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## Original Article

# Low accuracy of predictive equations for resting metabolic rate in overweight women after weight loss

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## SUMMARY

**Background & Aims:** The better equation to predict resting metabolic rate (pRMR) to use for overweight individuals mainly after weight loss is unclear. This study aimed to identify the best equation to pRMR in overweight adult women before and after a nutritional intervention aiming to lose weight.

**Methods:** Thirty overweight adult women were enrolled in this study. Subjects underwent 6-month energy-restricted diet intervention. Anthropometrics and body composition measures were evaluated. Nine equations that are widely used to pRMR were performed based on anthropometric and body composition parameters. Measured RMR (mRMR) was obtained by indirect calorimetry. A new equation to predict resting metabolic rate (npRMR) was also developed by multiple regression analysis based on anthropometric and body composition variables. The validity of the equations was investigated through comparisons, accuracy, and agreement tests.

**Results:** Before the nutrition intervention, only the Mifflin equation was similar to mRMR, with a mean difference of 12kcal and 83% of accuracy. The mean weight loss was 4.2% after 6 months. Following weight loss, only the Owen equation was similar to mRMR, with a

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mean difference of –33 kcal. However, this equation showed low accuracy (63%). All the others equations showed reduction of accuracy, increase of bias and overestimation of RMR. A npRMR was calculated, and this showed a lower mean difference to mRMR with 70% of accuracy.

**Conclusions:** Out of the nine resting metabolic rate equations evaluated only the Mifflin equation was similar to mRMR before intervention. Interestingly, after weight loss none of the assessed equations were reliable to pRMR. Based on that we propose a new equation that showed greater accuracy and lower mean difference when compared with mRMR.

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## 1. Introduction

Overweight and obesity are global health public problems defined as abnormal or excessive fat accumulation, being important risk factors for chronic diseases [1]. Indeed, excessive body fat represents a major threat to the health of people due to the associated increase in the incidence of medical co-morbidities, such as diabetes, cardiovascular diseases and cancer [1,2]. The world prevalence of adult women obesity has increased over the last decades, rising from 22.7% in the 1970's to 39.0% in 2016 [2]. Conversely, intervention programs for weight loss reach on average 20% of success rate, defined as the loss of 5 to 10% of the initial weight, for at least one year [3–5].

Any nutritional intervention program based on a dietary planning requires the knowledge of individual energy requirements, which depends on accurate methods for the calculation of total energy expenditure (TEE) [6]. In this regard, to know precisely the resting metabolic rate (RMR) is fundamental since it is the major component of TEE [7]. However, indirect calorimetry (IC), the gold standard technique that more accurately evaluates the RMR, is an expensive method requiring a complex protocol and trained staff to handle it, hampering its wide use in the clinical practice [8,9].

In this context, many equations were developed to predict RMR (pRMR) in distinct populations with different sex, nutritional status, presence of medical conditions, age or body composition [10]. While the limitations of predictive equations are clear, their use is still common in clinical practice. Indeed, in 2019, a Scientific Symposium was organized by the European Society for Clinical Nutrition and Metabolism (ESPEN) to commemorate the centenary of the publication of Harris and Benedict's equations for estimating energy expenditure, highlighting the progress of energy expenditure assessment measures and what is currently known about these [11].

Therefore, to select a single formula of pRMR that can be widely used is a challenge. Furthermore, the estimate of TEE based on anyone of these formulas may not be accurately, which consequently will influence the weight loss treatment and the achievement of the expected goals [12,13]. Previous studies evaluating equations to estimate RMR after weight loss in overweight individuals [12,14] showed low accuracy values and absence of a precise formula to assess RMR after weight loss.

The objective of the current study was twofold: (i) to analyze the validity of predictive formulas to estimate the resting metabolic rate in overweight women before and after weight loss; (ii) to develop a predictive equation to estimate RMR in overweight adult women after weight loss.

## 2. Materials and methods

This cross-sectional study conducted in Hospital das Clínicas at the Universidade Federal de Minas Gerais (UFMG) from January 2016 to March 2018. This research was approved by the Ethics Committee

at the UFMG (CAAE: 30409114.8.0000.5149). All volunteers signed the informed consent prior the beginning of the research.

