between TEE predicted by different equations and measured by indirect calorimetry. The predicted TEE values obtained using equations were considered unbiased if the 95% confidence interval of the mean difference between the predicted and measured TEE included zero. The agreement limits were mean bias ± 2 SD between predicted and measured TEE. A p value less than 0.05 was considered significant. Statistical analyses were performed using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

From the cohort of 340 mechanically ventilated patients we finally included 270 patients. The new predictive equation of TEE was developed with 179 patients in the prediction cohort and validated in the remaining 91 patients (validation cohort) (Fig. 1).

Prediction and validation cohorts did not differ in clinical characteristics: age, gender, weight, height, BMI, SAPS II, type of injury, calorimeter used to measure REE, body temperature, caloric and nitrogen intake and mean value of REE measured and predicted by

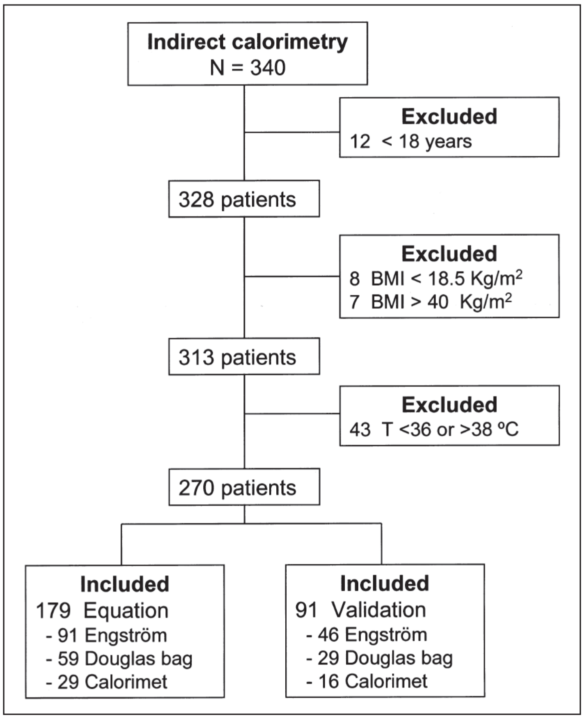

Fig. 1.—Flowchart of patients entered in the study.

Harris-Benedict equation (Table I). Measured REE was 122% of predicted by Harris-Benedict in both groups.

Predictive equation

In the linear regression analysis, the independent variables that entered in the new TEE predictive equation (in kcal/Kg/d) were age, BMI and gender (r^2 = 0.37, $p < 0.001$). The type of injury dropped of the equation (Fig. 2 and Table II). The new TEE predictive equation used in the validation cohort was simplified without decimals as shown below:

TEE = 33 - (3 x A) - (3 x BMI) - (1 x G)

Where: A (years): ≤ 50 = 0; > 50 = 1

BMI (Kg/m²): 18.5 – 24.9 = 0
25 – 29.9 = 1
30 – 34.9 = 2
35 – 39.9 = 3

G: Gender: male = 0; female = 1

Validation of predictive equations

Difference between measured and predicted TEE by the new equation, the Harris-Benedict equation and

Table I
Clinical and calorimetric characteristics of patients, distributed by equation and validation cohorts

