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Total Body Electrical Conductivity Measurements: An Evaluation of Current Instrumentation for Infants

MARTA L. FIOROTTO, NIELS C. DE BRUIN, YVES W. BRANS, HERMAN J. DEGENHART, AND HENK K. A. VISSER

USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas 77030 [M.L.F.]; Department of Pediatrics, Erasmus University and University Hospital Rotterdam, Sophia Children's Hospital, Rotterdam, The Netherlands [N.C.D., H.J.D., H.K.A.V.]; and Departments of Pediatrics and Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Michigan 48201 [Y.W.B.]

ABSTRACT

Quantitation of the body's fat and lean masses is an important component of nutritional assessment. Such measurements, however, are difficult to conduct routinely in infants due to the numerous limitations of traditional methods. The application of total body electrical conductivity measurements for quantitating fat-free mass (FFM) overcomes many of these limitations. The instruments required to perform these measurements in pediatric patients (HP-2) have recently become commercially available, but their measurement performance has not been evaluated. In these studies, we compared the precision, day-to-day variability, and magnetic field profile of three HP-2 instruments. We also derived a new calibration equation that relates the FFM to the total body electrical conductivity measurement in piglets, and compared it with an equation (provided currently by the manufacturer) derived on a prototype instrument. The performance of the instruments was generally similar, although a significant difference in the magnetic field of one instrument was identified. The coefficient of variation of inanimate phantom measurements varied from ±0.2 to ±0.5%, and the day-to-day variability was generally similar. Such measurement error is significant (±0.035 to ±0.078 kg FFM) for small subjects. The new calibration equation was similar to the original equation; therefore, all the data were pooled to generate a new equation that is linear at least to 10 kg. Thus, the HP-2 total body electrical conductivity instruments, which can be safely and easily used to measure FFM and fat in infants through 1 y of age, proved to be reliable and precise, and results obtained from different instruments can be confidently compared. (***Pediatr Res* 37: 94–100, 1995**)

Abbreviations

E#, TOBEC unit
E#$_{cor}$, E# corrected
FFM, fat-free mass
L_{con}, conductive length
MIC, Perinatal Metabolism Laboratory, Detroit, MI
NDL, Sophia Children's Hospital, Rotterdam, The Netherlands
SEE, standard error of the estimate
TOBEC, total body electrical conductivity
TX, Texas Children's Hospital, Houston, TX

The quantitation of the body's fat and lean masses are fundamental for the assessment of an individual's nutritional status. The adequacy of fat stores and the FFM traditionally are assessed indirectly from measurements such as skinfold thicknesses and arm muscle area. These latter methods are relatively insensitive, and their accuracy in predicting fat and lean masses is questionable in infants. Although measurements of total body FFM and fat mass are preferable, they are difficult to conduct on a routine basis in infants due to the numerous limitations of traditional methods. The application of TOBEC measurements for quantitating FFM overcomes many of these limitations.

TOBEC measurements have been used to estimate the conductive mass of human subjects and animals *in vivo* (1–4). The conductive mass of the body corresponds to that compartment occupied by total body water and the conductive, fat-free solids of the body throughout which the water is distributed (3, 4). This compartment corresponds to the FFM. The instrument consists essentially of a cylindrical measurement chamber encompassed by a solenoidal coil through which a low-frequency oscillating electrical current (2.5 MHz) is passed to

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Correspondence: Dr. Marta L. Fiorotto, Children's Nutrition Research Center, 1100 Bates St., Houston, TX 77030.
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generate a magnetic field within the chamber. A conductive object placed in the field dissipates some of the field's energy and in doing so changes coil impedance. The magnitude of this change in impedance is a function of the instrument's magnetic field characteristics, as well as the object's conductivity, and total conductive mass. Because fat is nonconductive, it does not change coil impedance and therefore is not measured; fat mass, however, can be calculated as the difference between body weight and FFM.

The technique is ideally suited for infants because it is safe, noninvasive, requires no active participation by the subject, and can be rapidly performed (in approximately 5 min); hence, it provides immediate estimates of FFM and body fat mass. In addition, the accuracy of the FFM measurements is compromised minimally by isotonic variations in the hydration of the FFM (4). This feature is advantageous for studying populations, such as the pediatric population, in which the hydration of the FFM can be widely divergent even under normal circumstances.