2.1. Subjects

Overweight and obese women (body mass index – BMI  $\geq 25 \text{ kg/m}^2$  and  $< 35 \text{ kg/m}^2$ ) aged between 18 to 59 years old were recruited via online advertisements. Exclusion criteria were: (i) any chronic health conditions (e.g. diabetes, hypertension, chronic renal failure, heart and liver diseases, endocrine diseases); (ii) previous surgery for weight loss; (iii) medications known to affect TEE (total energy expenditure); (iv) pregnancy and breastfeeding; (v) smoking and (vi) alcohol abuse (>2 doses/day).

The sample size was estimated in 27 participants to detect a difference in resting metabolic rate of 221 kcal after 5% of weight loss, with standard deviation of the change of 169 kcal [12], considering the confidence level of 95.0% and statistical power of the 90.0% test [15].

2.2. Study procedures

After 12 h of fasting, volunteers were assessed in the outpatient clinic where body composition and anthropometric parameters were assessed. The analyses included body weight, height and waist circumference (WC), bioelectrical impedance and indirect calorimetry (IC).

Weight and height were obtained by a mechanical balance (Filizola®), with a stadiometer coupled. Participants used light clothes without shoes. This measure was used to calculate BMI (weight/height<sup>2</sup>). WC was measured in the smallest abdominal circumference [16].

Body composition was measured by bioelectrical impedance, using the device Quantum X® (RJL Systems), after 12 hours of fasting. Resistance and reactance measurements were used to determine fat-free mass (FFM) and fat mass (FM) in kilogram and percentage, with the device formula. The test was performed in a comfortable and calm room. The patients were instructed to: (i) do not exercise at least 8 hours previously; (ii) do not drink alcohol in the previous 12 hours and (iii) remain lie quietly during the entire test.

RMR was defined by indirect calorimetry using the QUARK-RMR® device (COSMED, Rome, Italy) as validated by previous studies [17,18]. The volunteers were instructed to fast for 12 hours and to refrain from drinking coffee or black tea and exercising 24h before assessment. The VO<sub>2</sub> and VCO<sub>2</sub> were measured during 15 minutes with the participants lying down and awoken, after a 30-minute resting period in the supine position. RMR was calculated by the Weir’s equation: [(3.941xVO<sub>2</sub>) + (1.106xVCO<sub>2</sub>) x 1440] [19].

Measured RMR (mRMR) was compared to predicted RMR (pRMR) considering equations shown in Table 1. These equations were selected by screening previous publications based on their frequency of use and clinical relevance [8,10,20–27]. These equations rely on weight, height and age parameters, and one of them also includes body composition data [13]. The actual body weight was used in the calculations of all the equations.

**Table 1**  
Equations used to predict resting metabolic rate in overweight adult women.

Mifflin (1990)	$9.99 \times \text{weight} + 6.25 \times \text{height} - 4.92 \times \text{age} - 161$
Harris-Benedict (1918)	$665 + 9.56 \times \text{weight} + 1.84 \times \text{height} - 4.67 \times \text{age}$
Owen (1986)	$795 + 7.18 \times \text{weight}$
WHO/FAO/UNU (1985)	$8.7 \times \text{weight} + 829$
WHO/FAO/UNU (wt+ht) (1985)	$8.7 \times \text{weight} + (25 \times \text{height}^2) + 865$
Liu (1985)	$(13.88 \times \text{weight}) + (4.16 \times \text{height}) - (3.43 \times \text{age}) - 112.4 + 54.34$
Müller (2004)	$(0.08961 \times \text{FFM} + 0.05662 \times \text{FM} + 0.667) \times 238.84$
Schofield (1985)	$14.8 \times \text{weight} + 487$
Schofield (wt + ht) (1985)	$13.6 \times \text{weight} + 283 \times \text{height}^2 + 98$

wt + ht = weight + height; FFM = fat free mass; FM = fat mass.

