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Estimation of body fat from anthropometry and bioelectrical impedance in Native American children

TG Lohman[1]*, B Caballero[2], JH Himes[3], CE Davis[5], D Stewart[5], L Houtkooper[1], SB Going[1], S Hunsberger[4], JL Weber[1], R Reid[2] and L Stephenson[6]

[1]*University of Arizona, Tucson, AZ, USA;* [2]*Center for Human Nutrition, Johns Hopkins University, Baltimore, MD, USA;* [3]*University of Minnesota, Minneapolis, MN, USA;* [4]*National Heart, Lung and Blood Institute, Bethesda, MD, USA;* [5]*University of North Carolina, Chapel Hill, NC, USA; and* [6]*Gila River Indian Community, AZ, USA*

OBJECTIVE: Obesity, as measured by body mass index, is highly prevalent in Native American children, yet there are no valid equations to estimate total body fatness for this population. This study was designed to develop equations to estimate percentage body fat from anthropometry and bioelectrical impedance as a critical part of Pathways, a multi-site study of primary prevention of obesity in Native American children.
DESIGN: Percentage fat was estimated from deuterium oxide dilution in 98 Native American children (Pima/Maricopa, Tohono O'odham and White Mountain Apache tribes) between 8 and 11 y of age. The mean fat content (38.4% ± 8.1%) was calculated assuming the water content of the fat-free body was 76%. Initial independent variables were height, weight, waist circumference, six skinfolds and whole-body resistance and reactance from bioelectrical impedance (BIA).
RESULTS: Using all-possible-subsets regressions with the Mallows C (*p*) criterion, and with age and sex included in each regression model, waist circumference, calf and biceps skinfolds contributed least to the multiple regression analysis. The combination of weight, two skinfolds (any two out of the four best: triceps, suprailiac, subscapular and abdomen) and bioelectrical impedance variables provided excellent predictability. Equations without BIA variables yielded r^2 almost as high as those with BIA variables. The recommended equation predicts percentage fat with a root mean square error = 3.2% fat and an adjusted $r^2 = 0.840$.
CONCLUSION: The combination of anthropometry and BIA variables can be used to estimate total body fat in field studies of Native American children. The derived equation yields considerably higher percentage fat values than other skinfold equations in children.

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Introduction

The prevalence of obesity in US children has increased at an alarming rate over the past 30 y.[1] This trend is particularly evident in Native American children. A recent survey of 9000 Native American school-aged children from 19 states found that 39% had a body mass index (BMI) above the 85th percentile of reference data.[2,3] Few surveys, however, have measured total body fat, which is considered a more accurate estimate of obesity in children. It has been suggested that BMI may overestimate the prevalence of obesity in Native American populations because they tend to have a higher proportion of fat-free mass than the national reference values.[2] In contrast, other studies suggest that body fat quantity and distribution may differ among ethnic groups, and that estimates of body fat based on national BMI reference data may underestimate obesity in adult Native American populations.[4,5] Therefore, it is important to evaluate obesity in Native American children using better estimations of body fat than BMI and to develop valid equations for body composition in this population suitable for large surveys.

Pathways is a multi-site study of primary prevention of obesity in Native American children. A critical component of Pathways is the development of a means to evaluate body fat in children suitable for large scale data collection under field conditions. This paper describes the development of an equation for estimating total body fat in Native American children and its validation using total body water from deuterium dilution as the criterion method. A carefully selected set of anthropometric predictor variables, appropriate for field applications, is presented and the results from these equations are compared with those from selected equations derived on non-Native American US children.

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*Correspondence: TG Lohman, 114 Ina Gittings Building, University of Arizona, Tucson, AZ 85721, USA.

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Methods

Subjects

The subjects included 98 third-, fourth- and fifth-grade Native American children (39 boys and 59 girls) from the Gila River and Tohono O'odham communities ($n = 60$) and White Mountain Apache Indian community ($n = 38$) in Arizona. Informed consent was obtained from a parent or legal guardian for each child, and the study protocol was approved by school and tribal authorities and University Human Subject Committees.

