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Energy Expenditure in Mechanically Ventilated Patients: Indirect Calorimetry vs Predictive Equations

Mekanik Ventilasyon Uygulanan Hastalarda Enerji Tüketimi: İndirek Kalorimetri Tahmin Ettirici Eşitlik Karşılaştırması

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ABSTRACT

Background & Objectives: Indirect calorimetry(IC) is used in the calculation of energy consumption (EE) in critical care patients. In this study, it was aimed to compare the frequently used equations with IC in different body weight and disease classes and to determine relationship between them and disease severity.

Materials & Methods: 100 mechanically ventilated critical care patients were prospectively included in the study. Measurements were done on 3th, 4th and 5th days of ICU stay with IC and Harris Benedict (HB), Penn State 2003(PS), Schofield(SCH), Swinamer (SW) and Ireton-Jones(IJ) equations were calculated and APACHE II and SAPS II scores were determined. Bland-Altman limits of agreement analysis was done to determine the range of error with each predictive equation compared to the measured IC.

Results: The mean age±standard deviation was 66,10 ± 14,98 years and mean body mass index was 24,91 ± 4,45 kg.m-2 for the study group. Mean±standard deviation for APACHE II score and SAPS II were 23,42 ± 8,47 and 42,23 ± 10,62. Measured EE was 1828, 580 ± 436, 272 kcal/day. Correlation analysis between equations and IC showed that all equations were moderately correlated with IC. For all weight categories and equations , the limits-of agreement range was large. For the patient group, the bias was lowest with the PS predictive equation (mean error 14 kcal/ day). HB and PS equations have better agreement with IC than others do. No correlation was observed between severity scores and EE.

Conclusion: Predictive formulas for EE is not reliable in determining the energy, confidence intervals are wide in ICU patients necessitating mechanical ventilation.

Key words: indirect calorimetry, energy expenditure, predictive equations, intensive care, nutrition

ÖZ

Giriş ve Amaçlar: Yoğun bakım hastalarında enerji tüketiminin (EE) hesaplanmasında indirek kalorimetre (IC) kullanılır. Bu çalışmada, farklı vücut ağırlıkları ve hastalık sınıflarında olan hastalarda sık kullanılan eşitliklerin IC ile karşılaştırılması ve hastalık şiddeti ile bunların arasında ilişkinin belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya mekanik ventilasyon uygulanan 100 yoğun bakım hastası prospektif olarak dahil edildi. Yoğun bakım ünitesi yatışının 3. 4. ve 5. günlerinde IC ölçümleri yapıldı ve Harris Benedict (HB), Penn State 2003 (PS), Schofield (SCH), Swinamer (SW) ve Ireton-Jones (IJ) denklemleri hesaplandı, APACHE II ve SAPS II skorları belirlendi. Bland-Altman limit analizi, ölçülen IC'ye kıyasla her bir tahmin denklemi ile hata aralığını belirlemek için kullanıldı.

Bulgular: Çalışma grubunun yaş ortalaması ± standart sapması 66,10 ± 14,98 yıl ve vücut kitle indeksi ortalaması 24,91 ± 4,45 kg.m-2 idi. APACHE II skoru ve SAPS II için ortalama ± standart sapma 23,42 ± 8,47 ve 42,23 ± 10,62 idi. Ölçülen EE 1828, 580 ± 436, 272 kcal / gündü. Denklemler ve IC arasındaki korelasyon analizi, tüm denklemlerin IC ile orta derecede ilişkili olduğunu göstermiştir. Tüm ağırlık kategorileri ve denklemleri için, anlaşma aralığının sınırları büyüktü. Hasta grubu için bias PS tahmin denklemi ile en düşüktü (ortalama hata 14 kcal / gün). HB ve PS denklemleri, IC ile diğerlerinden daha iyi bir uyuma sahiptir. Hastalık şiddet skorları ile EE arasında bir korelasyon gözlenmedi.

