

Accuracy of a User-Friendly Centrifuge for Measuring Creamatocrits on Mothers' Milk in the Clinical Setting

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ABSTRACT

The creatocrit (CRCT), a simple, accurate, and inexpensive technique for the estimation of lipid and caloric content in mothers' milk, has been used extensively in lactation research, but has not been integrated into the routine management of clinical lactation problems such as slow weight gain in mothers' milk-fed preterm and term infants. The Creamatocrit Plus™ is a lightweight, noiseless centrifuge with an embedded reader that automatically calculates lipid and calories from the CRCT value, making it ideal for use in the clinical setting. This study compared intra-user and inter-user reliability, the equivalence of the CRCT values obtained with the Creamatocrit Plus to the two standard techniques for performing CRCTs: the standard laboratory centrifuge with a hematocrit reader and the standard laboratory centrifuge with digital calipers, and the predictive accuracy of the Creamatocrit Plus for estimating the lipid and caloric content in mothers' milk. CRCTs were performed using the three techniques on 36 milk specimens from 12 women. Laboratory analyses of lipid and calories were performed by investigators blinded to CRCT values. The mean absolute intra-user and inter-user differences were all <1% CRCT, and the mean CRCT measures were nearly identical for the three measurement techniques. Linear correlations between CRCT and laboratory measures for lipid ($r = 0.95$) and calories ($r = 0.94$) were very high. The authors conclude that the Creamatocrit Plus can replace cumbersome laboratory equipment for measuring CRCTs in the clinical setting.

INTRODUCTION

UNLIKE COMMERCIAL FORMULAS, the lipid content in mothers' milk is highly variable, and depends upon individual variation, the stage of lactation, degree of breast fullness at the time of emptying, and the completeness of milk removal.^{1,2} The lipid content in human milk is strongly correlated with the caloric den-

sity of the milk.³ For the healthy term infant, this variability does not cause problems, because the infant will self-regulate volume in order to consume adequate lipid and calories.⁴ However, when mothers provide expressed milk, or breastfeed in complicated situations, the infant's consumption of lipid and calories may be inadequate, resulting in slower than desired weight gain.

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The creatocrit technique has been used extensively to estimate the lipid and caloric content in milk since it was first described by Fleet and Linzell in 1964,⁵ and later validated for human milk by Lucas in 1978.⁶ The procedure involves placing milk in glass capillary tubes, which are then spun in a hematocrit centrifuge, a process that separates the lipid, or cream, from the aqueous phase of the milk.^{2,6-8} Then, the size of the cream layer and the total column of milk (including the cream) in the capillary tube are measured with calipers or a hematocrit reader.^{2,6-8} The creatocrit value is calculated as a percentage [crematocrit = (cream layer/total milk column)*100] and typical creatocrit readings are usually in the range of 3% to 10%.⁶⁻⁸ The creatocrit value can then be used to estimate the lipid (g/L) and caloric (kcal/L) content by using either a regression graph or a conversion chart based on the regression equation.^{2,6-8} The technique provides an accurate estimate of lipid and caloric content in mothers' milk, yet is simple, quick, and inexpensive to perform.^{2,6-8}

Until the last several years, the creatocrit was used almost exclusively in the research setting, either in field studies in which more complicated human milk analyses were not feasible,⁹⁻¹⁵ or clinical investigations that sought to measure milk lipid changes in response to an independent variable.¹⁶⁻²² For example, Mitoulas et al.¹⁹ used the creatocrit to compare the completeness of breast emptying for different milk expression patterns with an electric pump, Brennan-Behm et al.²¹ used the creatocrit to determine which type of infusion tubing resulted in the least lipid loss during continuous gavage infusion of mothers' milk for premature infants, and Askit et al.²⁰ used it to measure the association between the lipid in mothers' milk and infant sucking characteristics.

The factors that make the creatocrit attractive as a research technique—accuracy, simplicity, and low cost—also make it ideal for the routine clinical management of common lactation and infant feeding problems. However, few published studies could be located in which practitioners used the creatocrit to diagnose and manage clinical problems. Meier et

al.,^{8,23} Vasan et al.,²⁴ and Slusher et al.²⁵ have used the creatocrit in the neonatal intensive care unit (NICU) to guide the fractionation of high-lipid, high-calorie hind milk for infant feedings, and Griffin et al.²⁶ demonstrated that the technique can be performed accurately by infants' mothers. Although there are several plausible explanations for this limited clinical application, the cumbersome equipment needed to perform the creatocrit is a likely factor.