### 2.3. Nutritional intervention

Overweight volunteers were followed up during six months. Nutritional intervention included energy-restricted diet and evaluation of anthropometric and body composition measures. The diet plan was based on the total energy expenditure obtained by the formula: mRMR (obtained by indirect calorimetry) x MET Compendium + 10% of Thermal Effect of Food [4]. Restriction of 513 to 770 kcal was proposed to favor a loss of 2 to 4 kg per month [4]. Macronutrient intake ranges were 55–65% carbohydrate, 20–25% protein and 25–30% lipid as recommended by the Dietary Reference Intake [28].

### 2.4. Statistical analyses

Data were organized in the Microsoft Office Excel version 2007 and analyzed using the software Statistical Package for the Social Sciences® (SPSS) version 19.0 and Stata® version 11.2. After consistency analysis, normality of the quantitative variables was tested by the Kolmogorov-Smirnov test. Estimates of frequency (absolute and relative), central tendency (means and medians) and dispersion (standard deviation, minimum and maximum values) were performed. In addition, Pearson or Spearman correlations and Wilcoxon test or paired samples t-test were applied.

Wilcoxon test or paired samples t-test were used to assess differences between the mRMR and pRMR by equations for non-parametric and parametric variables, respectively. The 95% confidence interval (95% CI) of the difference between pRMR and mRMR was calculated. Predictions between 90% and 110% of mRMR were considered accurate measurements at the individual level. Predictive values less than 90% or more than 110% of mRMR were classified as underestimates and overestimates, respectively [10,29–31]. The mean percentage difference between pRMR and mRMR (bias) was the measurement of accuracy at the group level [9,31]. Bland Altman analysis for all the equations vs indirect calorimetry were used before and after weight loss.

The new equation to predict RMR (npRMR) was developed by multiple linear regression, with the mRMR obtained by indirect calorimetry as reference. The variables with value of  $p \leq 0.20$ , obtained in the bivariate analysis, were tested in the model. The backward method was used. The significance of the final model was evaluated by the F test of the analysis of variance and the quality of the fit by the coefficient of determination ( $R^2$ ). The residues were evaluated according to the assumptions of normality, homoscedasticity, linearity and independence. In addition, the verification of multicollinearity between the variables included in the model was performed.

## 3. Results

Anthropometric measures before and after the intervention of 30 overweight women are shown in Table 2. The mean age was  $43 \pm 1.7$  years old. The percentage of overweight was 33,3% and 66,7% of

**Table 2**  
Characteristics of a cohort of 30 Brazilian overweight women before and after the weight loss intervention program.

Variable	Before intervention	After intervention	P-value
	Mean ± SD	Mean ± SD	
Weight (kg)	79.9 ± 8.0	76.1 ± 7.2	<0.001 <sup>a</sup>
BMI (kg/m <sup>2</sup> )	31.1 ± 2.7	29.5 ± 2.5	<0.001 <sup>a</sup>
WC (cm)	89.5 ± 5.7	86.2 ± 5.4	<0.001 <sup>b</sup>
FFM (kg)	46.1 ± 5.1	44.7 ± 4.2	<0.001 <sup>b</sup>
FFM (%)	57.7 ± 3.1	58.9 ± 2.9	0.012 <sup>a</sup>
FM (Kg)	33.8 ± 4.3	31.3 ± 4.1	<0.001 <sup>a</sup>
FM (%)	42.3 ± 3.1	41.0 ± 2.9	0.012 <sup>a</sup>
mRMR (kcal)	1,422.9 ± 163.3	1,300.0 ± 135.0	<0.001 <sup>b</sup>

BMI = Body Mass Index; WC = Waist Circumference; mRMR = measured Resting Metabolic Rate; FFM = Fat Free Mass; FM = Fat Mass.

<sup>a</sup> Paired Sample T-test.

<sup>b</sup> Wilcoxon test.

obesity. All anthropometric variables reduced after six months of dietary treatment including reduction of  $4.2 \pm 1.1\%$  in body weight,  $3.6 \pm 2.9\%$  reduction in waist circumference and  $1.3 \pm 1.2\%$  reduction in free fat mass. After the intervention, the measured RMR also decreased 8.6%, totaling an average reduction of  $122.9 \pm 29.1$  kcal.