Sonuç: Enerji tüketiminin belirlenmesinde tahmin edici formüller güvenilir değildir, mekanik ventilasyon gerektiren yoğun bakım hastalarında güven aralıkları geniştir.

Anahtar kelimeler: indirek kalorimetre, enerji tüketimi, tahmin ettirici denklemler, yoğun bakım, beslenme

Introduction

Patients with critical illness requiring hospitalization in intensive care units (ICU) are at high risk for malnutrition. Patients in ICU have undesirable effects of both overnutrition and malnutrition. Disease state creates a hypermetabolic state and increases EE. If the patient's energy need does not meet, patient's lean body mass losts very quickly. Lost of lean body mass in intensive care patients has been reported to reduce the chances of survival. On the other hand, overnutrition can be harmful and cause complications such as hyperglycemia, azotemia and hypercapnia (1). The determination of the energy requirements associated with a clinical assessment of nutritional support is an important condition in these patients (2).

Total EE includes resting energy expenditure (REE), physical activity, disease process and growing issue. Many methods and equations have been defined to calculate EE in criticall care patients; on the other hand, all of these methods have some weakness. The development of a more practical tool for identifying the EE have emerged in an effort to predictive equations (3). Most predictive equations come from studies carried out typically healthy, non-hospitalized patients, but only a few studies has been approved in patients on mechanical ventilation. There is large variability in EE regardless of which weight and equation are used, so many predicted values will differ from the measured values (4,5). Indirect calorimetry (IC) is a noninvasive gold standart method that calculate EE via oxygen expenditure (VO_2) and carbon dioxide production (VCO_2) (6). However, IC devices for determining estimated energy requirement are not still commonbecause of being expensive and being time consuming.

The aim of this study was to compare predictive equations (Harris-Benedict (HB), IretonJones (IJ), Schofield (SCH), Swinamer (SW) and Penn State (PS) 2003 predictions) with IC measurements in a mechanically ventilated critically ill patient population.

Materials and Methods

This prospective study was done after written informed consent was obtained from the patient or an authorized legal guardian. The Local Ethics Committee of the, Medical School approved the study. 114 mechanically ventilated patients admitted to ICU aged over 18 were included in the study.

Patients were excluded if they were younger than 18 years old, had hyperthemia (>38^{0}C) or hypothermia (<35°C), had intolerance to IC procedures, were pregnant, had an FiO_2 requirement > 60% or a positive end-expiratory pressure requirement greater than 20 cm H_2O, intolerant to mechanical ventilation, had a bronchopleural fistula or chest tube leak, required continuous renal replacement therapy or continuous ambulatory peritoneal dialysis, or were receiving neuromuscular blockade, were intoxicated, hemodynamically unstable. Patients were not admitted to the study with possible edema, cardiac and/or renal failure.

The variables for each patient were obtained such as admission height, admission weight, primary diagnosis, calculated body mass index (BMI), age and sex by standardized chart abstraction. Severity of disease scores (eg. Acute Physiology and Chronic Health Evaluation II (APACHE II), Simplified Acute Physiology Score II (SAPS II)) were calculated from patient's data within 24 hours of ICU admission. The Subjective Global Assessment (SGA) was recorded at admission. Enteral and/or parenteral nutrition were given during the study period according to the patient's status. Total parenteral nutrition (TPN) were administered via multilumen central catheters or peripheral catheters. Enteral nutrition were given via nasogastric tube or gastrostomy tube. We use standard nutritional protocol in our clinic. For starting enteral or parenteral feeding, physician should fill standardized order sheet. Enteral or parenteral nutrition should be started at low rates and gradually advanced to an hourly goal rate. We did not accounted nutrition for feeding interruptions. Patients receiving enteral nutrition are assessed for gastric residual volume every 4 hours. Gastrointestinal motility agents are received only displays of delayed gastric emptying. Immune-enhancing formulas such as the amino acids glutamine or lipids like omega-3 fatty acids; micronutrients, such as antioxidant vitamins A, C and E and the minerals selenium and zinc has not been used during the study period.