Because the creatocrit originated as a research technique, it has traditionally been performed with laboratory-type equipment that does not transfer easily to the clinical setting. For example, the standard laboratory centrifuge weighs over 10 pounds, must be plugged into a power source, and makes a loud noise during the entire 5 minutes that spinning occurs. After centrifuging, the cream and total volume columns in the capillary tubes must be measured using vernier or digital (electronic) calipers^{2,6,7} or a hematocrit reader.⁸ Although accurate, all three measuring devices present limitations in a clinical environment. Vernier calipers are sharp, easily misplaced, and involve the additional step of measuring the caliper spread against a millimeter ruler. Digital calipers eliminate the need for the millimeter ruler, but are easily broken or lost, and require calibration repeatedly. Both types of calipers require mathematical calculation of the creatocrit. The hematocrit reader, while more practical than calipers, is space-consuming and requires several measurement steps to determine the creatocrit value. For all three of these methods, the actual creatocrit value must be determined first. Then, another chart, graph, or a calculator is used to convert this value to the estimate of lipid and caloric content.⁶⁻⁸ These equipment limitations make it difficult for clinicians to justify the noise, time, and space that creatocrit measures have required.

The Creatocrit PlusTM is a new centrifuge that weighs two pounds, can be battery powered, is noiseless, and centrifuges the milk in only 3 minutes. It is equipped with an embedded reader system that eliminates the need for either calipers or a hematocrit reader. The em-

bedded reader is programmed with software that prompts the user to press a button at specific interfaces along the capillary tube, and then calculates and digitally displays the creatocrit value. Further presses of the button convert the creatocrit value to the estimated lipid and caloric content, eliminating the need for regression graphs or conversion charts. These features make this device feasible for clinical use, especially in the NICU or other hospital and office settings, in which space is limited and sound levels are closely monitored and controlled. However, the Creatocrit Plus is a new instrument, so its reliability, equivalence to the standard equipment, and accuracy all must be established before it can replace traditional measurement methods.

The purpose of this study was to compare intra-user and inter-user reliability, and the equivalence of the creatocrit values obtained with the Creatocrit Plus to the two standard techniques for performing creatocrits: the standard laboratory centrifuge with a hematocrit reader and the standard laboratory centrifuge with digital calipers. The authors also sought to determine the predictive accuracy of the Creatocrit Plus for estimating the lipid and caloric content of mothers' milk.

MATERIALS AND METHODS

Sample

A total of 36 freshly expressed milk specimens were acquired from 12 mothers whose infants were cared for in a 52-bed Level III NICU. To be eligible for the study, the women were at least 7 days postpartum to ensure that specimens would consist of higher-fat mature milk rather than lower-fat colostrum. Mean gestational age and birthweight for the mothers' infants were 31.0 weeks (SD = 5.1), and 1629 g (SD = 854), respectively. The mothers' milk samples were obtained a mean of 19.8 days postbirth (SD = 10.2). Of the 12 mothers, five, five, and two, respectively, were black, white, and Latina. To be included in the study, mothers also needed to document a daily milk volume that exceeded the infant's requirements by ≥ 120 mL, so that infants could receive exclu-

sive mothers' milk feedings and sufficient extra milk could be stored for future use.

These 36 milk samples provided an estimated power of 0.85 to detect a difference of 2% in the actual creatocrit values, and a power of ≥ 0.80 to detect a 20% difference between the actual and estimated lipid and calories with an α of 0.05. This project was approved by the institutional review board for the medical center in which the study was conducted. Women provided written informed consent in either English or Spanish before enrollment in the study.

Procedures

At a mutually agreed upon time, the mother expressed her milk with an electric breast pump (Symphony; Medela, Inc., McHenry, IL) using a double collection kit, while one of the investigators directed the milk expression and collected milk samples. For each mother the investigator collected three separate milk specimens to provide a heterogeneous sample of milk for analysis: the first 20 mL removed from one breast (low-fat, low-calorie foremilk); the last 20 mL removed from that same breast (high-fat, high-calorie hind milk), and a 20-mL sample of the entire milk volume removed from the opposite breast (moderate-fat, moderate-calorie composite milk). Milk expression was not interrupted by the sampling procedures, because specimen containers were screwed onto and removed from the breast shield by the investigator when the 20-mL volumes were attained.