Use of TOBEC in pediatric clinical or research applications has been limited because pediatric instruments were not commercially available until 1989, and the initial evaluations of this technique were conducted exclusively on prototype instruments (models EMME M60 and HP-1) (2, 5). The current, commercially available instrument (5) (model HP-2) differs from the prototypes in the shape, length, and homogeneity of its magnetic field; the units in which the TOBEC values are expressed also differ.

Despite these developments, three concerns still potentially limit the use of the technique in pediatrics. First, each HP-2 instrument is assembled individually. Thus, there is no assurance that all instruments perform similarly. If the instruments differ, then estimates of FFM determined on one instrument would not be directly comparable with those obtained from another. Although the manufacturer standardizes the HP-2 instruments, there has been no evaluation to establish whether variations in performance regarded acceptable by the manufacturer (because they are within the design tolerances) are acceptable in practice.

Second, there is the concern of calibration (6). Adult TOBEC instruments were calibrated by measuring the FFM of a reference population with an alternative technique (usually hydrodensitometry) and relating this to TOBEC measurements of the same individuals (3). A similar approach could be used for the pediatric TOBEC instrument; however, methods currently available for the estimation of FFM of infants (such as total body water and potassium) do not measure the same body compartment as does TOBEC. A calibration equation based on these methods therefore would provide estimates of FFM that would only be as accurate as body water and potassium in their prediction of FFM. Thus, an alternative approach for calibration was used (7, 13). The conductance of animals (infant miniature pigs) with a chemical composition and size similar to that of human infants was measured, and this was related to the piglets' true FFM measured by chemical analysis (7, 13). These TOBEC measurements were made on prototype instruments, and the calibration equation so derived is currently used to estimate FFM from TOBEC measurements made on the new HP-2 instruments. In view of the changes in magnetic field characteristics, it is essential to compare the original calibration equation with a calibration equation derived directly on an HP-2 instrument.

The final concern relates to the size range of the subjects over which the calibration equation is applicable. The original calibration was confined to piglets weighing less than 5.6 kg; the validity of linear extrapolation for larger subjects has not been tested.

Our studies were designed to address these three concerns. Our first objective was to assess the variability in measurement precision and magnetic field characteristics among three HP-2 instruments. The measurement precision determines the smallest change in FFM that can be discerned with confidence. The evaluation of the magnetic field profiles would allow us to determine whether a universal calibration equation can be used for all instruments to derive FFM or whether each instrument must be separately calibrated. If each instrument must be separately calibrated, it would materially reduce the usefulness and widespread use of the technique in pediatrics.

Our second objective was to compare a calibration equation derived from direct measurements of miniature piglets on one of the three HP-2 instruments with the original equation derived from measurements on an HP-1 instrument. This comparison essentially tests the practical consequences of the modifications associated with the upgrade of the HP-1 to the HP-2 instrument. The use of an animal model that can be subjected to chemical analysis enabled us to circumvent concerns that arise about the accuracy of the reference method for FFM determination.

Finally, our third objective was to determine whether the relationship between FFM and the TOBEC measurement is linear over a wider range of sizes.

METHODS

Comparison of Three HP-2 Instruments

Our first objective, to compare measurement precision and magnetic field profiles among three (designated as MIC, NDL, and TX) HP-2 instruments (model HP-2, EM-SCAN Inc., Springfield, IL), was accomplished with the use of inanimate standards (phantoms) provided with each instrument. One phantom is a copper hoop with a resistor in series and provides a measurement at a single point in the magnetic field. The other is a 45-cm long cylinder that contains a conductive coil, and provides a measure of the average conductance integrated over 45 cm. Each phantom has an E# determined using standard operating conditions on a reference HP-2 instrument maintained by the manufacturer. Each new instrument is then adjusted (using a normalization constant) so that the phantom E# is the same value as that measured on the manufacturer's reference instrument. This constant therefore is meant to correct for instrument-to-instrument variations in a magnetic field. The correction procedure, however, adjusts only for differences at the center of the measuring range and does not identify discrepancies at the two ends.

Measurement precision (within-measurement variability) was assessed from the average SD of 10 individual, consecu-

tive readings of a phantom measured on numerous separate occasions (Table 1). Day-to-day variability was assessed from the variation in the average E# of either phantom over a period of 18 to 24 mo. All measurements were made with the phantoms placed in the center of the measurement chamber (in the user-determined position, *i.e.* "fixed" mode). Empty carrier (background) measurements were made concurrently, and this value was subtracted from the gross E# of the phantom to give a net E#.