*The predictive equation*s: Resting energy expenditure has been measured from actual EE measurements using IC. At the same time with IC measurements, the predictive equations were recorded during study period (3th, 4th and 5th days of ICU stay). In addition, patients' nutritional values and temperature also recorded. The prediction accuracy of these equations was also accepted as prediction values that were inside the range of 80% to 110% of the measured value by IC. All other predictions that outside this range were considered inaccurate.

*Indirect Calorimetry protoco*l: Energy expenditure was measured after a 30-minute rest, with no movement by the patient in a thermoneutral environment for the 30-minute duration. According to the protocol, in the early morning (05:30 to 07:30), 30 minutes IC measurements were taken and the average of the measurements recorded from monitor. Patients have waited at least 2 hours after the administration of general anesthetic agents or hemodialysis, and have an administered fraction of inspired oxygen of 0.6 or less. Patients should be hemodynamically stable during measurements. During measurements, patients should not be given drugs like vasopressors, inhaler steroid, and bronchodilatators. Enteral or parenteral nutrition was stopped during the IC measurements.

Patients ventilated-assisted controlled as a pressure or volume controlled mode to be stable and comfortable in accordance with the cause of respiratory failure via multiprocessed ventilators (Savina or Evita XL, Dräger medical). Indirect calorimetry was performed using Datex Ohmeda M-CAiOVX module (Datex-Ohmeda, Finland) in accordance with IC protocol.

Body mass index (kg/m^2) was calculated for all patiens using their admission height and weight. Patients were classified into weight categories such as BMI less than <19,9 kg/m2, 20-24.9, 25-29.9 and 30 or greater. The ideal body weight (IBW) and adjusted body weight (AdjBW) was calculated for predictive equations(7). Data for Long correction factor was recorded at the patient's file.

Statistical Analysis

A statistical software program (SPSS 16.0 for Windows, SPSS Inc., Chicago, IL, USA) and MedCalc12.7.0.0 were used for statistical analysis. Descriptive statistical methods (frequency, percentage, mean, standard deviation) were used for data analyzing. Correlation analysis conducted to determine the relationship between equations and IC and between equations itself. Pearson correlation coefficient was calculated to assess agreement inside the method. Bland-Altman limits of agreement analysis was undertaken to determine the extent of error with each predictive equation compared to the measured IC. The limits of agreement show the range of differences between the IC measurement and the EE predicted by the equations. Data are presented as mean±SD, with P values, and 95% confidence intervals. P values <0.05 were accepted statistically significant.

Results

In the present clinical study, 114 patients were included. Fourteen patients were eliminated from the study (having tube thoracostomy, exitus during the study, need high FiO_2 and having out of range of RQ ratio (<0.7 or> 1.3)). The study was conducted with 100 patients (58 men and 42 women). Underweight patients accounted for 12%, whereas 45% were normal-weight, 30% were over weight, and 13% were obese. According to SGA, 60% of the patients were in A, 27% B and the others were in class C. Patients were given 78% enteral nutrition where as 10% parenteral and 12% combined nutrition. Since we have a mixed intensive care unit, we accept all types of patients. Diagnoses of acute lung injury (n = 28, 28%), cardiac failure (n=19, 19%) and malignancies (n = 30, 30%) predominated in this population. Demographic variables for the patient population are given in Table 1.

All of the equations were moderately correlated among themselves and showed good agreement with each other (p <0.05). Based on the correlation analysis SW (r=0,913, r^2= 0.834) and PS (r=0,897, r^2=0.805) equations were found to be most correlated with the IC (Table 2).

The limits-of agreement range was large for all equations and in all weight categories. Bias is the predicted value (by equation) minus measured value (by IC). For the patient group, the bias was lowest with the PS predictive equation. HB and PS equations have better agreement with IC than others. SCH, IJ and SW did not show agreement with IC in this patient population (Table 3, Figure 1).