Tables 3 and 4 shows the measured resting metabolic rate (mRMR) and the results of the nine equations to predict metabolic rate (pRMR) in overweight women before and after the nutritional intervention. Before the intervention, the Mifflin was the only equation that had similarity with the measured RMR (Table 3). All the other equations did not have a good accuracy and, apart from the Owen equation, overestimated the measured RMR in a range from 47% to 97%.

After weight loss associated with dietary intervention, only the Owen equation was good to estimate RMR (Table 4). Even the Mifflin equation, that had a good accuracy to predict RMR before intervention, did not have a good accuracy when the women lost weight, with the accuracy dropping from 83% to 57%. Although the Owen equation was similar to mRMR after the intervention, its individual accuracy was less than 70%. All the other equations presented with even lower values of accuracy, also overestimating the RMR in a range from 43 to 100%.

Fig. 1 shows the Bland Altman analyses between predictive equations and indirect calorimetry before and after weight loss. It is clear that the measures do not agree, showing statistically significant bias in all analyzes, except for Mifflin equation before weight loss ( $p = 0.234$ ) and Owen after weight loss ( $p = 0.312$ ).

In this context, it is important to propose new equations to estimate RMR after weight loss. Accordingly, we suggest a new predicting equation to be used after weight loss. For this purpose, we considered variables that were positively correlated with mRMR: baseline weight ( $p = 0.024$ ), final weight ( $p = 0.059$ ), BMI ( $p = 0.049$ ), WC ( $p = 0.004$ ) and FFMkg ( $p = 0.015$ ). In the final model, the variables BMI, WC and FFMkg remained, explaining 35.2% of the RMR value variability (Fig. 1). This new equation (Table 5) showed higher accuracy (70%), and also a smaller difference to the mRMR ( $-0.0004$  kcal,  $\pm 108.6$  kcal) in comparison with the other equations after weight loss (Table 4).

#### 4. Discussion

Obesity is a pandemic health problem. One of the biggest challenges during obesity treatment is the maintenance of weight loss. Besides that, with weight loss, the estimation of energy requirement

**Table 3**

Comparison between the predicted and measured resting metabolic rate for a cohort of 30 Brazilian overweight women before intervention.

Overweight women before intervention	Kcal/day	P <sup>a</sup>	Difference			Prediction		
			Kcal/d	95% CI	Bias <sup>b</sup> (%)	Accuracy <sup>c</sup> % (n)	Under estimation % (n)	Over estimation % (n)
Measured RMR	1,422.9 ± 163.3	-	-	-	-	-	-	-
Mifflin	1,429.4 ± 100.7	0.262	-6.5 ± 131.4	-55.5; 42.6	-1	83 (25)	7 (2)	10 (3)
Harris-Benedict	1,524.2 ± 83.8	<0.001	-101.2 ± 133.9	-151.2; -51.2	-8	50 (15)	3 (1)	47 (14)
Owen	1,369.3 ± 57.8	0.041	53.7 ± 130.2	5.0; 102.3	3	80 (24)	17 (5)	3 (1)
WHO/FAO/UNU	1,529.1 ± 72.1	<0.001	-106.2 ± 127.6	-153.9; -58.5	-8	47 (14)	6 (2)	47 (14)
WHO/FAO/UNU (wt+ht)	1,524.1 ± 70.4	<0.001	-101.2 ± 127.2	-148.7; -53.7	-8	47 (14)	6 (2)	47 (14)
Liu	1,684.6 ± 120.7	<0.001	-261.6 ± 123.2	-307.7; -215.6	-19	13 (4)	-	87 (26)
Muller	1,603.4 ± 146.2	<0.001	-180.4 ± 117.2	-224.1; -136.6	-13	37 (11)	-	63 (19)
Schofield	1,670.8 ± 119.1	<0.001	-247.8 ± 118.3	-291.9; -203.7	-18	20 (6)	-	80 (24)
Schofield (wt+ht)	1,915.2 ± 136.8	<0.001	-492.2 ± 120.2	-537.1; -447.3	-35	3 (1)	-	97 (29)

WT: weight; HT: height; CI: confidence interval; WHO/FAO/UNU: World Health Organization/Food and Agriculture Organization of The United Nations/United Nations University.