Each milk specimen was initially aliquoted into five 11-mL containers: one for immediate study (this investigation); one for study after refrigeration for 24 to 48 hours; one for study after freezing at -20°C for ≥ 24 hours; one for direct measurement of lipid and caloric concentrations (stored at -80°C until studied for this purpose); and one stored at -80°C to serve as a replacement in the event that any specimens were spilled or otherwise unusable. The refrigerated and frozen specimens were collected for future research to determine the effect of routine NICU milk storage procedures on the creatocrit value.

Two researchers, who were blinded to each other's measures, performed creatatocrits as follows. Using the milk specimen aliquoted for immediate study, each researcher filled four glass capillary tubes, gently shaking the milk specimen between each filling to ensure that milk lipid was well mixed. The four capillary tubes for each researcher were centrifuged and measured as follows. Each researcher placed two capillary tubes in the standard laboratory centrifuge, centrifuged them for 5 minutes, and then measured each tube using both the hematocrit reader and the digital calipers. Separately, each researcher centrifuged the other two tubes for 3 minutes in the Creatatocrit Plus; these tubes were measured using the embedded reader, as described. The researchers recorded their measurements on preprinted, color-coded index cards, which were turned over immediately after each measurement was completed. All index cards for each milk sample for the two researchers were placed into an envelope, which remained sealed until data collection had been completed.

Direct laboratory measures of lipid and caloric content were performed by investigators who were blind to creatatocrit measures and whether the samples were foremilk, hind milk, or composite milk. Milk lipid, lactose, and protein concentrations were determined by commonly used colorimetric spectrophotometric methods detailed in previous research by Mitoulas et al.²⁷ Then, the caloric content of the milk was calculated using the conversion fac-

tors for these lipid, lactose, and protein measures, as described in previously published research.²⁸

Data analysis

Data were processed and analyzed using Microsoft Excel (www.microsoft.com) and SPSS-PC Version 12.0 (Chicago, IL). Data analysis examined three aspects of the reliability and validity of the data: intra-user and inter-user reliability of both the new and standard methods of measuring the creatatocrit; equivalence of the new method to the standard methods; and the accuracy with which the new method estimated the actual lipid and caloric content of the mothers' milk.

Data were analyzed using procedures appropriate for evaluating the reliability, equivalence, and accuracy of physical measures, which included means, mean absolute differences, standard deviations, percentages of differences $\leq 1\%$ and $\leq 2\%$ creatatocrit, and Bland and Altman plots.³⁰⁻³² Data were described using frequencies and univariate statistics. Paired *t*-tests were used for statistical comparisons involving two dependent measures. Correlations were performed using Pearson's correlation coefficient. The relationship between creatatocrit measures and the actual lipid and caloric content were examined using linear and nonlinear regression analyses to determine the line of best fit. These nonlinear models included logarithmic, quadratic, cubic, growth, and exponential

TABLE 1. DESCRIPTION OF THE MILK

Measurement	n	M + SD	Range
Creatatocrit (%)			
Foremilk	12	7.09 ± 3.09	3.01-12.40
Hind milk	12	12.82 ± 3.92	7.34-19.76
Composite milk	12	8.68 ± 2.53	5.43-13.32
All specimens	36	9.53 ± 3.98	3.01-19.76
Lipid content (g/L)			
Foremilk	12	46.74 ± 20.90	18.30-79.30
Hind milk	12	80.11 ± 27.79	33.20-119.40
Composite milk	12	56.37 ± 18.64	29.10-85.30
All specimens	36	61.07 ± 26.30	18.30-119.40
Caloric content (kcal/L)			
Foremilk	12	787.91 ± 203.44	506.02-1106.84
Hind milk	12	1107.38 ± 260.44	651.47-1452.62
Composite milk	12	872.44 ± 177.22	604.26-1097.77
All specimens	36	922.58 ± 250.97	506.02-1452.62

models. A Type 1 error of 5% was used for all tests of statistical significance.

RESULTS

The standard laboratory measures for creatmatocrit, lipid content, and caloric content are presented in Table 1. The lipid and caloric content of the foremilk, hind milk, and composite milk specimens varied widely within and between women.

