Body Fat Content Determination in Premenopausal, Overweight, and Obese Young Women Using DXA and FT-NIR

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Even though BMI is the most commonly used method for assessing and monitoring obesity, it does not take into account the individual's body fat content assuming instead that body mass is closely associated with body fat, which is a tenuous assumption. The aim of this study was to make a direct comparison between measurements of body fat content using a convenient and rapid Fourier transform near-infrared (FT-NIR) spectroscopy and dual-energy X-ray absorptiometry (DXA). We recruited 52, premenopausal women (age range 19–45), all of whom had a BMI that classified them as either overweight or obese (range: 27–40 kg/m^2, mean: 31.1 ± 3.7 kg/m^2) and indicated a statistically significant linear relationship between the fat content in kilograms measured by FT-NIR and DXA (r = 0.95, P < 0.001). Bland–Altman analysis showed that almost all the differences between two measurements fell within 2 s.d. We report here that the FT-NIR method provided comparable measurements of subcutaneous body fat content similar to those of total fat obtained using DXA. The FT-NIR method is a lower cost, easy to use and transport, and, based on comparison with DXA, an accurate method to measure body fat content. We propose that FT-NIR is an ideal method for safe repeat measurements in large trials or in screening and monitoring individuals during interventions in which changes in body fat will occur.

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INTRODUCTION

BMI is the most common variable calculated and reported as an estimate of overweight and obesity (1–4). However, it is body fat, and not body weight that is the primary physiological risk factor for morbidity and mortality (5). BMI does not take into account the individual's body composition and its guidelines assume that body mass is closely associated with body fat (6), which is not always the case. For example, individuals with a BMI of 19–24.9 kg/m^2 have been reported to have a total body fat content varying from ~23 to 42% (measured by magnetic resonance imaging (MRI)) (7). Thus, BMI is not a good indicator of fat content and there is a substantial range of body fat content in people who, according to the BMI guidelines, are considered at low risk. Interestingly, BMI is also not as strong of a predictor for cardiac mortality as either waist-to-hip ratio or waist circumference (8), both are proxy markers for visceral body fat, which is a strong predictor of disease risk. In light of this finding and the fact that body fat and not body weight is the primary risk factor for morbidity and mortality (5,8), a reliable measure of body fat is needed to rapidly assess risk.

Accurate methodologies are currently available to measure body fat content including dual-energy X-ray absorptiometry (DXA) and MRI, which show good agreement and would both correctly classify adiposity phenotype and estimate risk (9). However, neither the MRI nor DXA method would be suitable for routine fieldwork in large-scale studies where a quick and easily transportable method would be desirable. On the other hand, the bioelectrical impedance analysis method is considered to be easily transportable, although not as accurate as either MRI or DXA. Kim *et al.* (10) reported that bioelectrical impedance analysis overestimated body fat and underestimated lean body mass when compared to DXA in a 6-week herbal diet intervention program in 50 premenopausal obese (by BMI) women.

Azizian *et al.* (11,12) reported a rapid and noninvasive method to measure subcutaneous body fat using Fourier transform near-infrared (FT-NIR) spectroscopy, where the reflectance of near-infrared light from the upper part of the ear was compared to reference materials with known fat content. They also demonstrated that the results can be validated by an indirect comparison with results obtained by MRI in different individuals, or by the use of equations that incorporate body surface area, NIR response, as well as MRI data to convert subcutaneous to total body fat (11). In the present communication, we

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report a direct comparison between FT-NIR and DXA measurements in the same subjects; FT-NIR obtained a measure of subcutaneous body fat, which was based on the comparison to a reference material. Subjects underwent a 16-week diet- and exercise-induced weight loss program and we compared body fat content and percent body fat obtained by these two methods at various time points throughout the study.

METHODS AND PROCEDURES

Subjects

All subjects were recruited from McMaster University (Hamilton, Ontario, Canada) and the surrounding Hamilton city area through posted advertisements or word of mouth. Otherwise, healthy, premenopausal, nonpregnant women between the ages of 19–45 years, with a BMI between 27 and 40 kg/m^2 were selected. These overweight and obese women were all part of a larger clinical trial that was designed to assess the effect of a particular hypoenergetic diet- and exercise-induced weight loss program. The majority of subjects who took part in this FT-NIR vs. DXA substudy were scanned more than once and up to three times throughout their participation in the larger clinical trial. The full study protocol, which included (optional) FT-NIR scanning, was approved by the McMaster University Medical Research Ethics Board and conformed to all standards of Canada's Interagency Panel on Research Ethics for conducting human research (http://www.pre.ethics.gc.ca/english/index.cfm). All subjects gave their written consent to participate in the study having read and understood all of the risks and procedures.