The magnetic field profiles of the instruments were compared using two procedures. First, the hoop phantom was placed at the distal end of the carrier (furthest from the handle); the carrier was then slowly inserted into the measurement chamber and a reading was taken every 2 cm over the length of the measurement chamber, *i.e.* 200 cm. This is the method recommended by the manufacturer. In the second procedure, we compared the average E# obtained for the tube phantom when it was placed at the proximal (closest to the handle) and distal ends of the subject carrier with the E# in the center (reference) position (Fig. 1). In all instances, the subject carrier was positioned in the center of the measurement chamber. This procedure provided a quantitative measure of how the magnetic field of each instrument varied over its length and enabled the magnetic fields of instruments to be compared without the need for the same phantom to be measured on all instruments.

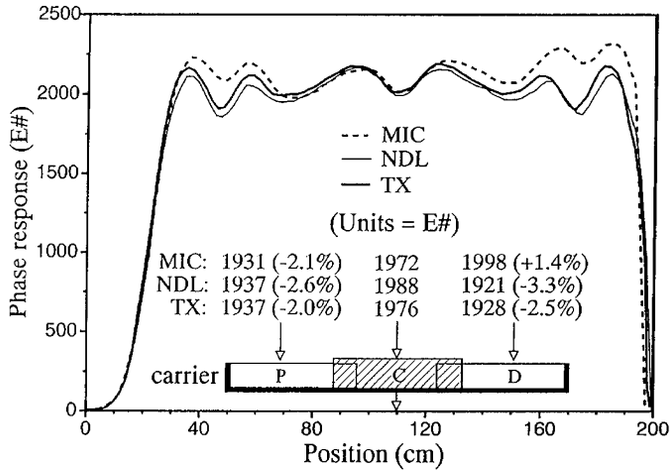

Figure 1. Magnetic field plots of the MIC, NDL, and TX HP-2 instruments showing the position of the subject carrier when it is centered in the measurement chamber. *Boxes* on the subject carrier represent the location of the tube phantom in the chamber when measurements were made at the proximal (*P*), center (*C*), and distal (*D*) positions. The values are the average E# of the respective phantoms at these positions for the three laboratories; all measurements were made in the "fixed" mode. The values in parentheses are percent differences between the values of E# in the P or D positions and their respective value in the center position.

Relationship between TOBEC Measurements (E#) and FFM

To address our second objective, we compared the relationship between the chemically determined FFM and the TOBEC measurement of two groups of piglets: for one group of piglets (TX), the E# was determined on an HP-1 instrument, and for the second (NDL), measurements were made on the NDL HP-2 instrument. The general procedures used have been described previously (7) and essentially involved measuring piglets in the TOBEC instruments and then determining their FFM by chemical analysis. The two laboratories, however, differed in certain details.

Animals. The NDL laboratory studied 12 miniature piglets of the Gottingen strain (University of Dusseldorf) ranging in age from 7 to 99 d and weighing from 1.03 to 10.10 kg. The TX laboratory studied 26 miniature piglets of the Hanford strain (Charles River Laboratories, Wilmington, MA) ranging in age from 7 to 33 d and weighing from 1.87 to 5.53 kg. With one exception, all piglets were healthy and had been stabilized after transport to the respective laboratories; during this time piglets had free access to a swine milk replacer. One NDL piglet refused feedings between arrival and measurement time. All piglets were fasted (but provided with water) for at least 6 h before TOBEC measurements were made.

Table 1. *Comparison of average day-to-day and within-measurement variability of TOBEC measurements made on three HP-2 instruments*

Laboratory	Reference†	*n*	Mean	Variability (SD)* Day-to-day	Variability (SD)* Within-measurement
MIC (0006)‡	2012	49	2007	13.8	3.6 ± 1.2
NDL (0011)	1944	50	1942	9.8	8.8 ± 2.9
TX (0010)	2032	50	2037	4.1	4.7 ± 1.2

* All units are E#.

† Manufacturer's specified net E# for phantom.

‡ Instrument serial number.

The animal protocols were reviewed and approved by the respective institutional review boards.