The mean absolute differences for both intra-user and inter-user reliability for creatmatocrit measures were $<1\%$ creatmatocrit for all three measurement techniques (Fig. 1). The percentages of intra-user differences that were $\leq 1\%$ and $\leq 2\%$ creatmatocrit were comparable for the three measurement techniques (Creatmatocrit Plus = 93.5% and 98.4%; standard centrifuge with hematocrit reader = 95.8% and 100%; standard centrifuge with digital calipers = 85.9% and 98.6%). The percentages of inter-user differences $\leq 1\%$ and $\leq 2\%$ creatmatocrit also were comparable for the three measurement techniques (Creatmatocrit Plus = 86.1% and 94.4%; standard centrifuge with hematocrit reader = 75% and 94.4%; standard centrifuge with digital calipers = 75.0% and 97.2%).

The equivalence among the creatmatocrit values obtained using the three measurement techniques is depicted in Figure 2 for foremilk, hind milk, and composite milk. The means and standard deviations are nearly identical for the three measurement techniques.

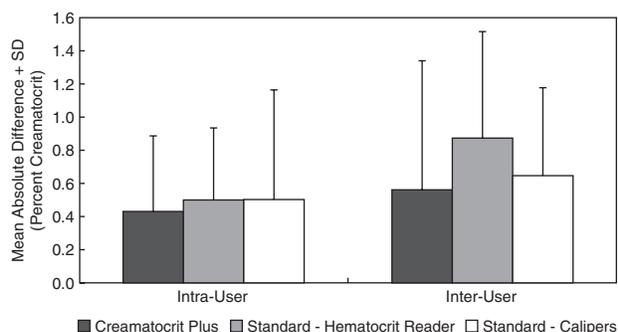


FIG. 1. Intra-user and inter-user reliability comparisons for the three Creatmatocrit methods. (Values denote mean absolute difference \pm SD.)

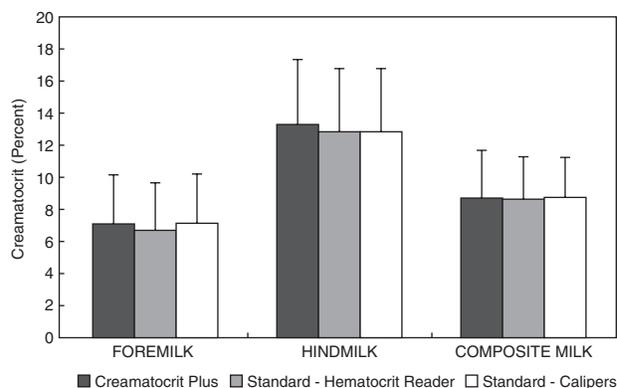


FIG. 2. Comparison of Creatmatocrit values obtained with the three measurement methods for foremilk (low-lipid, low-calorie), hind milk (high-lipid, high-calorie), and composite milk (moderate lipid, moderate-calorie). (Values are mean percent \pm SD.)

The linear relationship between the laboratory measures of lipid content and corresponding creatmatocrit values obtained with the Creatmatocrit Plus is depicted in Figure 3, and demonstrates a very high correlation ($r = 0.95$; $p < 0.001$) between the measures. Non-linear regression models did not significantly increase the percentage of variance explained by the linear model. Thus, the linear regression equation (lipid = $3.968 + [5.917 \times \text{Creatmatocrit}]$) was used to predict lipid content for the 36 milk specimens. The mean difference between the actual and predicted lipid content was very small (0.003 g/L , $SD = 7.93$) and not statistically significant. The mean absolute difference between the actual and estimated lipid content was 6.80 g/L ($SD = 3.91$). A Bland and Altman plot of the differences between the actual and estimated lipid contents is displayed in Figure 4.

The linear relationship between laboratory measures of caloric content and corresponding creatmatocrit values obtained with the Creatmatocrit Plus is depicted in Figure 5, and demonstrates a very high correlation ($r = 0.94$; $p < 0.001$) between the measures. Nonlinear regression models did not significantly increase the percentage of variance explained by the linear model. Thus, the linear regression equation (calories = $385.422 + [55.656 \times \text{Creatmatocrit}]$) was used to predict caloric content for the 36 milk specimens. The mean difference between actual and predicted caloric content was very