Best prediction among the equations was 86% with the SCH. For the study patients, HB and PS predicted accuracy in 83% while IJ predicted accuracy in 78%. Correlation analysis between prediction equations and measured IC data in the patient groups according to their BMI showed that all predicted equations were correlated with IC (p <0.05). PS equation was found to be well correlated with the IC in the overweight patients (r^2=0,667) and obese patients (r^2=0,605). HB, SW and PS equations moderately correlated with IC data in thin patients. According to the Blandt-Altman analysis, PS equations have well agreement in overweight and obese patients (Table 4). According to the correlation analysis,

Table 1. Demographic variables for the patient

	Mean	SD	Min.	Max.
Age (year)	66,1	15,0	20	89
BMI (kg.m^{-2})	24,91	4,45	16,6	40,6
APACHE II	23,42	8,47	6	40
SAPS2	42,23	10,62	18	70
IC (kcal.day^{-1})	1 828,58	436,27	908	3711
HB (kcal.day^{-1})	1 716,97	404,19	1090	3393
SCH (kcal.day^{-1})	1 692,25	340,44	1167	2758
IJ (kcal.day^{-1})	1 577,61	262,9	1053	2510
SW (kcal.day^{-1})	1 792,04	346,36	955	3280
PS (kcal.day^{-1})	1 842,7	409,43	1075	3400

BMI: body mass index, **APACHE II:** Acute Physiology and Chronic Health Evaluation II, **SAPSII:** Simplified Acute Physiology Score II, **IC**: indirect calorimetry, HB: Harris Benedict, **SCH:** Schofield, **IJ:** Ireton –Jones, **SW:** Swinamer, **PS:** Penn-State, **Min:** minimum, **Max:** maximum, **SD:** standard deviation

Table 2. Correlation analysis between equations and IC, and between equations itself

		IC	HB	SCH	IJ	SW	PS
IC	r^2	1,00	0,526	0,598	0,540	0,834	0,805
	p	0,00	0,00	0,00	0,00	0,00	0,00
HB	r^2	0,726	1,00	0,760	0,659	0,733	0,929
	p	0,00	0,00	0,00	0,00	0,00	0,00
SCH	r^2	0,598	0,760	1,00	0,605	0,612	0,773
	p	0,00	0,00	0,00	0,00	0,00	0,00
IJ	r^2	0,540	0,659	0,605	1,00	0,602	0,658
	p	0,00	0,00	0,00	0,00	0,00	0,00
SW	r^2	0,834	0,733	0,612	0,602	1,00	0,806
	p	0,00	0,00	0,00	0,00	0,00	0,00
PS	r^2	0,805	0,929	0,773	0,658	0,806	1,00
	p	0,00	0,00	0,00	0,00	0,00	0,00

IC: indirect calorimetry, **HB:** Harris Benedict, **SCH:** Schofield, **IJ:** Ireton –Jones, **SW:** Swinamer, **PS:** Penn-State,

Table 3. Summary of limits of agreement, bias and p value for predicted energy expenditure (by equations listed), and measured energy expenditure (by IC)

	BIAS ± SD (%95 CI)	Limits of agreement (upper-lover)	p
HB	111,61±45,82 (65,79-157,43)	341,00-564,22	0,1518
SCH	136,33±54,94 (81,39- 191,27)	-406,33-678,98	**0,0002**
IJ	250,97±59,77 (191,20- 310,74)	-339,42-841,36	**<0,0001**
PS	-14,12±38,46 (-52,58 - 24,34)	-394,07-365,83	0,1583
SW	36,54±36,77 (-0,23 - 73,31)	-326,71-399,79	**<0,0001**

HB: Harris Benedict, **SCH:** Schofield, **IJ:** Ireton –Jones, **SW:** Swinamer, **PS:** Penn-State,