TOBEC measurements. Measurements were made on anesthetized piglets. NDL piglets were fitted with ear vein catheters before being measured. The piglets were centered on the subject carrier in a lateral recumbent position and the L_{con} was measured as described previously (7). Ten consecutive readings were taken (in "peak" mode) and an average net E# was calculated for each pig after subtracting the background reading of the empty subject carrier. The HP-1 E# units were subsequently converted to HP-2 E# units using an instrument-specific conversion equation. A phantom measurement was also made to adjust for short-term variations in instrument performance. For both NDL and TX pigs, the net E# was then corrected by a factor that reflected the extent by which the concurrent phantom reading deviated from its predicted value ($E\#_{cor}$). The square root of the product of $E\#_{cor}$ and L_{con}, $\sqrt{[E\#_{cor} \cdot L_{con}]}$, was calculated for each pig; this term was used as the independent variable in the regression analysis against FFM.

Chemical analysis. On completion of the TOBEC measurements, the pigs were killed with an overdose of anesthetic and weighed. The analytical procedures used by the two laboratories were similar and have been previously reported (7). Total body water was estimated by desiccation of the whole carcass (at 97°C). Complete desiccation was verified by the absence of weight change with further drying. The fat content was measured by carrying out an initial extraction with methylene

chloride (TX laboratory) or hexane (NDL laboratory), followed by a diethyl ether extraction in a Soxhlet apparatus. Both laboratories verified that fat extraction was complete by the absence of further weight change on repeated extraction. The coefficient of variation for the replicate fat analyses were ±1.4% and ±1.1% for the NDL and TX laboratories, respectively. FFM was calculated as the difference between body weight and the analyzed value for total body fat.

Statistics

All statistics were carried out using Minitab statistical software (Minitab Inc., State College, PA). Regression analysis techniques were used to derive calibration equations; dummy variables were used to categorize the equations in the comparison procedures. Only values of $p < 0.05$ were considered statistically significant.

RESULTS

HP-2 instrument characteristics. The sources of instrument variability that influence the precision and accuracy of TOBEC measurements are shown in Table 1. The measured mean E# for the phantoms were within 0.3% of the reference value. The day-to-day variability was the SD of mean phantom readings for the 18- to 24-mo period over which data were collected and in all cases was <1% of the mean value. Day-to-day variability (±0.7%) was almost 4-fold higher than the within-measurement (±0.18%) variability for the MIC instrument and was more than could be accounted for by within-measurement variability alone. The within-measurement variability was ±0.5% for the NDL instrument and ±0.2% for TX HP-2 instruments. The day-to-day was not different from the within-measurement variability for the NDL and TX HP-2 instruments. The average SD for the piglet (NDL) measurements was 7.8 ± 3.9 E#, a value that was almost identical with that for the phantom measurements and that was not influenced by the absolute value of E#.

The magnetic field profiles for the three instruments (Fig. 1) were generally similar in form, but quantitative differences were discerned at the two ends of the measurement chamber. These differences were largely in portions of the magnetic field that are outside of that part of the coil where subjects are positioned for measurement. A quantitative measure of the between-instrument differences in magnetic field profiles is given by the tube phantom measurements obtained with the phantom placed at the two ends of the subject carrier relative to the reading in the middle (Fig. 1). At the proximal end, the signal generated was similar for all instruments, and on average was 2.2% less than the value obtained in the center. At the distal end, however, the NDL and MIC instruments differed by approximately 5%, which was anticipated in view of the relative difference in magnetic field strength at the distal ends of the measurement chambers.

Relationship between $\sqrt{[E\#_{cor} \cdot L_{con}]}$ and piglet FFM. The characteristics of the two sets of piglets analyzed by the NDL and TX laboratories are summarized in Table 2. The chemical compositions were similar for animals of similar ages. The exception was the piglet that had refused to eat. The total body water (72.2% FFM) of this animal was substantially less than that of a littermate (84.3%), which, in turn, was appropriate for its age (7 d old) (9); this suggests that the fasted piglet was substantially dehydrated. The dehydration, however, did not adversely influence the TOBEC-FFM relationship, and thus the measurements of this piglet have been included in the analyses.