DXA

Subjects underwent whole-body DXA scans (QDR-4500A; Hologic, Waltham, MA) three times throughout the study (week 0, 8, and 16) at the McMaster University Medical Centre to determine body composition with a specific focus on fat mass and %body fat. All scans were performed by the same investigator (A.R.J.). Study participants were scanned at the same time of day wearing either a standard hospital gown or light clothing (the same for each scan) and were asked to refrain from vigorous exercise that day. As per protocol, the DXA machine underwent quality control testing daily to ensure no significant deviations existed in the day-to-day variability. On some occasions, corrections had to be made to DXA measurements for subjects with higher body weights because the scanning field was slightly smaller than the subject's body. In these cases, adjustments were made whereby the side of the upper body (fingers/arm) that was in full view was used as a surrogate for the other side that was cutoff (**Figure 1**). Similar adjustments have been made previously in other studies where subjects' body dimensions exceed the length or width of the scanning bed (13).

FT-NIR methods and procedures

A matrix-F FT-NIR spectrometer equipped with a standard fiber optic probe (Bruker Optics, Milton, Ontario, Canada) in combination with signal processing software (OPUS; Bruker Optics) was used to obtain the spectra. The fiber optic probe carrying the He-Ne laser (Class IIIA, 4 mW) focused a near-infrared light that was held up to the back of the subjects' upper ear (to avoid eye contact); see Azizian and Kramer (14) for details on the technique of scanning. Several measurements per subject were taken from various parts of the upper ear (cartilage area) and sometimes from the ear lobe. The whole measure took <3 minutes. Absorption spectra were averaged and the average spectrum was used in conjunction with the calibration curve which was based on a synthetic reference material that resembled human tissue in water, fat, and protein content (**Figure 2**), to determine the subcutaneous fat content of each subject at various times in the study, generally at the beginning (0 weeks), between weeks 7 and 9, and again between weeks 15 and 16. We did not consider it necessary in this study to validate our results using the reference material with the use of the equations since we had already demonstrated the accuracy of data obtained using the reference material in a previous publication (11). In fact, we converted the subcutaneous fat content obtained using FT-NIR to total body fat using the previously determined MRI ratio of subcutaneous to total body fat (11), but the calculation only showed a small increase (data not shown), which was within the experimental error of FT-NIR and DXA. Thus, only the subcutaneous FT-NIR results were used in this study.

Some minor adjustments to this procedure were made in a few cases. The FT-NIR method is based on the reflectance of the light from the cartilage (14), therefore, in the first instance a much lower than expected reading for a subject was obtained due to some scarring on the ear cartilage from a prior ear piercing. This cartilage damage caused light scattering, therefore a new measurement was collected from a different area of the ear, avoiding the scarred part of the cartilage. In a separate instance, again due to reflectance of the light, the measurements within a person differed when the upper part of the ear was scanned closer to the outer border of

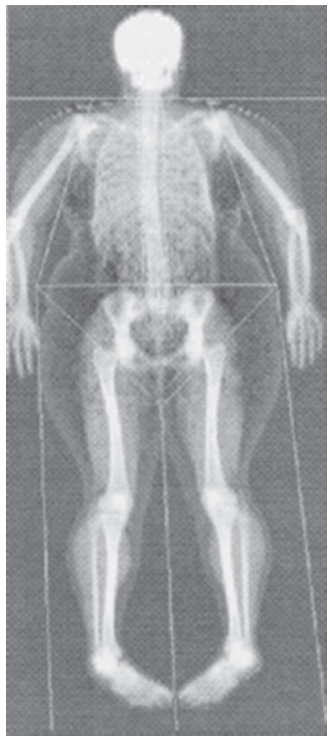

Figure 1 Dual-energy X-ray absorptiometry (DXA) scan of one subject who did not completely fit on the DXA scan bed.