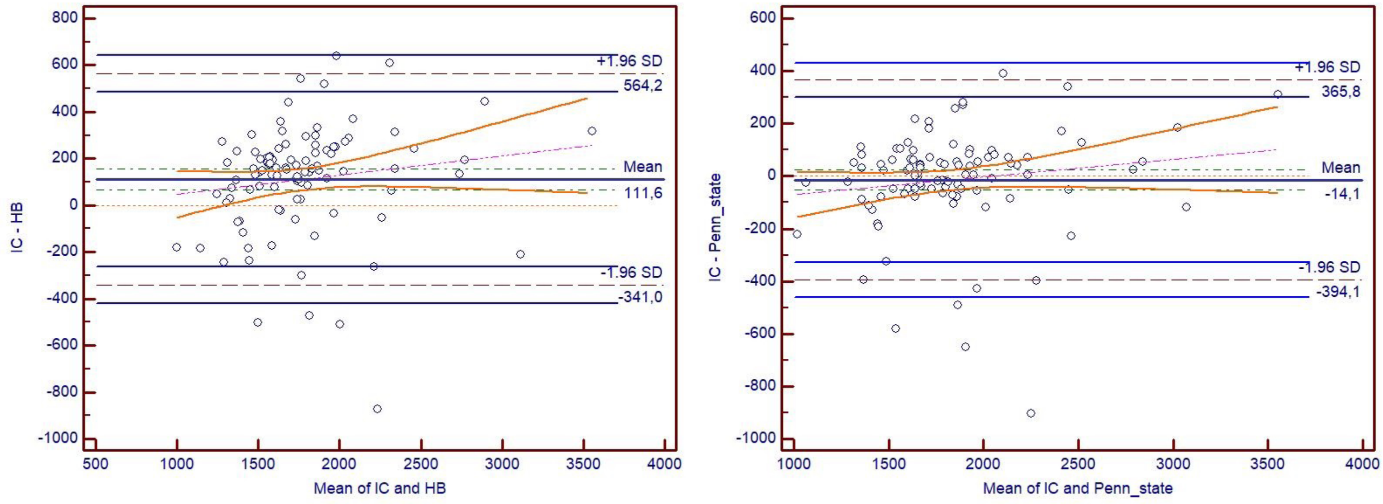

Figure 1. Bland-Altman plot for all patients using HB equation and PS equation compared with measured energy expenditure by IC
The thick straight line in the center: Bias, dotted lines above and below Bias ± SD. Dashed lines: limits of agreement. Solid lines above and below dashed lines: 95% CI for limits of agreement.

Table 4. Correlation analysis between equations and IC for subgroup patients for BMI

IC	underweight (n=12)		normal (n=45)		overweight (n=30)		obese (13)	
	r^2	p	r^2	p	r^2	p	r^2	p
HB	0,578	**0,014**	0,411	**0,000**	0,585	**0,000**	0,507	**0,002**
SCH	0,297	**0,005**	0,547	**0,000**	0,262	**0,000**	0,372	**0,008**
IJ	0,078	**0,049**	0,448	**0,000**	0,365	**0,001**	0,303	**0,040**
SW	0,548	**0,001**	0,584	**0,000**	0,612	**0,000**	0,549	**0,004**
PS	0,533	**0,002**	0,548	**0,000**	0,667	**0,000**	0,605	**0,000**

IC: indirect calorimetry, **HB:** Harris Benedict, **SCH:** Schofield, **IJ:** Ireton –Jones, **SW:** Swinamer, **PS:** Penn-State,

SW equations were found to be suitable in groups with respiratory failure (r^2 = 0.71), malignancy (r^2 = 0.69) and cardiac disease (r^2 = 0.94). In the neurological and trauma group, PS equations (r^2 = 0.96) were the highest correlations.

Mean±standard deviation for APACHE II score and SAPS II were 23,42 ± 8,47 and 42,23 ± 10,62. Measured EE was 1828, 580 ± 436, 272 kcal/day. The energy consumption measured by the IC method was not correlated with disease severity scores such as the APACHE II and SAPS II scores (p> 0.05).