Table 2. *Characteristics of piglet body composition and TOBEC measurements*

	NDL	TX
n	13	26
Length (L_{con}) (cm)*	37.8 ± 10.6†	37.2 ± 4.3
	(22.0–53.0)	(30.5–47.1)
Body weight (kg)	4.45 ± 3.00	3.02 ± 1.00
	(1.03–10.10)	(1.87–5.54)
FFM (kg)	3.78 ± 2.45	2.62 ± 0.84
	(0.94–7.68)	(1.61–4.73)
Fat (% body wt)	14.2 ± 5.0	12.8 ± 3.7
	(7.0–24.0)	(6.6–19.9)
$E\#_{cor}$‡	606 ± 536	283 ± 154
	(47–1606)	(120–733)
FFM/L_{con} (g/cm)	90 ± 40	67 ± 14
	(41–145)	(49–103)
Total water (% FFM)	77.3 ± 2.8‖	79.3 ± 1.1
	(74.4–84.3)	(76.9–81.8)

* Conductive length, *i.e.* rump to lateral canthus of the eye, with the pig lying in a lateral recumbent position on the instrument carrier.

† Values are means ± 1 SD; ranges are given in parentheses.

‡ A mean of 10 readings (made in the "peak" mode) was obtained per piglet and the empty subject carrier reading subtracted. The resulting value was adjusted by a factor that corrected for the deviation of the net phantom reading obtained on the same day from the manufacturer's specified value.

‖ Value for dehydrated piglet, 72.2%, omitted.

The ratio between FFM and length provides an index of the geometry of the FFM (Table 2). FFM increased logarithmically with length (NDL: $r = 0.99$; TX: $r = 0.96$), and the slope of the relationship did not differ significantly between the two groups of piglets. The apparent difference suggested by the mean values in Table 2 therefore reflected the different range of sizes studied by the two laboratories rather than differences in geometry of the piglets.

The relationship between FFM and $\sqrt{[E\#_{cor} \cdot L_{con}]}$ (Table 3) was linear for both TX HP-1 and NDL HP-2 instruments. The use of polynomial equations with the inclusion of higher power functions did not improve the fit significantly (significance of higher order power functions, $p > 0.18$; Δ SEE = 0.001 kg). The SEE of equation 1 was markedly larger than that of equation 3. Closer examination of the NDL data revealed an outlier with a standard residual of 2.8. Omission of the data from this animal reduced the SEE of the NDL equation by 40% (equation 2). The data for this animal were not included subsequently.

Neither the intercepts nor the slopes ($p = 0.285$ and 0.493, respectively) of equations 2 and 3 (Table 3) were significantly different from each other. There were no differences between equation 2 and 3 in the variability of the residuals about the regression line, nor was there any bias in the distribution of the residuals: the mean values (0.019 ± 0.079 kg for equation 2 and −0.009 ± 0.075 kg for equation 3) were not significantly different from each other or from 0. Thus, the data sets were

Table 3. *Coefficients for linear regression of FFM versus* $\sqrt{[E\#_{cor} \cdot L_{con}]}$ *derived from measurements on infant miniature pigs*

Instrument* (laboratory)	*n*	Intercept (1 SD) (kg)	Slope (1 SD)	SEE (kg)	r^2 (%)	Equation
HP-2 (NDL)†	13	−0.0361 (0.0754)	0.0269 (0.0005)	0.143	99.7	1
HP-2 (NDL)‡	12	−0.0047 (0.0441)	0.0264 (0.0003)	0.082	99.9	2
HP-1 (TX)‖	26	0.0261 (0.0506)	0.0258 (0.0005)	0.078	99.1	3
HP-2‡ + HP-1	38	−0.0213 (0.0274)	0.0264 (0.0002)	0.077	99.7	4

* Instrument model on which original TOBEC measurements were made.
† Regression based on all data points from NDL laboratory.
‡ Regression omitting one data point with a standard residual of 2.8.
‖ Calibration equation currently provided by instrument manufacturer; the units for the HP-1 E# have been converted to HP-2 units.

homogeneous. Equation 4 was therefore derived from the pooled data.

We also assessed the level of agreement of equations 2 and 3 (Table 3) by using the equation derived on one instrument to predict the FFM from the TOBEC measurements obtained on the other. The difference between predicted and measured estimates of FFM are summarized in Table 4. Absolute differences between predicted and measured values were as large as 0.213 kg for the NDL piglets and 0.145 kg for the TX piglets. In neither case was the average significantly different from 0. For both sets of piglets, the percent error was randomly and equally distributed around 0; it was greatest for the smallest piglets and decreased nonlinearly as absolute FFM increased. For either group, the prediction using equation 4 (Table 3) was better than that using the equations derived from the alternate instrument. The difference between measured and predicted values was less than 5% for all values of FFM of more than 2.0 kg.