Total body fat

To develop a prediction equation for percentage fat in Native American children, selected anthropometric measures and bioelectrical impedance (BIA) variables were tested in the field setting. These measures included height, weight (wt), waist circumference, six skinfolds (biceps, triceps, subscapular, suprailiac, abdomen and calf) and bioelectrical resistance (R) and reactance. Total body water (TBW), measured by deuterium dilution, was used as the criterion method for the estimation of total body fatness. The water content of the fat-free mass was assumed to be 76%, based on results from studies of prepubescent non-Native American children.[6,7] Boileau *et al*[7] found no significant difference between prepubescent and post-pubescent children in the water content of fat-free mass (75.6% prepubescent and 75.5% pubescent). Percentage fat was calculated as:

$$\frac{\mathrm{wt} - \mathrm{TBW}/0.76}{\mathrm{wt}} \times 100$$

where wt = body weight in kg and TBW = total body water in kg.

Total body water

Saliva samples for measuring deuterium concentration were collected between 8:00 am and 8:30 am after the children finished school breakfast. The children were asked to chew on a special cotton pellet for saliva collection. After 45–60 seconds, the pellet was removed and placed in a special tube used to separate the liquid phase of saliva. Samples were stored in ice, and later frozen at −28°C until analysis.

After the baseline saliva sample was obtained, each child received a fixed, 30 g dose (BCI) of deuterium oxide (99.9% APE, Cambridge Isotopes, Cambridge, MA), followed by a 20 cm^3 tap water rinse. After 150 min, another saliva sample was obtained following the same procedure. Children did not eat or drink during this period of time. The 30 g dose was shown to maximize reliability of infrared measurements in previous studies.[8] Previous studies have also shown that D_2O isotopic enrichments in saliva equilibrate at around 120 min, and remain stable for 240–300 min.[8] The model used in the present study measured TBW by dilution at the time of dose. Performing the study after food intake results in an expansion of the TBW pool by metabolic water produced during the postprandial state. Assuming children are resting during the study and that breakfast produced a respiratory quotient (RQ) of 0.85, metabolic water formation during the equilibration period would expand the TBW pool by approximately 0.04% per hour. We consider this figure sufficiently small to be acceptable, considering the practical difficulties in performing the study in children in the fasting state.

Deuterium concentration was determined in saliva samples by infrared spectroscopy, as previously described.[9] Water was extracted by vacuum sublimation followed by condensation in a dry ice–methanol trap. Deuterium absorbence was measured in duplicate aliquots at 2500 cm^{-1}, in a Miran-1 fixed filter, single beam infrared analyzer (Foxboro Analytical Co., South Norwal, CT). All samples were analyzed at the Johns Hopkins Center for Human Nutrition Core Laboratory. Inter-assay coefficient of variability was 2–3%. No deuterium was detected in rinses from cotton pellets, and the deuterium enrichment of tap water samples was also below detectable levels. This is consistent with the very low levels of deuterium enrichments in water reported using the more sensitive isotope ratio method.[10] The deuterium dilution space was calculated as D_2O space $= (D/C)/wt$, where D is the isotope dose, C is tracer concentration in saliva at equilibrium, and wt is body weight in kg. The isotope dose was converted to ml using a density of 1.1044 g/ml at 25°C for D_2O. TBW was calculated from D_2O space assuming that the D_2O dilution space is 3.8% larger than total body water.[11]

Anthropometry

All anthropometric measurements were obtained with the children wearing light-weight clothing, and barefoot or wearing socks. All measurement procedures followed the protocol described in the Anthropometric Standardization Reference Manual.[12] Height was measured to the nearest 0.1 cm using the Schorr measuring board.[13] The measurements were repeated in sets of two trials until the difference between the two height measurements was ≤ 1.0 cm. The average of two acceptable trials was used as the final height value for each subject. Body weight was measured using the Seca Model 770 scale. This is a strain-gauge portable digital scale with a capacity of 180 kg and a precision of 0.1 kg. The weight measurement was recorded to the nearest 0.1 kg. The measurements were repeated in sets of two trials until the difference between the two weight measurements was ≤ 0.5 kg. The average of two acceptable trials was used as the final value for each subject. The waist circumference was measured 1 cm above the iliac crest, in a horizontal plane. The measurement was recorded to the nearest 0.1 cm. The average of three trials was used as the final value for each child. Extremity skinfolds were measured at the triceps, biceps and medial calf sites and truncal skinfolds were measured at the abdomen, suprailiac and subscapular sites using the Lange skinfold caliper.[12] The measurements were repeated in sets of three trials until the range for the three measurements was $\leq 20\%$. The average of three acceptable trials was used as the final value for each subject for each site.