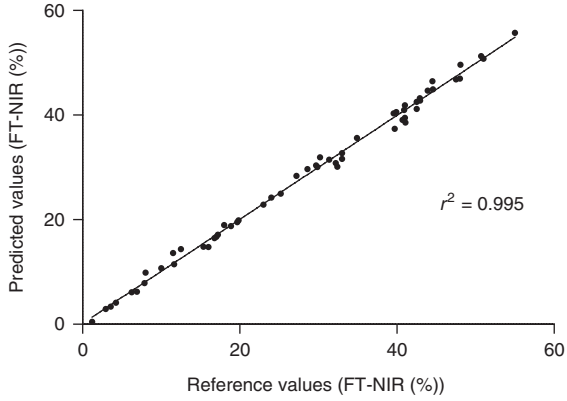

Figure 2 Fourier transform near-infrared (FT-NIR) spectroscopy calibration curve for fat content determination.

the ear vs. the middle. This was due to variations in the thickness of the skin layer near the edge of the ear. The maximum light penetration depth is about 2 mm and in this case, the edge of the ear was thicker. This would be akin to scanning without cartilage where reflectance is significantly reduced. Figure 3 shows the first-derivative spectrum of this subject's scan near the edge of the ear (solid line) and the back middle part of the ear (dotted line), which predicted a subcutaneous body fat content of 33.5 and 44.6%, respectively. These two measurements represented a substantial difference in the fat content of this subject; the comparable measurement by DXA was 43.0%. The lack of proper reflectance on the edge of the ear was confirmed by repeating the scans for several other subjects with similar results.

Statistics

Descriptive statistics of age and body composition measurements are presented as the mean ± s.d. (Table 1). We used Pearson's correlation coefficient (r) to examine the correlation between fat content (kg) determined by DXA and FT-NIR and the correlation between subjects' body weights and %body fat from both DXA and FT-NIR. We also used the Student's t-test to compare the fat content values (kg) obtained by DXA and FT-NIR. The Bland–Altman analysis (15) was used to evaluate the validity of comparing the body composition results between the two methods (FT-NIR and DXA). Data were analyzed using SIGMASTAT statistical software (version 3.10, 2004; Systat Software, San Jose, CA). P values of <0.05 were considered statistically significant.

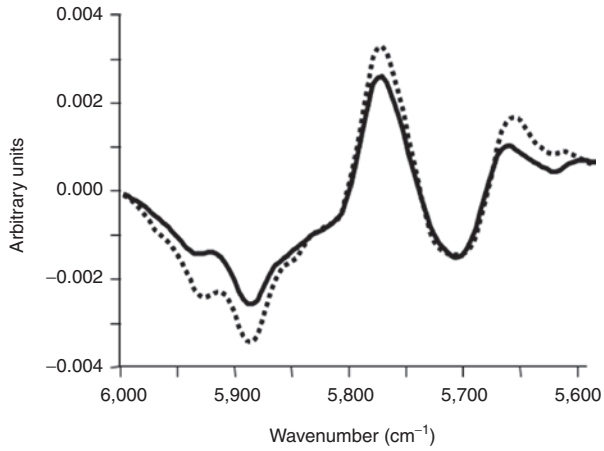

Figure 3 First-derivative spectrum of one subject measured on the back of the ear in the middle (dotted line) and closer to edge (solid line). The more accurate reading (the one that more closely matched that obtained by dual-energy X-ray absorptiometry (DXA)) was taken from the back middle part of the ear.

Table 1 Subject characteristics and DXA and FT-NIR-based fat mass variables of 91 separate measurements of individuals

	Multiple measurements per subject
Variable	**Mean ± s.d.**
Number of subjects	$n = 52$
Number of FT-NIR and DXA measurements	$n = 91$
Age (years)	30.0 ± 7
Weight (kg)	83.6 ± 14
Height (m)	1.6 ± 0.1
BMI (kg/m^2)	31.1 ± 4
Fat—DXA (%)	37.9 ± 5
Fat—DXA (kg)	32.1 ± 9
Fat—FT-NIR (%)	37.8 ± 5
Fat—FT-NIR (kg)	32.0 ± 8
Difference ((FT-NIR) – DXA) (kg)	0.13 ± 2.6
Mean ((FT-NIR) + DXA)/2 (kg)	32.6 ± 9

DXA, dual-energy X-ray absorptiometry; FT-NIR, Fourier transform near-infrared spectroscopy.