Figure 2 shows the relationship between FFM and $\sqrt{[E\#_{cor} \cdot L_{con}]}$ for the pooled data set (equation 4; Table 3), together with the 95% confidence interval for the regression and the 95% prediction interval for an individual observation.

The residuals from the regression of $\sqrt{[E\#_{cor} \cdot L_{con}]}$ against FFM were calculated and then used to determine the extent to which variability in $\sqrt{[E\#_{cor} \cdot L_{con}]}$ was attributable to factors other than FFM; any such factors would increase the uncertainty in the prediction of FFM. The residuals were regressed against those factors which we identified as possibly contributing to variation in the E# on the basis of theoretical considerations, *i.e.* fat (absolute or as % body weight), body geometry (L_{con}, chest circumference, weight/L_{con}, chest circumference/L_{con}, weight/L_{con}^2, chest circumference/L_{con}^2), or degree of maturity (age, hydration of the FFM). None of these variables made any significant contribution to the variability in the $\sqrt{[E\#_{cor} \cdot L_{con}]}$.

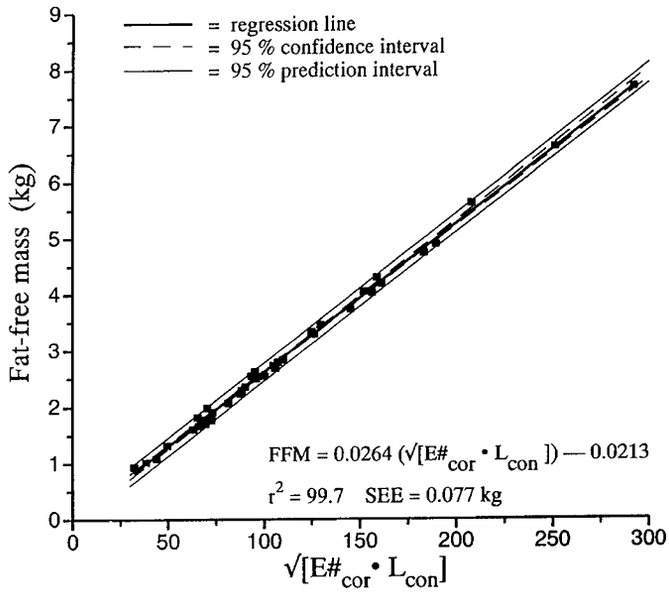

Figure 2. Regression line (equation 4, Table 3) of FFM determined by chemical analysis *versus* the $\sqrt{[E\#_{cor} \cdot L_{con}]}$, showing the 95% confidence and prediction intervals. ■, values for individual piglets.

Table 4. *Comparison of FFM of piglets determined by chemical analysis with values predicted from TOBEC measurements*

Method	FFM (kg)	Δ† (kg)	Δ‡ (%)
NDL piglets (*n* = 12)			
Chemical analysis	3.521 ± 2.362 (0.938–7.675)		
TOBEC‖			
Equation 3	3.478 ± 2.325 (0.842–7.592)	0.043 ± 0.086 (−0.064–0.213)	1.5 ± 3.8 (−5.8–10.2)
Equation 4	3.504 ± 2.361 (0.828–7.681)	0.017 ± 0.079 (−0.084–0.158)	1.4 ± 4.2 (−5.0–11.8)
TX piglets (*n* = 26)			
Chemical analysis	2.619 ± 0.841 (1.609–4.732)		
TOBEC‖			
Equation 2	2.646 ± 0.850 (1.651–4.832)	−0.027 ± 0.075 (−0.145–0.136)	−1.1 ± 3.4 (−8.2–6.8)
Equation 4	2.630 ± 0.850 (1.641–4.815)	−0.011 ± 0.075 (−0.129–0.152)	−0.4 ± 3.4 (−7.3–7.7)

* Values are means ± 1 SD; ranges are shown in parentheses.
† Measured FFM − predicted FFM.
‡ [(Measured FFM − predicted FFM)/measured FFM] × 100.
‖ Equations used to predict FFM are as described in Table 3.