Bioelectrical impedance

Measurements of resistance (*R*, ohms) and reactance (*Xc*, ohms) were made on the right side of the body using a four-terminal, single-frequency ($800\,\mu A$ at 50 kHz) impedance plethysmograph (Valhalla Scientific Model 1990B). All children were measured between 9 a.m. and 12 noon at each field center, 2–4 h after their last meal in a classroom or cafeteria. Two trials were performed on each subject within 1 min, and the average of the two trials was used as the final value for each subject.[14] Before each test session, the analyzer was calibrated using 500 (± 1) ohm external resistors and by using the internal calibration system of the analyzer. The range of values this device can measure accurately is from 0 to 1023 ohms.

To standardize measurement procedures, centralized training for bioelectrical impedance (BIA) and anthropometry was held for the staff from the participating field centers.

Data analysis

The candidate predictor variables for the development of a regression equation for estimating percentage fat were age, sex, height, weight, waist circumference, each of six skinfolds and whole-body resistance and reactance from BIA. Initially, higher order polynomial terms were included in a regression model to test for nonlinearity. This was done univariately for each independent variable with percentage fat as the dependent variable. Nonlinearity was also tested using a spline model. Both analyses showed the linear model was appropriate. All-possible-subsets regression was used with Mallows C (p) criterion, and age and sex included in each model. All possible two-way interactions were tested and only those which were statistically significant were retained in the model.

Cross validation was accomplished by the bootstrap or, a 'leave-one-out' method. In this method one data point at a time is left out, the model is fit to the remaining data points and then is applied to the excluded point to determine how well the excluded point is predicted. The average of the prediction errors, each point being left out once, is the cross-validated measure of the prediction error. Statistical theory shows that this estimate is approximately equivalent to an independent sample validation, if the original sample and the validation sample are random samples of the same population.[15] The computed mean square residual error can thus be interpreted the same as one that would be obtained with independent validation.

For comparison of results from previously published equations on children with this sample, the following equations of Guo *et al*,[16] Slaughter *et al*,[17] and Houtkooper *et al*[14] were selected:

1. Guo *et al*[16] for females only,

   fat-free mass (FFM) kg $= 4.34 + 0.68$ (wt, kg) -0.18 (calf skinfold) -0.24 (triceps) skinfold -0.20 (subscapular skinfold) $+ 0.18\ S^2/R$.
2. Slaughter *et al*,[17]
   for females, percentage fat $= 0.610$ (triceps and calf skinfolds) $+ 5.0$ for males, percentage fat $=$ 0.735 (triceps and calf skinfolds) $+ 1.0$.
3. Houtkooper *et al*[18] for males and females, FFM, kg $= 0.61\ S^2/R + 0.25$ wt $+ 1.31$ where $S =$ standing height (cm) and $R =$ resistance (ohms).

Results

Means ($\pm$ standard deviation) and the ranges for age, the anthropometric variables, and BIA variables for the 98 subjects are presented in Table 1. The mean body water of the sample was 18.2 l, or 46.8% of body weight. The mean fat content was estimated at 38.4% with a range of 22.7–60.5%. The mean skinfold thicknesses ranged from 10 to 26 mm, depending on the site. The resistance and reactance values ranged from 497 to 999 ohms and 60 to 123 ohms, respectively.

From a theoretical perspective, we expect S^2/R and reactance to be related to TBW (l), the best measure of the volume of the conductor.[14,16] To verify this

Table 1 Mean, standard deviation and range for body size and composition ($n = 98$)

Variable	*x*	*S*	*Minimum*	*Maximum*
Age (y)	9.1	1.0	7.0	11.0
Weight (kg)	40.4	12.4	21.0	82.6
Height (cm)	139.7	7.6	118.3	157.8
BMI (kg/m^2)	20.4	4.8	13.5	35.5
TBW (l)	18.2	3.5	12.3	30.5
Percentage of TBW	46.8	6.2	30.0	58.7
Percentage fat[a]	38.4	8.1	22.7	60.5
Skinfolds (mm):				
Biceps	9.7	4.3	3.2	24.0
Triceps	16.5	6.2	7.3	32.5
Suprailiac	26.3	14.0	6.3	58.8
Subscapular	16.9	10.5	4.3	52.0
Abdomen	22.6	11.8	5.2	51.8
Calf	16.0	6.5	5.0	31.5
Waist circumference (cm)	69.5	12.0	52.9	102.8
Resistance (ohms)	710.3	86.9	496.5	999.0
Reactance (ohms)	90.5	14.0	60.0	122.5
S^2/R (cm^2/ohms)	28.1	5.5	17.3	50.2