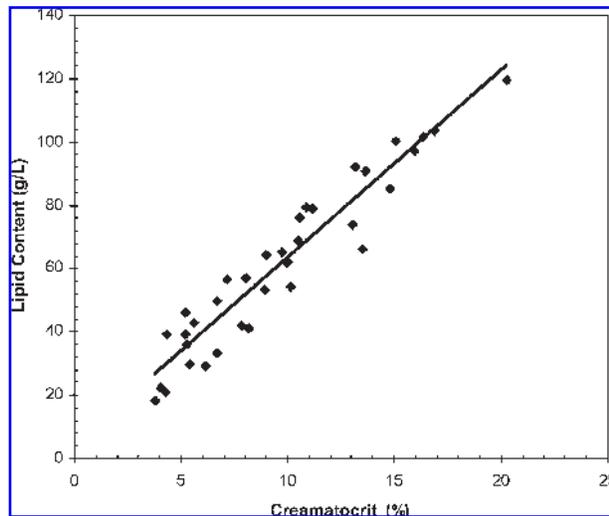


FIG. 3. Linear correlation between Creamatocrit and lipid content. ($r = 0.95$; $p < 0.001$; regression equation for lipid: $\text{lipid} = 3.968 + [5.917 \times \text{Creamatocrit value}]$).

small (0.004 kcal/L, SD = 85.79) and not statistically significant. The mean absolute difference between the actual and estimated lipid content was 73.61 kcal/L (SD = 42.26). A Bland and Altman plot of the differences between the actual and estimated caloric contents is displayed in Figure 6.

DISCUSSION

This study indicates that the Creamatocrit Plus performs comparably to conventional laboratory equipment with respect to intra-user and inter-user reliability, equivalence to creamatocrit values obtained by conventional methods, and predictive accuracy for lipid and caloric content. These findings are based upon a heterogeneous sample of milk specimens, with respect to creamatocrit, lipid, and calories (see Table 1). This distinction is important for both research and practice because previous studies have used specimens consisting primarily of colostrum, drip (collected from one breast while the infant suckled at the other), and banked milk,^{6-8,33,35-38} all of which have much lower lipid and caloric contents than occurs in routine clinical practice.¹⁵ The technique of securing foremilk, hind milk, and composite milk from each mother during a single milk expression used in this study resulted in a sam-

ple of specimens that reflects the range of lipid and caloric values commonly encountered in routine clinical practice.

Previous investigators have reported only the predictive accuracy of the creamatocrit for estimation of lipid and/or caloric contents,^{6-8,33-38} probably reflecting its primary utility as a research technique. However, in the clinical setting it is important to understand the differences in creamatocrit values that might occur with the same practitioner over time, or more commonly, between practitioners when measuring the same milk specimen. These findings serve as the basis for clinical protocols that delineate whether the same person should perform all creamatocrits, or whether the measuring instrument is sufficiently robust that different clinicians can perform the procedure and obtain comparable results.

Figure 1 demonstrates high intra-user and inter-user reliability for all three measurement techniques. The mean absolute differences obtained between users (inter-user) are only slightly higher than the mean absolute differences obtained by the same user for duplicate measures (intra-user). Overall, the magnitude of mean absolute differences for intra- and inter-user comparisons and for the three creamatocrit techniques is very small, <1.0% in all instances, which is not clinically appreciable. Thus, these findings indicate creamatocrit measures can be performed by more than one person, with the confidence that the measures obtained by different clinicians are comparable.

As apparent from Figure 3, the Creamatocrit

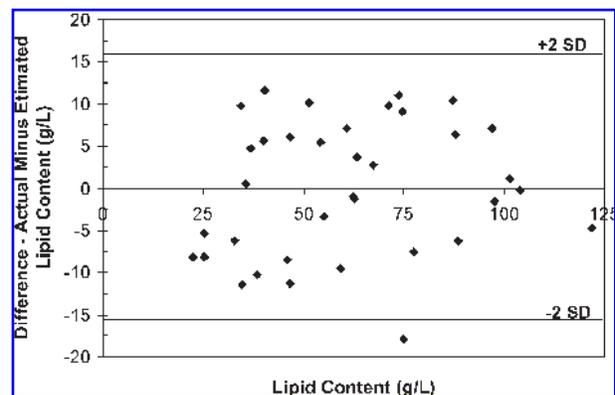


FIG. 4. Bland and Altman plot of differences between actual and estimated lipid content.