RESULTS

Fifty-two subjects were assessed in this study. Fat content was measured between one and three times in each subject throughout the study using both the FT-NIR and DXA methods. The subject characteristics are shown in Table 1. Figure 4 shows the plot of weight (kg) for each individual in relation to their fat content (%) showing both DXA (open circles) and FT-NIR (closed circles) results. The Pearson correlation coefficients obtained on comparison of the subjects' body weight (kg) with fat content (%) by both DXA and FT-NIR were 0.65 ($P < 0.01$) and 0.51 ($P < 0.01$), respectively. It is evident from this graph that individuals with the same body weight show a wide range of body fat content.

Placing the subjects into three groups (Figure 5) based on currently used BMI categories (group 1 BMI 19–24.9 kg/m^2; group 2 BMI 25–29.9 kg/m^2; group 3 BMI 30–39.9 kg/m^2) showed the extent of variation within each group. This phenomenon was observed for both DXA (open circles) and FT-NIR (closed circles). The three measurements in group 1 (BMI: 19–24.9 kg/m^2) were taken from subjects following the completion of the program. There was a wide variation in the fat content of individuals within each BMI group, with some individuals in the lower BMI range having similar body fat contents to those in higher BMI groups. This was particularly evident with the degree of overlap in body fat content in groups 2 and 3 (Figure 5).

Figure 6 shows a statistically significant relationship between DXA (kg) and FT-NIR (kg) fat content measurements for all subject scans, $r = 0.95$ ($P < 0.001$). There was no statistical dif-

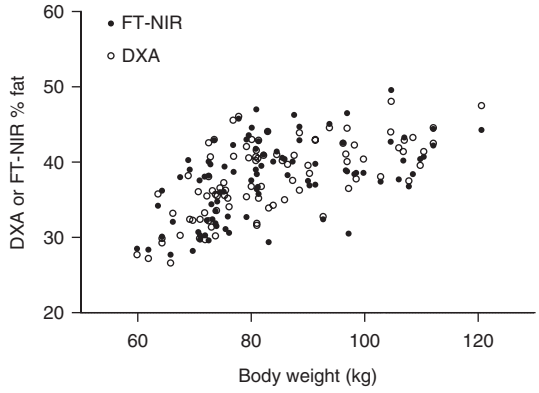

Figure 4 Relationship between body weight (kg) and fat content (%) in the same individuals determined by both Fourier transform near-infrared (FT-NIR) spectroscopy (closed circles) and dual-energy X-ray absorptiometry (DXA) (open circles). R values for body weight vs. DXA and FT-NIR were 0.65 and 0.51 ($P < 0.01$), respectively.

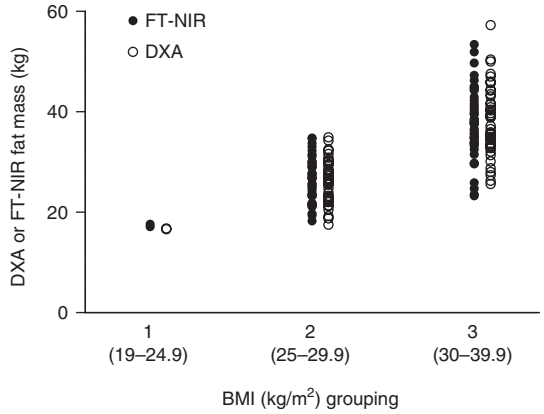

Figure 5 Relationship between weight and fat content based on BMI subgroups. Body fat (kg) determined by Fourier transform near-infrared (FT-NIR) spectroscopy (closed circles) and dual-energy X-ray absorptiometry (DXA) (open circles) for each individual grouped according to BMI: group 1, BMI 19–24.9 kg/m^2 (n = 2); group 2, BMI 25–29.9 kg/m^2 (n = 41); group 3, BMI 30–39.9 kg/m^2 (n = 48). The numbers of individuals (91) in each group are given in brackets. The FT-NIR and DXA results within each BMI group are presented on separate lines for clarity.