[a] Estimated from total body water.

expected relationship, we first used age, sex, S^2/R and reactance together to estimate TBW and found an adjusted r^2 of 0.90 and a root MSE of 1.01. Both S^2/R and reactance were significant predictors in this model and these results are comparable with other investigations.

Bivariate correlations between the predictor variables and percent fat are presented in Table 2. As expected, all correlations with percentage fat were statistically significant ($P < 0.05$), although correlations with height and with biceps and abdomen skinfolds and with reactance were lowest.

Multiple regression equations were developed to compare BMI, BIA, waist circumference, and selected skinfolds (triceps, suprailiac and subscapular) in estimating percentage fat, while controlling for age and sex (Table 3). Including other skinfolds did not account for significant additional reduction in residual variance. The partial regression coefficients are included (Table 3) so that other researchers may select from a variety of measures to estimate total body fat. The best models use two skinfolds (model 4) or a combination of skinfolds and BIA (models 5, 6 and 7). These equations yielded root mean squares (root MSE) of 3.2–3.4% fat and accounted for 81.9% or more of the variation in percentage fat. S^2/R (height squared over resistance) was a significant predictor when used in the model with reactance (model 3), but not when included with skinfolds (models 5, 6 and 7). Models using BMI or waist circumference alone yielded higher prediction errors (4.1% fat) than other models.

Table 2 Correlations between percentage fat (TBW) and anthropometry ($n = 98$)

Variables	*(Correlations with percentage fat)*[a]
Weight	0.85
Height	0.53
BMI	0.84
Skinfolds:	
Biceps	0.77
Triceps	0.86
Subscapular	0.86
Suprailiac	0.84
Abdomen	0.78
Calf	0.85
Waist circumference	0.85
Reactance	−0.38

$r > 0.20$, $P < 0.05$.
[a]Percentage fat estimated from total body water (TBW).

Using all-possible-subset regression analysis, all combinations of skinfolds were tested for the highest multiple r. Results of various combinations of two and three skinfolds are summarized in Table 4. In general, measurements of three skinfolds give only slightly different estimates than two skinfolds, and the triceps and suprailiac skinfolds are the two best predictors for this sample. The interaction terms of skinfolds with S^2/R were tested and the triceps skinfold significantly interacted with S^2/R. No other skinfold interactions were found. The interaction term for triceps with S^2/R increased the R^2 from 81.9% (model 6) to 84.0% (model 7). This equation (model 7) with skinfolds and bioelectrical impedance was selected as the final body composition equation for Pathways, in part because the suprailiac was not well accepted by the children. The cross-validated mean square residual from the 'leave-out-one' method was close to the mean square residual of our final model (13.5 *vs* 10.5 model 7, Table 3).

Estimates of percentage fat for the present sample using previous equations developed on non-Native American children using skinfolds,[17] BIA[14] and skin-

Table 4 Multiple correlation coefficients for estimation of percentage fat from various combinations of two and three skinfolds with weight, age, sex, S^2/R and reactance

Skinfold variables	*Two skinfolds*[a]	*Three skinfolds*[a,b]
Triceps, suprailiac (abdomen)	0.837	0.838
Calf, suprailiac (triceps)	0.830	0.837
Calf, subscapular (suprailiac)	0.810	0.830
Triceps, subscapular (calf)	0.819	0.820

[a]Age, weight, sex, S^2/R and reactance included in each regression analysis.
[b]The third skinfold is given in parentheses.