<sup>a</sup> Wilcoxon test.

<sup>b</sup> The mean percentage difference between pRMR and mRMR (group level).

<sup>c</sup> Predictions between 90% and 110% of mRMR (individual level).

**Table 4**

Comparison between the predicted and measured resting metabolic rate for a cohort of 30 Brazilian overweight women after intervention.

Overweight women after intervention	kcal/day	P <sup>a</sup>	Difference			Prediction		
			Kcal/d	95% CI	Bias <sup>b</sup> (%)	Accuracy <sup>c</sup> % (n)	Under estimation % (n)	Over estimation % (n)
Measured RMR	1,300.0 ± 135.0		—	—	—	—	—	—
Mifflin	1,390.7 ± 92.9	<b>0.001</b>	-90.7 ± 130.9	-139.6; -41.7	-8	57 (17)	—	43 (13)
Harris-Benedict	1,487.1 ± 76.5	<b>&lt;0.001</b>	-187.1 ± 123.1	-233.0; -141.1	-15	37 (11)	—	63 (19)
Owen	1,341.5 ± 52.2	0.084	-41.4 ± 126.6	-88.7; 5.8	-4	67 (20)	3 (1)	30 (9)
WHO/FAO/UNU	1,491.2 ± 63.3	<b>&lt;0.001</b>	-191.1 ± 127.6	-238.7; -143.4	-16	40 (12)	—	60 (18)
WHO/FAO/UNU (wt + ht)	1,487.0 ± 62.7	<b>&lt;0.001</b>	-187.0 ± 127.4	-234.6; -139.5	-15	43 (13)	—	57 (17)
Liu	1,630.7 ± 109.2	<b>&lt;0.001</b>	-330.7 ± 136.9	-381.8; -279.6	-26	10 (3)	—	90 (27)
Muller	1,538.2 ± 129.7	<b>&lt;0.001</b>	-238.2 ± 150.1	-294.3; -182.1	-19	30 (9)	—	70 (21)
Schofield	1,613.4 ± 107.6	<b>&lt;0.001</b>	-313.4 ± 140.3	-365.8; -261.0	-25	7 (2)	—	93 (28)
Schofield (wt + ht)	1,862.3 ± 125.8	<b>&lt;0.001</b>	-562.3 ± 155.7	-620.4; -504.1	-44	—	—	100 (30)
npRMR	1,300.0 ± 80.1	1,000	-0.0004 ± 108.6	-40.6; 40.6	0.68	70 (21)	13 (4)	17 (5)

WT: weight; HT: height; CI: confidence interval; npRMR: new equation to predict RMR; WHO/FAO/UNU: World Health Organization/Food and Agriculture Organization of The United Nations/United Nations University.

<sup>a</sup> Paired Sample T-test.

<sup>b</sup> The mean percentage difference between pRMR and mRMR (group level).

<sup>c</sup> Predictions between 90% and 110% of mRMR (individual level).