Discussion

Optimal nutrition in criticallcare patients is a vital part of intensive care unit therapy in reducing morbidity and mortality (8). It is important to know that the actual EE to decide the most appropriate calorie need (9). IC is accepted like the gold standard for the assessment of the EE of patients who receive mechanical ventilation therapy (1). For patients undergoing mechanical ventilation therapy, 3 systems can be used in the IC measurement (10). The Deltatrac metabolic monitor (Datex-Ohmeda, Finland) system is the most extensive used system for IC in recent years. The other two new systems are Quark RMR (Cosmed, Rome, Italy) and CCM Express (Medgraphics Corp., St Paul, Minneapolis, USA). Previous studies comparing different systems to predict EE in patients undergoing mechanical ventilation with IC have concluded to be the Deltatrac superiority (11). In recent years, measurement of gas exchange technologies have been integrated in both mechanical ventilators and patients monitors and in our study, M-CAiOVX module was used which a new technology is belonging to the same manufacturer of Deltatrac Monitor.

Indirect calorimetry can be performed intermittently or continuously. The number of studies in which EE is continuously measured for 24 hours is very limited. Most of the study showed that measurements done in 30 minute. In clinical practice, it is not feasible to perform 24-hour measurements with an IC in each patient, so shorter measurements are being made. Most measurements of studies in the literature have been accepted to reflect the 24-hour EE for these 30-minute measurements (12,13). We made 30-minute measurements because it was more feasible to work with it. Our measured values were consistent with the literature.

Subramaniam et al. (14) compared HB and SCH equations with EE measured using the Weir equation in 60 ventilated patients (systemic inflammatory response syndrome and sepsis). Bland–

Altman analysis were done to compare the two equations. They found that measured energy expenditure was strongly correlated with HB among severe sepsis patients (r=0.9) and moderately correlated with HBE among septic shock patients (r=0.43). Correlation was found to be better in patients with severe sepsis and an APACHE 2 score below 25. They reported that, HB and SCH equations have sufficient validity for use in clinical practice. Our result also agreement with their sudy. We found that the bias was lowest with the PS predictive equation. HB and PS equations have better agreement with IC than others. Long et al (15) emphasized that variables such as fever or type of the injury or illness influence the energy expenditure of surgery patients. In another study conducted by Faisy et al. (16), predicted equations was compared with the IC measurements. The values measured with IC were found to exceed the values calculated with 25% HB, and it was emphasized that the addition of long factors significantly reduced this difference but also they reported that values obtained by adding long factors to patients with mechanical ventilator treatment were not reliable. The addition of long factors has been emphasized in numerous studies that have improved compatibility (14-16). In our study, the correlation between the predicted equation values obtained by adding long factors and the IC measurements was good.