Table 3 Regression models for estimation of body fat

	Regression coefficient						
Variable	*Model 1*	*Model 2*	*Model 3*	*Model 4*	*Model 5*	*Model 6*	*Model 7*
Age	0.38	−0.28	−0.71	−0.08	−0.28	−0.18	−0.49
Sex	2.92[a]	3.12[a]	0.83	2.4[a]	1.9[a]	1.19	0.61
BMI	1.40[a]						
Weight			0.83[a]	0.21	0.21	0.31[a]	0.44[a]
Triceps skinfold				0.38[a]	0.44[a]	0.51[a]	1.55[a]
Suprailiac skinfold				0.20[a]	0.21[a]		
Subscapular skinfold						0.17[a]	0.15
S^2/R			−0.60[a]		0.10	−0.09	0.54
Reactance			0.04		0.10[a]	0.09[a]	0.13[a]
Waist, circumference		0.58[a]					
Triceps×S^2/R							−0.04[a]
Intercept	4.53	−1.03	23.64	17.66	6.22	9.71	−10.91
Adjusted r^2	0.737	0.740	0.776	0.827	0.843	0.819	0.840
Root mean square error, (%fat)	4.15	4.13	3.83	3.36	3.21	3.44	3.24

[a]Partial regression coefficient significantly different than zero ($P < 0.05$).

Table 5 Comparison of prediction equations from other studies of children

	Girls (n = 59)				Boys (n = 39)			
Variable/equation	*x*	*S*	r^2	*RMSE%*[a]	*x*	*S*	r^2	*RMSE%*
Percentage total body fat (measured)	39.5	8.1			36.8	8.6	—	—
Predicted percentage fat:								
Houtkooper *et al* (1992)	28.7	6.9	0.644	4.8	25.7	7.4	0.835	3.5
Guo *et al* (1992)	33.4	8.8	0.623	5.0	—	—	—	—
Slaughter *et al* (1988)	25.3	7.0	0.731	4.2	24.1	10.1	0.830	3.5

[a]Percentage root mean square error.

folds and BIA[16] are shown in Table 5 for each sex. For both boys and girls, these equations consistently underestimate percentage fat in the Native American children by 5–14% (Table 5).

Discussion

Only a small number of body composition studies have been completed in Native American children,[2,3,19] and body composition equations using anthropometric dimensions or BIA in this population have not previously been published. The present study was designed to estimate percentage fat in 8–11-y-old Native American children. Body water estimated from deuterium dilution was selected as the reference method for assessing percentage fat, assuming the water content of the fat-free body was 76%.[6,20–22] Another approach, using a multicomponent model, could not be done in this population. While it is well known that the hydration level of the fat-free body is higher in prepubescent children than at later ages, it has not been determined if ethnicity would effect this level for this age group. In a comparison of Black and White children, where known differences in adult fat-free body density have been established, no significant differences in hydration were found in prepubescent children.[7,22] It is unlikely that Native American children would be at a different level of hydration of the fat-free body than other children for whom there are data.

Prediction errors and equations are summarized in Table 3 using different prediction variables. These prediction equations should be appropriate for other Native American children of similar body fatness levels and enable other investigators to use different combinations of variables to estimate body fatness. The prediction errors (root mean square error) for percentage body fat are similar to those found by others.[14,16–18] We developed equations to predict percent fat rather than total body water from a combination of variables, because we were interested in developing a prediction equation for this measure of body composition as an outcome for the Pathways Study. Other body composition equations,[18,24,25] using a combination of methods, to predict percentage fat have been summarized by Houtkooper *et al*[18] in their review article on bioelectric impedance and body composition. The standard error of estimations ranges from 3.0% to 5.0% fat. The prediction errors are similar in magnitude whether one uses a FFM equation or a percentage fat equation, as shown by Lohman[26] for skinfold-FFM equations (with body weight) and skinfold–percentage fat equations. For age, sex, weight and BIA variables alone (model 3), the derived equation is similar to that of Houtkooper *et al*;[14] however, as compared to the mean values for the Houtkooper sample, the new equation estimates percentage fat 9.8% higher for boys and 11.1% higher for girls.

A unique finding of this study is the significant interaction term for BIA and triceps skinfold. Previous studies by Graves *et al*,[27] Segal *et al*,[28] and Lohman[22] have shown that BIA variables underestimate fatness in non-Native American obese adults, and that level of fatness affects body composition estimates from BIA. Thus, it is not unexpected that a more direct measure of fatness (triceps skinfold) might interact with BIA variables, in our case S^2/R. This interaction may provide a means of accounting for change in relationship of BIA to body composition in fatter subjects. We have run the model including the three way interaction of sex, triceps skinfold and S^2/R (with all appropriate two-way interactions also in the model). This three-way interaction is not significant ($P = 0.56$), indicating that the association between percentage fat and the two-way interaction of triceps and S^2/R does not differ significantly by sex in our sample. The adjusted r^2 for this model is 0.839 indicating that the addition of the other interaction terms does not appreciably change the amount of variance explained by the model.