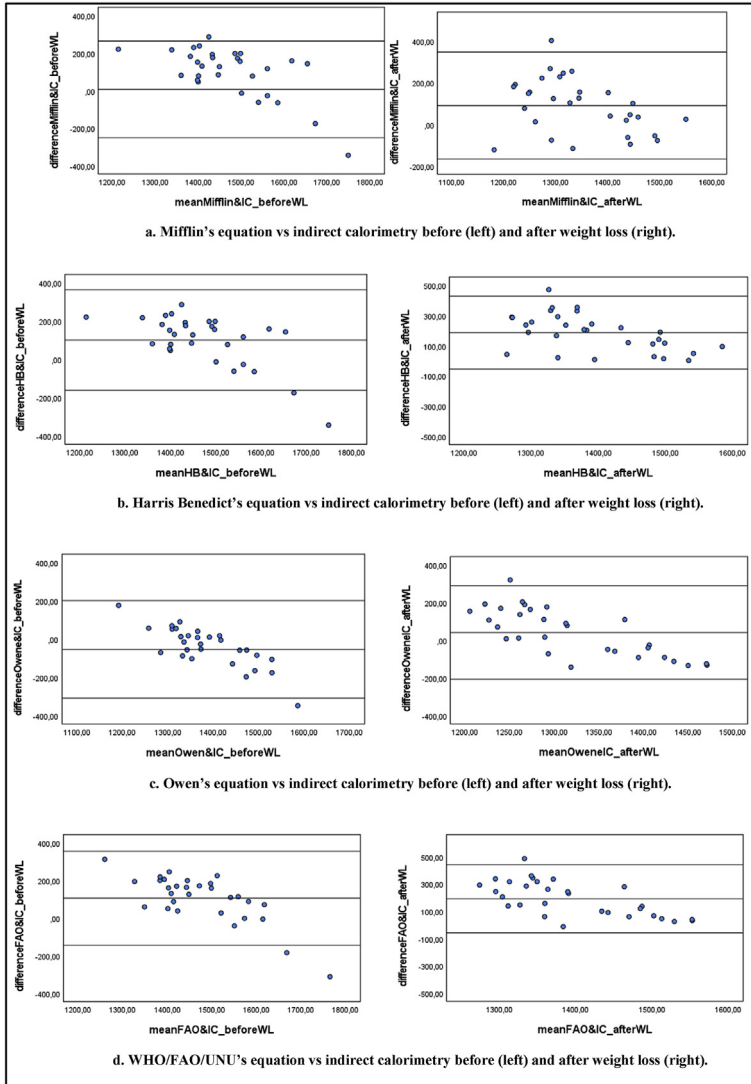
changes, and it has been complex to accurately establish this value. Actually, predictive equations of RMR are reliable in obese individuals, but it is debatable the best predictive equation for RMR estimation after weight loss [10,11].

Among the nine equations evaluated in this study, only the Mifflin equation was similar to mRMR in overweight women at baseline, i.e. before intervention. After weight lost, not even the Mifflin equation remained comparable to mRMR, presenting a considerable reduction of accuracy. These findings are clinically relevant, and indicate that the validity of the RMR predictive equations vary according to changes in the body weight and body composition. In addition, these data suggest the need to identify better estimates and/or equations to predict RMR in women undergoing an energy-restricted diet intervention in order to maintain weight loss or promote weight stabilization over time.

Before intervention, two equations presented individual accuracy greater than 70% ('ideal' value) [10] and after weight loss none of the equations presented individual accuracy above this value. In line with these observations, Weijs et al. (2010) and Krüger et al. (2015) found a reduction in the accuracy of the equations as the BMI reduces, while Siervo et al. (2003) pointed out that weight loss is responsible to increase the overestimation of the predictive resting metabolic rate equations [12,30,32].

Previous studies showed that the Mifflin equation was accurate in obese subjects before weight loss, although it did not reach predictions of 80% in most of them [10,29,33]. The Mifflin equation was adequate in overweight and obese Belgian women, but was less precise for Dutch obese subjects [29,30]. A systematic review by Frankenfield et al. (2005) reported that the Mifflin equation provides the best prediction of RMR for obese individuals compared to other equations [10]. More recently, Krüger et al. (2015) showed that RMR predictive equations overestimated RMR in overweight women, except the Mifflin equation, when compared with mRMR [32]. Despite the above mentioned evidence, there is no consensus regarding the use of a preferential RMR equation for obese individuals, and the conclusion may change according to BMI subgroups and analysis methods [29,33]. Therefore, caution must be taken when applying predictive equations for RMR in overweight and obese individuals.

