SPC 100x - -Sample S1 S2 S



Figure 1 Figure indicates the electrophoretic mobility shift assay (EMSA) band specificity testing. The p65/p50 DNA binding activity from nuclear extracts of different samples is shown (lanes 1 and 2). In the competitive assay, 100× specific cold competitor (SPC) was added to the reaction mixture (lane 3).

of weight loss in order to determine the utility of peripheral mononuclear cells for future human biomarker studies in obesity research.

DISCLOSURE

The authors declared no conflict of interest.

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Predictive Equations for Body Fat in Asian Indians

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TO THE EDITOR: We read with interest the article by Goel et al., titled "Predictive Equations for Body Fat and Abdominal Fat With DXA and MRI as Reference in Asian Indians," which offers predictive equations for body fat and abdominal fat in Asian Indians as functions of simple anthropometric measures in 171 apparently healthy North Indian respondents to a local advertisement (1). We noticed a possible error in the recommended equation for body fat percent. Correspondence with authors gave us the correct equations which we used for further analysis (%Body Fat = 42.42 + 0.003 × age (years) + 7.04 × gender $(M = 1, F = 2) + 0.42 \times \text{triceps skinfold (mm)}$ + 0.29 × waist circumference (cm) + 0.22 × weight (kg) $- 0.42 \times \text{height (cm)}$).

We tested Goel's recommended equation to calculate body fat percent in subjects of the Pune Maternal Nutrition Study (PMNS), a community-based study in six villages near Pune, Maharashtra, India (2). Detailed anthropometric measurements and body fat measurements (dual-energy X-ray absorptiometry (DXA), Lunar DPX-IQ) were available in 645 men (mean age 34 years, triceps skinfold 8.7 mm, waist circumference 80.2 cm, weight 57.1 kg, height 165.7 cm) and 681 women (27 years, triceps skinfold 9.7 mm, waist circumference 65.9 cm, weight 44.5 kg, height 152.9 cm), with a mean body fat percent of 17.7% (s.d. 8.44) and 25.6% (8.13), respectively. Mean error (predicted—DXA) for fathers (1.71 percentage points) was

PERSPECTIVES LETTERS TO THE EDITOR

significantly different from zero and also from mean error for mothers (-0.23% points) (P < 0.001 for both). Importantly, the Bland-Altman method indicates that Goel's equation produced a systematic bias in prediction of body fat percent such that there was overestimation at lower values and underestimation at higher values. Moreover, the bias for men and women were different (estimated by linear regression; slope for men -0.33 (99% CI -0.27, -0.39) and for women -0.48 (-0.42, -0.55); intercept for men 7.80 (6.56, 9.03) and for women 12.04 (10.30, 13.79)). When used as a classifier, the equation becomes progressively less sensitive at higher cut points of adiposity (Table 1). The equation fares poorly in children (at 6 years of age $R^2 = 0.15$, at 12 years $R^2 = 0.48$ with considerably stronger biases compared to adults).

Although statistical models are an attractive and potentially useful surrogate for measurements that are difficult to make, most statistical models fail to capture the complex relationships that exist between the dependent and predictor variables. This often leads to biases which vary across populations, i.e., populations differing because of gender (see Figure 1), race, lifestyle (rural-urban) etc. The relationship between anthropometric measures (BMI and waist circumference) and adiposity (body fat percent) is known to vary between populations of different ethnicities (3). Importantly, our analysis shows that this is also true of different populations from the same ethnic group. Our sample consists exclusively of rural Indians while Goel's presumably contains mostly urban volunteers. There is also a substantial difference in the body fat percent between PMNS subjects and Goel's volunteers. All of these factors (along with many unmeasured/ unknown factors) may contribute to the biases that Goel's equation produces.

Table 1 Sensitivity and specificity of Goel's equation when used as a classifier (for %Body Fat) on PMNS adults

	Males		Females	
	Sensitivity	Specificity	Sensitivity	Specificity
>25%	0.71	0.97	0.77	0.85
>30%	0.47	0.98	0.57	0.99
>35%	0.27	0.99	0.33	1

PERSPECTIVES LETTERS TO THE EDITOR



Figure 1 Bland–Altman plot showing limits of agreement between Goel's recommended equation for body fat percent (%BFG) and body fat percent measured by DXA (%BFDXA) in PMNS males (filled circles, solid line) and females (open circles, dashed line).

The arbitrary nature of statistical models coupled with the large number of predictor variables that are usually used, makes interpreting the model and its bias(es) difficult. An alternative is to derive population specific equations. Choosing population categories and deciding the number of categories is not always easy and practicality might dictate that we accept a degree of inaccuracy/error. However, a systematic bias might lead to misleading conclusions; for example it might reduce the adiposity difference between rural and urban subjects.

We believe that models based on known physiological or physical relationships between variables will fare better because such relationships should be invariant across populations.

DISCLOSURE

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Response to "Predictive Equations for Body Fat in Asian Indians"

Kashish Goel¹, Nidhi Gupta², Anoop Misra³, Pawan Poddar⁴, Ravindra M. Pandey⁵, Naval K. Vikram⁴ and Jasjeet S. Wasir³

TO THE EDITOR: Yajnik et al. in their letter entitled "Predictive Equations for Body Fat in Asian Indians" (1) used our proposed equation (2), which was derived from simple anthropometric measures, using the multiple linear regression model in north Indian urban population. They estimated percentage body fat (%BF) using this equation, in west Indian rural and semiurban population and reported a statistically significant difference in the values obtained. The use of predictive equation primarily requires that the populations in which the equation was developed and in which it has to be applied be similar. Our equation was developed in urban subjects in north India and Yajnik's data, on which the equation was tested came from rural and semiurban population from west India. Such differentials in almost all demographic, anthropometric, clinical, and other health outcomes are well known depending on the region and socioeconomic stratum. Ethnicity is not the only characteristic that one should look at while using a predictive

equation. Second, the concept of statistical significance is used in the situation of hypothesis testing and not in method comparison. In literature, it has been reported that using statistical testing and calculating correlation coefficient in method comparison situation are the two most common misuses of statistical methods. Comparing predicted and measured values of %BF is not a hypothesis testing situation but a method comparison situation. Therefore, commenting on the differences in predicted and measured %BF value using *P* value is incorrect.

Bias and random error are two different terms with distinct meanings. The difference between predicted and measured values of %BF should be seen as a random error and not as "bias" as mentioned repeatedly by Yajnik et al. Entire literature in epidemiology defines bias as a systematic error. Also, it is not possible to measure bias in a given study. Therefore, quantifying the "so called bias" using the equation is incorrect. Bias can never be eliminated, it can only be minimized during the conduct of the study, and no statistical methods have been developed so far which can quantify and adjust the outcome variable (predicted %BF in this case) for bias. None of the statistical models are expected to predict the exact values of the outcome variable for every subject. A model is labeled as good, if the residual term (i.e., the difference in predicted and measured values) is on an average minimum. The residual can never be zero for the simple reason that, no model can identify and include all the measurable and immeasurable predictors. This is the basis of all statistical models including the linear regression based models which we have used.

Yajnik *et al.* also commented that the bias in %BF values is different in men and women, based on the intercept value (i.e., constant term in multiple linear regression equation). We disagree with this conclusion. In any multiple linear regression model, the term intercept is uninterruptible. It is only to be used (along with other terms) to find the predicted value. The use of intercept term (95% confidence interval) to conclude that the predicted values of %BF are biased is incorrect. Two regression lines may have different intercept values, but may