	Total N = 270	*Prediction* N = 179	*Validation* N = 91	p *value*
Female, n (%)	61 (22.6)	39 (21.8)	22 (24.2)	0.65
Age, years	50 ± 20	50 ± 19	49 ± 21	0.50
Weight, Kg	71 ± 13	70 ± 12	72 ± 13	0.37
Height, cm	167 ± 9	167 ± 9	168 ± 10	0.36
BMI, Kg/m²	25.4 ± 4.5	25.3 ± 4.4	25.6 ± 4.8	0.68
BMI categories, n (%)				0.88
Normal	144 (53.3)	96 (53.6)	48 (52.7)	
Pre-obese	84 (31.1)	57 (31.8)	27 (27.9)	
Obese class I	33 (12.2)	21 (11.7)	12 (13.2)	
Obese class II	9 (3.3)	5 (2.8)	4 (4.4)	
SAPS II	38 ± 13	39 ± 13	37 ± 12	0.46
Type of injury, n (%)				0.89
Medical	61 (22.6)	41 (22.9)	20 (22.0)	
Surgical	78 (28.9)	50 (27.9)	28 (30.8)	
Trauma	131 (48.5)	88 (49.2)	43 (47.3)	
Calorimetric method, n (%)				0.96
Metabolic computer	137 (50.7)	91 (50.8)	46 (50.5)	
Douglas bag	88 (32.6)	59 (33.0)	29 (31.9)	
Spirometer (Calorimet)	45 (16.7)	29 (16.2)	16 (17.6)	
Temperature, ºC	37.2 ± 0.5	37.2 ± 0.5	37.3 ± 0.5	0.06
Caloric intake, kcal/d	1503 ± 605	1487 ± 594	1534 ± 631	0.60
Nitrogen intake, g/d	11.2 ± 6.1	11.1 ± 6.1	11.5 ± 6.1	0.64
Measured REE, kcal/d	1854 ± 343	1840 ± 343	1881 ± 343	0.36
Predicted REEHB, kcal/d	1526 ± 237	1515 ± 232	1548 ± 248	0.28
REE (%)	122 ± 18	122 ± 18	122 ± 18	0.96

BMI: body mass index. SAPS: Simplified Acute Physiology Score. REE: resting energy expenditure. HB: Harris-Benedict equation.

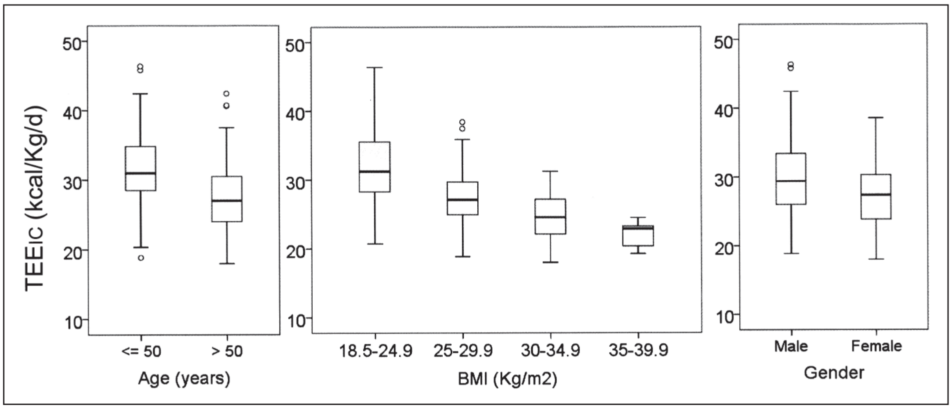

Fig. 2.—Total energy expenditure measured by indirect calorimetry (TEE_{IC}) stratified by BMI, age ≤ 50 years-old or > 50 years-old and gender in 179 patients of the prediction cohort. The horizontal lines within the boxes indicate medians, the lower and upper ends of the boxes the 25th and 75th percentiles.

Table II
Linear regression of predictive equation of total energy expenditure (TEE)

Variable	*B*	*95% CI for B*	p *value*
Constant	33.01	32.1 – 34.2	< 0.001
Age, (≤50 years-old = 0; > 50 years-old = 1)	- 2.97	- 4.4 – - 1.6	<0.001
Gender, (male=0; female =1)	- 1.3	- 2.6 – - 0.1	0.03
Body mass index *	- 3.1	- 3.9 – - 2.2	<0.001

(*) Body mass index in Kg/m^2 (18.5-24.9 =0; 25-29.9=1; 30-34.9=2; 35-39.9=3).

the Ireton-Jones 1997 equation were unbiased (95% CI did not include the zero), whether by the Ireton-Jones 1992 equation was biased (95% CI included the zero) (Table III). The agreement limits (mean difference ± 2 SD) between measured and predicted TEE were width for all equations, especially by the 2 Ireton-Jones equations (Table III).