After the intervention, there was a decrease in mRMR, similar as seen in the literature [14,34]. Concomitant with the mRMR reduction, the pRMR obtained by the Mifflin equation was no longer similar to the mRMR. The equation that showed similar results with the mRMR was the Owen equation, but with low accuracy. Actually, there was a reduction of accuracy, an increase of bias and overestimation of the RMR with all the equations after weight loss. Ruiz et al. (2011) investigated the



**Fig. 1.** Bland Altman analysis for all the predictive equations evaluated vs indirect calorimetry, before and after weight loss. a. Mifflin's equation vs indirect calorimetry before (left) and after weight loss (right). b. Harris Benedict's equation vs indirect calorimetry before (left) and after weight loss (right). c. Owen's equation vs indirect calorimetry before (left) and after weight loss (right). d. WHO/FAO/UNU's equation vs indirect calorimetry before (left) and after weight loss (right). e. WHO/FAO/UNU's equation (weight + height) vs indirect calorimetry before (left) and after weight loss (right). f. Liu's equation vs indirect calorimetry before (left) and after weight loss (right). g. Muller's equation vs indirect calorimetry before (left) and after weight loss (right). h. Schofield's equation vs indirect calorimetry before (left) and after weight loss (right). i. Schofield's equation (weight + height) vs indirect calorimetry before (left) and after weight loss (right).

validity of RMR predictive equations in obese Caucasian pre-menopausal women before and after interventions for weight loss. Similar to our results, the equation of Mifflin had the highest accuracy at baseline, but, this level of accuracy was lost after a 12-week energy-restricted diet. In addition, the lowest bias and larger accuracy was found with the Owen equation after the intervention [14].

The reduction in the accuracy of equations after weight loss in overweight subjects was also verified for Siervo et al. (2003) in young Caucasian women [12]. Poli et al. (2016), studying Brazilian obese women