DISCUSSION

HP-2 instrument performance. Our first objective was to characterize the accuracy and precision of the instruments that are now commercially available for measuring the TOBEC of pediatric subjects. The within-measurement variability (precision) defines the inherent minimum uncertainty for a TOBEC measurement on a given instrument. All three HP-2 instruments were very precise, and the value for the within-measurement variability was constant for each instrument. The measurement variability for the NDL instrument, however, was almost 2-fold higher compared with the MIC and TX HP-2 instruments. The greater precision of the MIC and TX HP-2 instruments was likely attributable to instrument and environmental factors. Indeed, we identified retrospectively the pres-

ence of an electrical motor in a position coaxial with the NDL HP-2 measuring chamber. Interference caused by the magnetic field generated by the motor would increase instrument noise, *i.e.* the within-measurement variability. The precision of the E# for piglets indicated that the uncertainty associated with the measurement of a live subject was no greater than for the phantom measurements. The practical consequence of the within-measurement variability for the estimation of FFM depends on the size of the subject. For example, for a 1-kg piglet an error of ±9 E# (NDL) *versus* ±4 E# (TX HP-2 and MIC) represents an uncertainty of ±0.078 kg FFM *versus* ±0.035 kg FFM, but for a 10-kg pig, an error of 9 E# translates into ±0.022 kg FFM. Thus, control of environmental conditions to minimize the within-measurement variability is important, especially when measuring subjects with a small conductive mass.

The low day-to-day variability in the phantom readings established the excellent degree of constancy of the instruments over time. Such long-term stability ensures that measurements of FFM made at different times can be compared with each other and assumed to be of equal accuracy and not influenced by differences in instrument performance. Under ideal circumstances, the variations in the TOBEC value of an inanimate phantom over time will reflect the within-measurement variability, and, indeed, this was found for the TX and NDL HP-2 instruments. The higher value for the MIC HP-2 was atypical, and in retrospect we noted that a significant increase (50–80 E#) in the phantom readings for several months after relocation of the instrument was responsible. Such long-term drift introduces bias and therefore compromises the accuracy of the FFM prediction. This observation underscores the necessity to document phantom calibrations and to ensure that they remain within a specified range.

Magnetic field characteristics. The TOBEC value of a conductive object is a function of both its conductive mass and the strength of the magnetic field within which it is placed. Thus, two subjects with identical FFM, but measured on different instruments, will have equivalent TOBEC values only if the magnetic fields of the two instruments also are identical. Although instruments are cross-calibrated by the manufacturer, this exercise is only performed in the center of the field. As can be seen from the field plots, this does not ensure that the instruments are equivalent over the whole measuring range. The practical consequence of discrepancies in magnetic field characteristics, such as those observed for the MIC instrument, would be the overestimation of FFM for a subject that extended into the distal end of the measurement chamber. Ideally, when magnetic field characteristics differ from those of the NDL or TX HP-2 instruments, and the user does not have the option of deriving their own calibration, the instrument should be adjusted to bring the magnetic field profile into an acceptable range over the full measuring range. A practical solution is to place subjects within the homogeneous sections of the magnetic field.

Calibration equation. A calibration equation is required to derive FFM from a TOBEC measurement. The primary measurements used to derive this equation were obtained on a prototype HP-1 TOBEC instrument, and its validity for the HP-2 TOBEC instrument previously had not been assessed. Our data show that the equation derived on the HP-2 was very similar to that derived on the TX HP-1 instruments and thereby indicate that the modifications to the design of the prototype HP-1 instrument had no tangible effects on the TOBEC measurements, other than the change in measurement unit. The similarity also gives confidence that differences between laboratories in the chemical analysis procedures, the E# determinations, and the geometry and composition of piglets of different strains were of little practical consequence. This conclusion was strengthened by the analysis of the residuals of the equations, which showed that factors related to geometry, composition, and maturity did not contribute to variation in E# to a greater extent than could be accounted for by FFM alone. The absence of an effect of dehydration on the relationship between FFM and $\sqrt{[E\#_{cor} \cdot L_{con}]}$ extended our previous observations (8) that isotonic variations in the hydration of the FFM do not compromise the accuracy of TOBEC-derived estimates of FFM.