Predicted TEE by the new equation and by the Harris-Benedict equation (predicted REE increased by 30%) were within 90%-110% of measured TEE in near 55% of patients and within 85%-115% in nearly 75% patients (Table IV and Fig. 3). The Ireton-Jones 1992 and 1997 equations had lower accuracy to predict TEE, within 90-110% of measured TEE in only

Table III
Bland and Altman analyses between predicted equations and measured total energy expenditure (TEE)

	TEE (kcal/Kg/d)	*Bias (kcal/Kg/d)*	*95% CI of bias (kcal/Kg/d)*	*Agreement (kcal/Kg/d)*	*Agreement (percentage)*
TEEIC	29.2 ± 5.0	–	–	–	–
TEEPE	29.1 ± 3.5	- 0.1	- 1.0 – 0.7	- 8.0 – 7.8	- 25.9 – 28.1
TEEHB	28.4 ± 3.7	- 0.8	- 1.7 – 0.01	- 9.0 – 7.4	- 28.7 – 26.1
TEEIJ92	33.8 ± 7.3	4.6	3.4 – 5.8	- 7.3 – 16.5	- 25.1 – 58.1
TEEIJ97	30.0 ± 6.9	0.8	- 0.4 – 1.9	- 10.5 – 12.1	- 35.3 – 41.7

TEEIC: TEE measured by indirect calorimetry. TTEPE: TEE predicted by the new equation. TEEHB: TEE predicted by Harris-Benedict equation increased by 30%. TEEIJ92: TEE predicted by Ireton-Jones 1992 equation. TEEIJ97: TEE predicted by Ireton-Jones 1997 equation. Bias: mean difference between predicted and measured TEE.

Table IV
Number and percentage of subjects whose predicted total energy expenditure (TEE) was over-predicted, accurate or under-predicted compared to measured TEE

	Over-predict > 110%	*Accuracy 90–110%*	*Under-predict < 90%*	*Over-predict > 115%*	*Accuracy 85–115%*	*Under-predict < 85%*
TEEPE	19 (20.9)	53 (58.2)	19 (20.9)	13 (14.3)	67 (73.6)	11 (12.1)
TEEHB	16 (17.6)	51 (56.0)	24 (26.4)	11 (12.1)	66 (72.5)	14 (15.4)
TEEIJ92	54 (59.3)	26 (28.6)	11 (12.1)	45 (49.5)	40 (44.0)	6 (6.6)
TEEIJ97	35 (38.5)	33 (36.3)	23 (25.3)	25 (27.5)	50 (54.9)	16 (17.6)

TTEPE: TEE predicted by new equation. TEEHB: TEE predicted by Harris-Benedict (REE increased 30%). TEEIJ92: TEE predicted by Ireton-Jones 1992 equation. TEEIJ97: TEE predicted by Ireton-Jones 1997 equation.