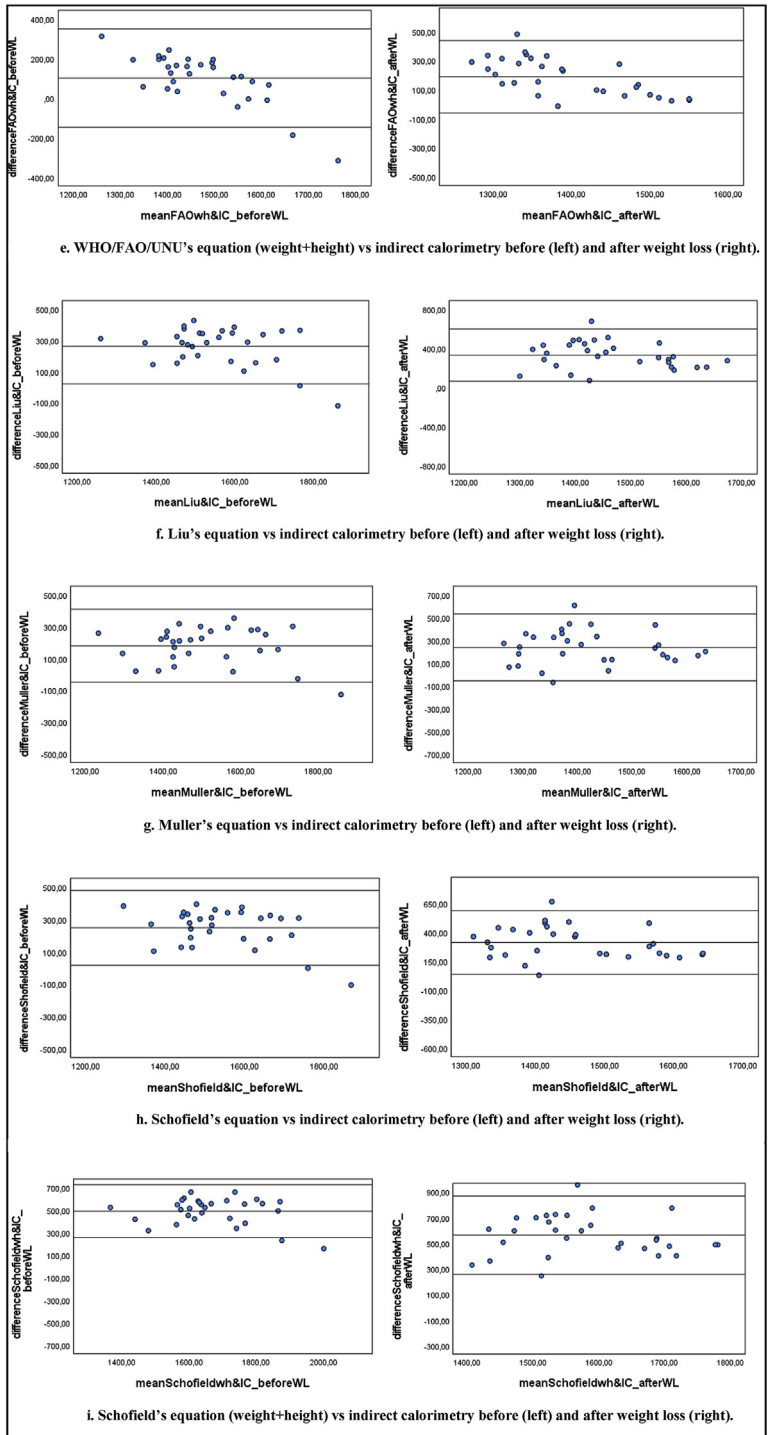


Fig. 1. (continued).



**Table 5**

Proposed equation for calculating RMR of overweight women after weight loss.

$$\text{npRMR (kcal)} = 1,064.69 + (24.18 \times \text{BMI}) - (12.81 \times \text{WC}) + (13.96 \times \text{FFMkg})$$

BMI: Body Mass Index ( $\text{kg}/\text{m}^2$ ); WC: Waist circumference (cm); FFMkg: Fat Free Mass, kilogram.Backward method:  $\beta_0$ :1,064.69;  $R^2$ : 0.3524;  $p$ :0.001.

engaged in an interdisciplinary approach to promote an active lifestyle, healthy nutritional habits and weight loss [34]. The authors showed a wide variation in the accuracy of the pRMR before and after weight loss. Conversely, no significant differences between mRMR e pRMR were reported by Harris–Benedict and FAO/WHO/United Nations University (UNU) with a bias <5% before and after the therapy [35,36]. The accuracy of these equations increased after weight loss, however with accuracy < 50% [34].

The new equation developed in this study had the highest accuracy (70%) among all predictive equations evaluated after weight loss. It also had the lowest value of overestimation (17%) comparing with the other equations. The overestimation of RMR may affect weight loss and contribute to withdrawal of nutritional interventional [10,37,38].

Oliveira et al. (2018) also proposed a new equation to predict RMR for women with class III obesity and found that weight, height, age, abdominal circumference, FFM and FM were positively correlated with RMR, but did not evaluate the similarity after weight loss [39]. The new equation proposed in our study included only 3 variables: BMI, WC and FFM (kg), being more useful in clinical practice.

Our study has some limitations. We studied only women, however we chose it to avoid the sex effect on RMR. We used bioelectrical impedance instead of dual energy X-ray absorptiometry to define body composition. Bioelectrical impedance allows reliable results and is more useful in clinical practice. On the other hand, the strength of the study includes duration of follow-up, over than other previously researches.

## 5. Conclusion

In conclusion, our data indicate low accuracy of RMR prediction equations after overweight women lose weight. Herein we highlight the real necessity to develop new formulas to accurately predict the RMR during weight loss treatment. In this sense, we propose an alternative predictive equation for overweight and obese class I women that are losing weight. Our research group is developing other study to validate the new equation proposed.

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## Author contributions

A.V.M.F. designed the research; N.M.M., A.M.S.R., G.B.P.F and B.L.M conducted research; N.M.M, M.I.T.D.C, L.C.S. and A.V.M.F analyzed data; N.M.M., A.M.S.R., L.C.S., A.L.T. and A.V.M.F wrote the paper; A.L.T. and M.I.T.D.C provided essential materials; A.V.M.F. had primary responsibility for the final content. All authors read and approved the final manuscript.

## Declaration of competing interest

No conflict to report.

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