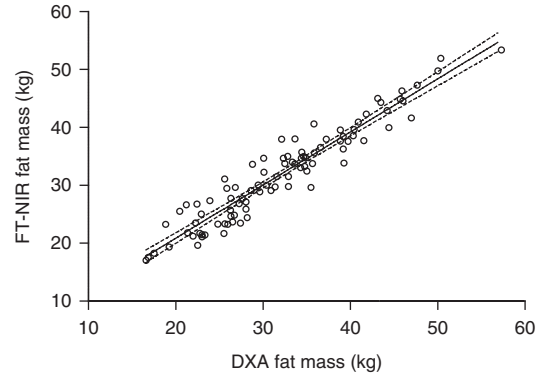

Figure 6 Correlation between individual subjects' fat content (kg) measured by dual-energy X-ray absorptiometry (DXA) and Fourier transform near-infrared (FT-NIR) spectroscopy. R value is 0.95, P < 0.001. This graph shows the line of best-fit by least squares linear regression and the 95% confidence interval.

ference between the fat content (kg) values obtained by DXA vs. FT-NIR as assessed using a paired t-test (P = 0.65).

We also performed a Bland–Altman analysis (15) of the differences between the FT-NIR and DXA (kg) results on each of the paired measurements (**Figure 7a,b**). Most of the measured differences were between ± 2 s.d. from the overall mean difference for the group (**Figure 7a**). **Figure 7b** shows the individual body fat measures from DXA and FT-NIR ± 1 s.d. The measurement bias was low (0.13, **Table 1**) indicating that the two methods produced similar results. Moreover, the plot shows no evidence of systematic bias at higher or lower body weights, which indicates no biased variability as the measured fat mass increased or decreased. The s.d. of the bias was 2.6 kg and the 95% confidence interval range was from −5.0 to 5.2 kg. This degree of variability is in line with the between-measurement technical error for % fat estimates (3–4%) by DXA reported by Boyer *et al.* (9).

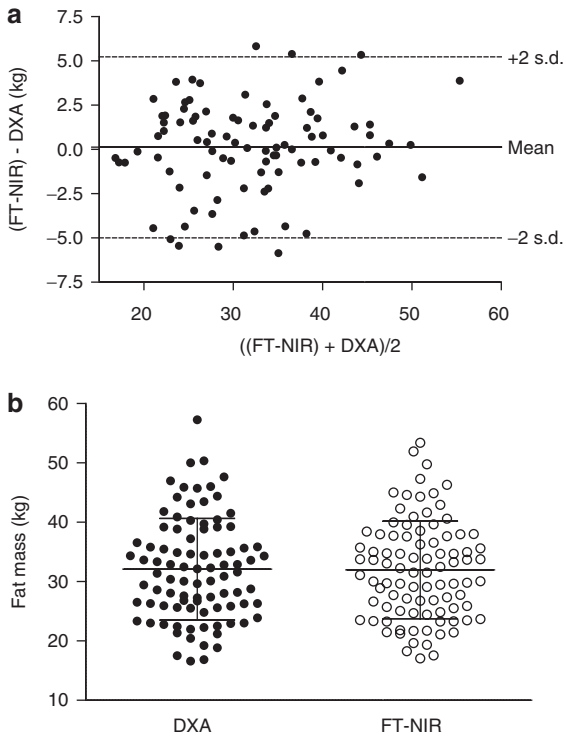

Figure 7 Agreement between the two methods for measuring fat mass. (**a**) Bland–Altman analysis of dual-energy X-ray absorptiometry (DXA) and Fourier transform near-infrared (FT-NIR) spectroscopy fat mass values (kg) showing the mean and 2 s.d. (**b**) Individual body fat content (kg) determined using FT-NIR and DXA showing the mean ± 1 s.d.

DISCUSSION

The primary purpose of the present study was to compare the FT-NIR method for body fat determination to the more established DXA method. DXA has been demonstrated as a reliable method to monitor fat mass reduction (9,16). However, DXA does have some disadvantages, which relate to a low-level radiation exposure, cost, and nonportability (13). These drawbacks make DXA somewhat impractical for large-scale trials with repeated measurements of body fat. Both DXA and FT-NIR fat content results showed a statistically significant linear relationship across a wide range of BMIs. In addition, subcutaneous and visceral fat decrease to a similar extent with diet and exercise (7). Hence, while FT-NIR only gives estimates of subcutaneous fat, its measurement alone should provide meaningful information for monitoring obesity in general and in particular during weight loss.