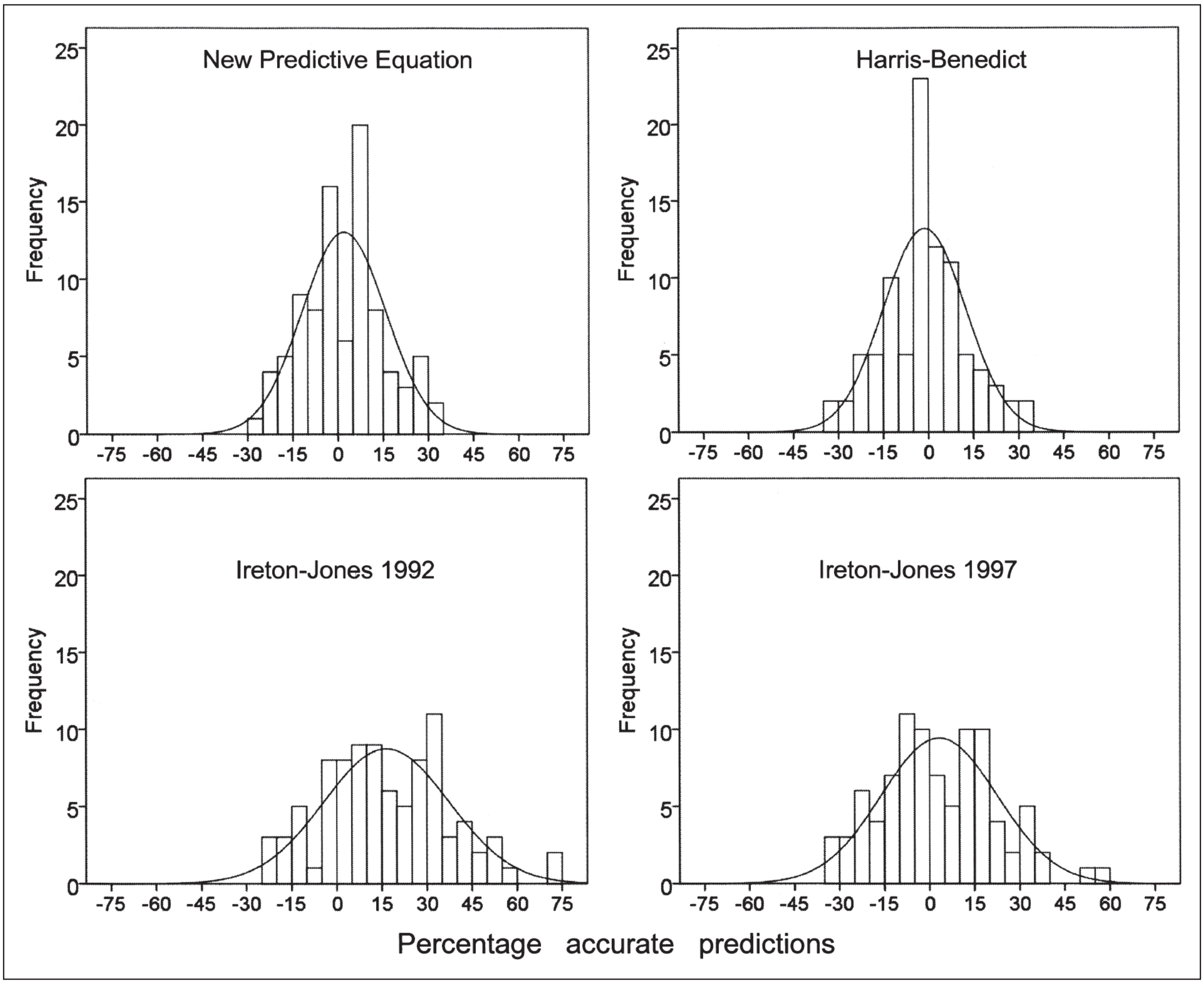

Fig. 3.—Percentage of accurate prediction of TEE (each bar represent a 5% of difference from measured TEE) with the new predictive equation, the Harris-Benedict equation and the 2 Ireton-Jones equations.

29% and 36% of patients and within 85-115% in 44% and 55% of patients respectively (Table IV and Fig. 3).

Discussion

In our cohort of patients the new predictive equation of TEE had acceptable accuracy in nearly 74% of patients. However, due to the large limits of agreement between measured TEE by indirect calorimetry and predicted TEE by our new equation, it should be used with caution in clinical practice. The same applies to the Harris-Benedict equation increased by 30%, while the TEE predicted by the 2 Ireton-Jones equations had poor accuracy and had very large limits of agreement and therefore, should not be used in mechanically ventilated critically ill patients.

A 10% accuracy of TEE predictive equations (within 90-110%) compared with measured TEE by indirect calorimetry would be ideal. However, considering that the widely accepted Harris-Benedict equation estimates REE of a normal subject with an accuracy of 14%[27], we consider acceptable in routine clinical practice an accuracy of 15% (within 85-115%).