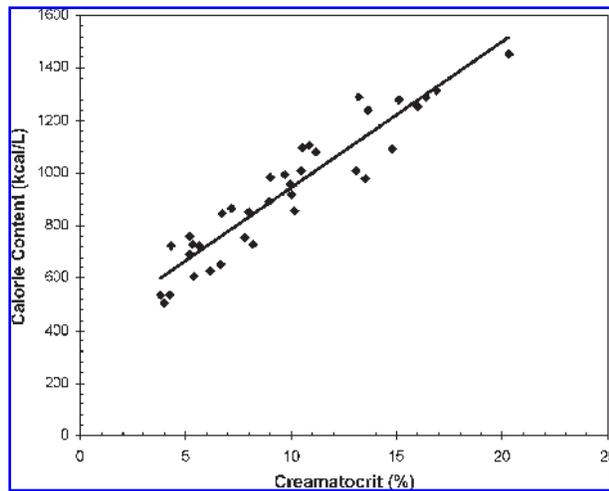


FIG. 5. Linear correlation between Creamatocrit and caloric content. ($r = 0.94$; $p < 0.001$; regression equation for calories: calories = $385.422 + [55.656 \times \text{Creatocrit value}]$).

Plus provided an accurate estimate of lipid content when creatocrit values were compared to direct laboratory measures. These findings are comparable to linear correlations of 0.92 to 0.99 that have been reported by previous investigators who used conventional laboratory equipment for measuring creatocrits.^{6-8,33-38} Similarly, the Creatocrit Plus provided an accurate estimate of caloric content when creatocrits were compared to direct laboratory measures, as depicted in Figure 5. The authors' linear correlation of 0.94 between actual and estimated calorie content was comparable to those reported for conventional laboratory techniques in previous studies.^{7,8,34} The Bland and Altman plots for both lipid (see Fig. 4) and calories (Fig. 6) did not reveal any systematic error in the relationship between estimated and actual measures.

Upon completion of this study, the software for the embedded reader in the Creatocrit Plus was programmed with the regression equations for prediction of lipid and calories, as reported in the authors' findings. Thus, after determining the actual creatocrit value (%), the estimated lipid and calorie contents of the milk sample can be displayed with subsequent presses of the button used to measure the creatocrit value. In the research setting, the investigator can choose either to use these programmed regression equations or establish lab-

oratory specific regression equations to estimate lipid and calories from the measured creatocrit value.

A limitation of this study is that the creatocrit measures reported here were performed exclusively with fresh milk specimens that were studied within 1 hour after removal from the breast. Little is known about the effect of temperature and storage on the creatocrit,^{35,37} and the freezing of milk specimens is routine in clinical and field research studies which incorporate the creatocrit technique. However, human milk lipases are not inactivated by storage at either -4°C or -20°C ,^{2,39} which correspond to refrigeration and freezing, respectively, in most home and hospital situations. Theoretically, the lipases would be capable of "digesting" milk lipids during storage, and subsequent creatocrits with these samples could slightly underestimate the actual lipid and calorie content. Whether or not these differences are clinically appreciable is unknown, but is under investigation by the authors' research team.

CONCLUSION

In summary, the authors conclude that the user-friendly Creatocrit Plus can replace cumbersome laboratory equipment for measuring creatocrits on mothers' milk in the clinical setting. The findings of high intra-user and inter-user reliability, equivalence of creatocrit

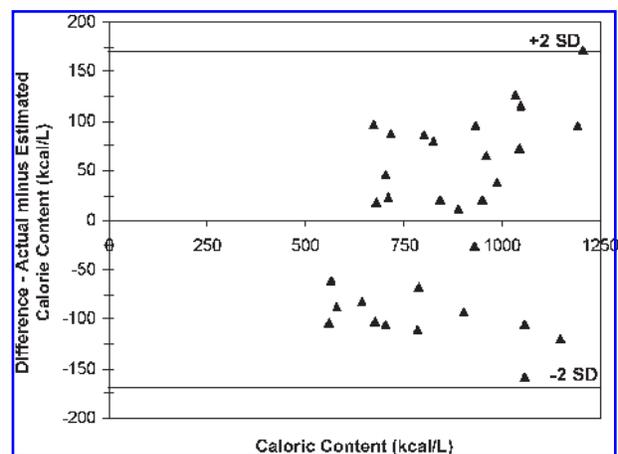


FIG. 6. Bland and Altman plot of differences between actual and estimated lipid content.

ocrit measures to those obtained with the standard laboratory equipment, and high predictive accuracy for lipid and caloric content demonstrate the clinical utility of this instrument.

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