The mean percentage fat in this sample was 36.8% for boys (± 8.3) and 39.5% for girls (± 7.8). The mean values are considerably higher than the 1986 national probability sample (National Children and Fitness Study) for 9-y-old children,[22] estimated from triceps and subscapular skinfolds and using the equations of Slaughter *et al*.[17] The mean percentage fat for the present sample of Native American boys and girls is comparable to the 95th percentile for the nation, thus indicating a high prevalence of obesity.

The mean BMI for 9-, 10- and 11-y-old Native American boys from a large survey was 19.4, 20.5 and

21.7, respectively,[2] compared to our sample mean of 20.5. For girls the BMI values for 9-, 10- and 11-y-olds were 19.6, 20.3 and 21.3,[2] respectively, as compared to our sample mean of 20.4. These comparisons suggest our sample is comparable in BMI to a larger, more general sample of Native American children.

The mean percentage fat content of this sample was 38.4%, which is systematically higher than that predicted from other anthropometric equations developed in non-Native American children. The BIA equation of Guo *et al*,[16] applicable only for girls, gave the closest estimate of 33.4% fat to our sample. The Houtkooper equation[14] using only BIA variables and wt underestimated percentage fat by 11.1% for boys and 10.8% for girls. Similar results were found for skinfolds using the triceps and calf skinfold equation of Slaughter *et al*.[17] For Slaughter *et al*[17] and Houtkooper *et al*[14] the criterion method was a multicomponent model using body density and body water. Thus, the discrepancy may be due to both biological and methodological sources of variation.

Kushner *et al*[29] developed an equation predicting total body water from BIA and weight in a combined sample of infants, children and adults. Using that equation, body water was estimated to be 19.0 kg (4.4% higher than our sample) and FFM of 26.0 kg (0.73 water content of FFM). These estimates yield a value of 35.6% fat from our sample. Most equations for TBW from Houtkooper review article[18] predict our sample will have a TBW of 19.2–21.1 kg. Thus, we see that our total body water values are lower than that predicted by various BIA equations and the Houtkooper *et al* equation[14] may over-estimate TBW (22.4 kg) compared to other samples. Skinfold equations of Williams *et al*[30] based on four different samples of children from the literature show similar results to the Slaughter *et al* equations for our sample.

One biological explanation for the discrepancies between previous models and the present results is that the water content of the FFM may be different in this population than the assumed 76%. However, to lower the fat content to 30% in our sample, the actual water content of the fat-free body would have to be about 67%, an extremely low and unlikely level. Increasing the water content by 1 or 2%, due to a hypothetically higher water content of FFM in obese children, would raise the estimate of fatness in this sample and further increase the differences among methods. A third, more plausible, biological factor is that Native American children have relatively more abdominal fat or more intra-abdominal fat as a proportion of total body fat than populations previously studied. For both skinfolds and BIA, variation in abdominal fat is not directly measured. For skinfolds, only subcutaneous fat is measured, so greater abdominal fat may have contributed to total body fat independently of the skinfolds used, and not be accounted for by the equations selected. For BIA, truncal resistance is much less than arm and leg resistance and it is underrepresented in the whole-body BIA results.[31] Consequently, BIA is not sensitive to differences in abdominal fat.

A final consideration is that the other equations were developed on a leaner sample of children or on children and adults. For example, the distribution in total body fatness in our sample has considerable overlap with that of the reference populations for the other prediction equations, even though mean fatness was higher. If the differences were due to levels of fatness *per se*, one would expect appreciable nonlinearity in relationships.[22] We examined our data and found no evidence for nonlinearity. Thus, it is unlikely that the observed underestimation of body fat by equations from non-Native American samples result from the level of fatness.

In summary, using a combination of anthropometric measurements and BIA, we developed equations for estimation of body fat in Native American school-age children. This approach is currently being used for the assessment of body composition in the Pathways study, and should permit more precise estimates of obesity than BMI in other studies involving Native American children.

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