The lack of improvement in the prediction of FFM with the addition of geometry variables contrasted with our previous finding, using an EMME M60 instrument, that the addition of a term to describe body geometry (weight/L_{con}^2) significantly improved the prediction (7). The difference is probably attributable to the improvement in the magnetic field characteristics and the elimination of the electrical field contribution to the measurement. The latter has minimized the contribution of the more geometry-sensitive dielectric component of the measurement (4).

The effect of the difference between equation 3 (Table 3), the equation currently used for all HP-2 instruments, and the new equation proposed (equation 4; Table 3) on the estimation of FFM varies according to the size of the subject. The effect is minimal for small subjects, *e.g.* a subject who weighs 2.8 kg has an L_{con} of 37.9 cm and a net E# of 283, equation 3 yields a FFM of 2.70 kg, whereas equation 4 yields a value of 2.71 kg. Equation 4 gives a slightly higher estimate of FFM for larger subjects: for a 9.5-kg subject with an L_{con} of 59.3 cm and a net E# of 1120, the estimates of FFM are 6.67 kg (equation 3) and 6.78 kg (equation 4). This represents only a 1.6% increase in the estimate of FFM but a 3.9% decrease in the estimate of fat. Great effort was spent to ensure that the TOBEC measuring procedure was similar between the two laboratories. Thus, the proposed equation 4 and the associated errors in estimates of FFM strictly apply to animals that are anesthetized and lying on their sides. E# are obtained in the "peak" mode. As discussed previously (5), if an investigator chooses to use equation 4 to interpret TOBEC measurements made on human infants, a similar measuring procedure should be followed. Infants should be swaddled to ensure that they are motionless, fully extended, and measured on their backs, thereby mimicking the position and geometry of the piglets as placed in the instrument. Additional factors that could influence the accuracy and precision of measurements in human subjects have been addressed previously (5).

Precision of FFM estimates. The uncertainty that should be anticipated in an estimate of FFM is dictated by the SEE. For an individual measurement of FFM, the uncertainty (reflected

by the 95% prediction intervals, Fig. 2) will be on average 2 SEE, or 0.154 kg of FFM. This is a fixed value and becomes ±5% or less above approximately 2.80 kg FFM. The magnitude of the uncertainty for individual measurements is one reason to emphasize that for small subjects the technique is more useful for assessing the average body composition of groups of individuals. Even for repeated measurements on the same individual, the uncertainty is dictated by the instrument precision, which, as discussed previously, could be significant for subjects with a small FFM. The uncertainty in the estimate of FFM of a mean value for a group of individuals is measured by the 95% confidence intervals. These varied from ± 0.041 kg FFM (±4%) for a FFM of 1.0 kg to ±0.085 kg FFM (±1%) for an average FFM of 7.7 kg.

Application of calibration equation to interpretation of measurements in human infants. On the basis of our data set, the proposed calibration equation can be used to interpret measurements from piglets with FFM at least within the 0.94 to 7.71 kg range. Its usefulness at the lower end of the range is limited for individual predictions by the precision of the instrument. Data on body composition determined from TOBEC measurement of human infants whose body weights range from 2.8 kg and up (10–14) are entirely consistent with body composition determined by chemical analysis (15–17), and reference data (18). Data on human infants from all three laboratories (19) (our unpublished observations), however, have indicated that the equation is inappropriate for infants less than 2.8 kg, in as far as the derived values of FFM were often greater than body weights. The exact cause of the discrepancy between piglets and human infants is not clear, and therefore it is difficult to give a set of parameters with precise limits outside which the calibration equation is no longer valid. Various factors could be responsible for the discrepancy between piglets and the very small human infant, including differences in the shape or density of their FFM and the exact nature of their conductive length (7, 10). Although there is no indication of nonlinearity to preclude extrapolation of the equation beyond 10 kg, there are no published data for infants of this size that would allow us to assess the validity of other assumptions inherent in the use of the proposed calibration equation. Strictly speaking, therefore, the use of equation 4 to interpret TOBEC measurements of human infants should be limited to infants between 2.8 and 10.0 kg. Calibration equation 4 (Table 3) thus is applicable to TOBEC measurements made in full-term infants from birth to 12 mo of age, at least. Nevertheless, the HP-2 instruments are sufficiently sensitive to measure groups of smaller infants provided some appropriate, new calibration method can be devised.

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