Even though BMI is the most commonly calculated variable in monitoring and categorizing overweight and obesity it does not take into account fat content or distribution, and clearly weight alone does not differentiate between body fat mass and lean mass. Other methods such as DXA (9,16), MRI (7,9), bioelectrical impedance analysis (10), and FT-NIR (11,12) do differentiate between fat and muscle mass and therefore should be implemented more readily in studies and clinics that assess the relationship between body composition and disease risk reduction. The wide variation in body fat content observed among the participants in this study within each of the BMI categories (**Figure 5**) is consistent with similar MRI findings

by Thomas *et al.* (7). Clearly, our data and that of others provide sufficient evidence to indicate that there is actually very little relationship between BMI and fat content making the continued use of BMI for categorizing risk as suspect.

There is substantial evidence indicating that a reasonable correlation exists between total fat, and subcutaneous and visceral fat in individuals. Thomas *et al.* (7) reported that of their total body fat, subject's subcutaneous fat content was ~85% and visceral fat content was ~15%. Azizian *et al.* (11) showed similar results based on MRI data and also found that the ratio of the two fat compartments was age-dependent with the ratio of subcutaneous to visceral fat decreasing as age increased particularly in women. In this study, we did calculate total body fat from our subcutaneous measures (data not shown) but the difference was very small so we report here our actual subcutaneous data only. We feel that this is appropriate considering the strong relationship that exists between total and subcutaneous fat contents (7).

Boyer *et al.* (9), when comparing several methods for measuring body composition, reported that the highest Pearson correlation coefficient obtained was between MRI and DXA. The variation in the differences between MRI and DXA was small and the correlation between the average and the difference was virtually zero. However, both of these methods have drawbacks that relate to nontransportability, cost, and limited accessibility. In the case of DXA, there is an additional concern of exposure to X-rays, and the physical limitations of the instrument size when conducting a whole-body scan of larger individuals (a limitation of MRI also). Although a single exposure may be around 0.3–2 μSv of radiation (17–20) (i.e., less than the amount acquired during a day from natural background radiation sources, which is ~2–8 μSv (19,20)), repeat measurements to monitor changes in body composition during weight loss may not be recommended and the use of DXA for repeat measurements in, for example, pediatric populations may also be unwarranted.

The burdgeoning prevalence of obesity and associated comorbidities such as type 2 diabeties and coronary artery disease needs to be addressed. To monitor, in large numbers, the increased prevalence and/or ability of interventions to affect change in fat mass, a rapid, safe and low cost method that accurately determines body composition is required. A common and effective recommendation for those with chronic disease is to lose body weight, but this recommendation is without recognition of the importance of lean mass. Thus, the recommendation should be to lose body fat. At this time, however, unless less expensive and more accessible technologies are available health-care practitioners and/or researchers are not routinely able to distinguish between weight loss as fat vs. weight loss as lean mass or body water. This poses a problem since disease risk reduction is strongly related to fat loss and inversely related to muscle loss, and not necessarily weight loss *per se* (21–23). In fact, the weight lost would actually be greater with greater loss of lean mass. Here, we report on a rapid, convenient, low risk methodology that uses a validated technique (11–12,14) of FT-NIR to measure body fat and we have directly compared this method with DXA methodology with good agreement. Results of the Bland–Altman analysis show very little and, importantly, no systematic bias indicating a good measurement capacity as compared to DXA. Further refinement of the FT-NIR method in this population, taking into account the newly identified measurement discrepancies outlined in this article, would likely improve the predictive capacity of this method.

We conducted our comparison in a population of overweight and obese young women who would be considered at risk for chronic disease and who also underwent a hypoenergetic diet- and exercise-induced weight loss program. FT-NIR was previously demonstrated to provide fat content measurements comparable to MRI, although these correlations were established using matched pairs of individuals (11) instead of the same subjects. It is evident from this and previous studies that the three methods, MRI, DXA, and FT-NIR, unlike BMI, provide an accurate measure of the fat content of individuals upon which meaningful health decisions can be made. The FT-NIR method is quick, easy to use, reliable, transportable and low-risk, and has been demonstrated to provide measures of fat mass that correlate strongly with those obtained using proven and reliable methods. Thus, FT-NIR technology may be able to provide the health-care community with an accurate means to repeatedly and safely monitor patients' body composition in clinics and those on weight/fat loss programs.

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DISCLOSURE

The authors declared no conflict of interest.

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