Reid et al (7) studied the accuracy of equations used to predict energy expenditure in 192 days of measurements, in 27 critically ill patients. They compared IC and equations with Bland–Altman analysis. The HB, SCH and American College of Chest Physicians equations provided estimates within 80% and 110% of EE values (66%, 66% and 65%, respectively). They suggested none of the prediction equations were sufficiently accurate for use in critically ill patients and would have resulted in approximately 35% of patients receiving excessive or inadequate energy consumption. Agreement between the equations and measured values was poor in their study. They reported Bias±SE (95%CI) for HB, IJ, SCH and American College of Chest Physicians equations are 111±27.7 (56–166), 141±33.2 (76–207), 85±27.9 (30–140) and 183±26.5 (131–236). In another study, EE was prospectively measured by IC and calculated with the HB in 70 mechanically ventilated patients (16). They used Bland Altman analysis to measure agreement between the HB and EE. The mean bias was 73 (±502) kcal/day with limits of agreement of 932 - 1078 kcal/day. The authors reported that there is a wide limit of agreement between the two methods and that the HB equation was unreliable in estimating energy expenditure in mechanically ventilated patients. We found a range of accurate energy prediction 78%-86% except SW equation (38%). We have different specialties of patients and older patients than Reid at al (7) and Faisy et al. (16) studies. In our study, according to Bland–Altman analysis Bias±SD (95%CI) for HB, IJ and SCH 111,61±45,82 (65,79-157,43), 250,97±59,77 (191,2-310,74) and 136,33±54,94 (81,39-191,27). Since the studies conducted in the literature are usually performed in a few critical cases, the disease diagnoses cannot be analyzed by subdividing the patients into subgroups such as old, young, obese, and weak. Frankenfield et al. (17) reported a comparative study of 202 intensive care patients was one of the most extensive investigations we have found. According to their study, the PS equation is the definite equation across all subgroups. It was reliable in trauma patients (r = 0.77), in surgical patients (r = 0.66), and in medical patients (r = 0.62). The correlation rate (r = 0.67) was the same in febrile and non-febrile patients. In a retrospective analysis, De Waele et al. (18) examined the agreement between EE measured by IC and predicted by equations in mechanically ventilated critically ill patients. They used eleven different prediction equations. This study had the largest and most different sample among the studies; there was also great variability in measured EE and prediction equations they used. They concluded that ten widely used equations for calculating resting EE failed to achieve acceptable accordance with resting energy expenditure measured by IC. In a comprehensive study conducted by Maday (1) in 2013, the agreement of IJ, PS 2003, SW, Brandi, Faisy, HB and MifflinSt.Jeor equations to IC measurements in obese and non-obese patients was examined. Patients were grouped as young obese, young non-obese, old obese and old nonobese. According to this study, in the whole population, the high accuracy of Penn State 2003 was found in young obese and elderly non-obese patients. In our study PS 2003 equation was used. SW equations were found to be suitable in groups with respiratory failure (r^2 = 0.71), malignancy (r^2 = 0.69) and cardiac disease (r^2 = 0.94). In the neurological and trauma group, PS equations (r^2 = 0.96) were the highest correlations. We may suggest SW or PS equations for accurate assessment of metabolic rate in critically ill patients if IC is not available. Best prediction among the equations was 86% with the SCH whereas HB and PS predicted accuracy in 83% while IJ predicted accuracy in 78% for the study patients with BlandAltman limits of agreement analysis.

IC measurements of patients receiving mechanical ventilator therapy were not affected by mechanical ventilator modes (19). In our study, we performed measurements using the most comfortable mechanical ventilation modes of the patients who were fit. Flancbaum et al. (3) reported that the difference in baseline EE might be due to disease severity. However, Brandi et al. (20) did not found any correlation between disease severity score and the basal EE measured by IC. In our study, in agreement with the Brandi et al study (20) no correlation was found between the APACHE II, SAPS II scores and the IC masurement.

There are some limitations of our study. The study was conducted on a heterogeneous group of patients and that the patient group consisted predominantly of elderly patients. The study is rather small to profound detailed subgroup analysis. An additional limitation is that several patient populations were not shown in the current study, spinal cord injury with quadriplegia or paraplegia, subgroups of children, burn injury, and penetrating trauma. The clinical benefit of these results should also be demonstrated.

In conclusion, in the intensive care patients who need mechanical ventilation, predictive equations are not reliable in determining EE; the confidence intervals are very high and can lead to inadequate feeding or overfeeding. In critically ill mechanically ventilated patients, there is a need for larger scale studies of which formula should be used in which group of patients in the absence of IC.

AUTHOR CONTRIBUTIONS:

Concept: HS, SK; **Design:** HS, SK; **Supervision:** HS, SK, SS; BB; **Resources:** HS, SK, SS; **Materials:** HS, SK, SS; **Data Collection and/or Processing:** SK; **Analysis and/or Interpretation:** HS, SK; **Literature Search:** SK; **Writing Manuscript:** HS

YAZAR KATKILARI:

Fikir: HS, SK; **Tasarım:** HS, SK; **Denetleme:** HS, SK, SS; **Kaynaklar:** HS, SK, SS; **Malzemeler:** HS, SK, SS; **Veri Toplanması ve/veya İşlemesi:** SK; **Analiz ve/veya Yorum:** HS, SK; **Literatür Taraması:** SK; **Yazıyı Yazan:** HS

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Pamukkale University, Faculty of Medicine (Approval Date: 2013 / Session No: x/x, Decision No: x).

Informed Consent: Written informed consent was obtained from relatives of patients or patients who participated in this study.

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