Thus, we found that calculated TEE with the new predictive equation and the Harris-Benedict equation increased by 30%[19,23] were accurate (within 85%-115%) in nearly 74% of patients. Unfortunately, TEE in 26% of patients was under- or over-predicted. This can be due to the variability of REE measurement throughout the day[28-30] and between days[15,31]. The two predictive Ireton-Jones equations[24,25] were acceptable (within 85-115%) only in nearly 50% of patients in our predictive cohort, a percentage similar to that found with other equations[12] non-tested in our study.

Many factors influence TEE in critically ill patients, and all of them have not been taken into account in our equation, because the aim of study was to describe a simplified equation for mechanically ventilated critically ill patients receiving sedatives.

The main factors that modify TEE in critically ill patients are:

- *Aging*. We simplified the effect of aging in our new TEE predictive equation using a cut-off of 50 years-old, since the progressive decline in REE has a breakpoint for a more rapid decline around 39 to 54 years-old depending on gender and BMI[32].
- *Gender*. We found that women had 1.3 kcal/Kg/d less than men, much lower than the 3.7 kcal/Kg/d found in the study of Drolz et al[33]. The underlying causes may include the few number of female and the potential interaction between age, BMI and gender with REE[32,34].
- *Weight, height and body mass index***.** We used the current weight to calculate the BMI, since previously we found that the REE in kcal/Kg/d were the same (27±3 kcal/Kg/d) with high and low weight[35]. Rapid variations in weight were attributable to acute changes in the volume of extracellular water.
- *Type of injury*. As expected, the type of injury dropped of the linear regression. Previously we found that critically ill patients with different types of injury undergoing mechanical ventilation had similar measured REE when they were matched according to age, gender, weight and body temperature[16], as Frankenfield et al[36] did.
- *Body temperature*. We did not include temperature in the new TEE predictive equation because we wanted to predict the TEE in normothermia conditions. However, given the influence on energy expenditure of the body temperature, we calculated the change produced in the TEE per ºC variation (data not shown). This corresponds to a variation of about 5-6% per degree Celsius (near to 100 kcal per ºC), similar to that observed in other studies in critically ill patients[31,37].
- *Diet induced thermogenesis*. Indirect calorimetries were performed without suspending continuous enteral and/or parenteral nutrition, because the thermogenic effect of continuous nutrition when caloric intake is similar to the energy expenditure is only around 3% of measured REE in patients with mechanical ventilation[38].
- *Activity*. We used an activity factor of 10% to estimate TEE in our new equation as well as the other predictive equations evaluated, according to Swinamer et al[39].

In our opinion, out of the 3 ways to determine the TEE in critically ill patients undergoing mechanical ventilation, the only one that allows achieving "the tight calorie control"[5] is through the measurement of TEE by continuous indirect calorimetry throughout all the time of mechanical ventilation. The measurement of REE with indirect calorimetry to estimate TEE can lead to under- or over-prediction due to the variability in energy expenditure over time[15,28-31] and therefore should not be recommended. We believe that the use of predictive equations of TEE, although they have been widely questioned[10-13], could be more accurate than the measurement of REE with indirect calorimetry to determine TEE during mechanical ventilation. We hypothesize that some predictive equations of TEE achieve a better cumulative energy balance in most patients than the measurement of REE once. In spite of it, at the best of our knowledge, only one study including 27 patients compared different equations with the measured TEE for 5 or more days. In this, study, the limits of agreement were unacceptably wide[40]. Therefore, prospective studies to confirm or refute this hypothesis will be needed.

Several limitations to our study must be noted. First, it was a secondary analysis of four studies. Second, we measured the REE and not the TEE, so we cannot properly assess the equations. Moreover, indirect calorimetry was performed with 3 different methods. Although it represents a drawback, it has the advantage of avoiding bias which involves the use of a single device. Third, in this cohort of patients there was a gender bias due to the relatively low number of women.

In conclusion, the new predictive equation was acceptable to predict TEE in clinical practice for most mechanically ventilated critically ill patients.

Conflict of interest

The authors declare no conflict of interest.

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