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# **In Vivo Body Composition Studies**

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K J Ellis, S Yasumura and W D Morgan

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## CHAPTER 25 Noninvasive Body Composition in Humans by Near Infrared Interactance

J M Conway and K H Norris

Energy and Protein Nutrition Laboratory and  
Instrumentation Research Laboratory,  
USDA/ARS, Beltsville, MD 20705, USA

### 25.1 Introduction

Since 1965, near infrared spectroscopy has been used to determine the chemical composition of forage and foodstuffs, i.e., the protein, fat, and moisture content<sup>1-4</sup>. Each of these major components has specific absorption characteristics in the near infrared region which are due to the stretching and bending of hydrogen bonds associated with carbon, nitrogen, and oxygen. These absorption characteristics determine the diffuse reflectance that enables composition measurement. This technology has been applied to the study of human body composition, in particular to the estimation of per cent body fat. Reported here are the results from two studies conducted at the Beltsville Human Nutrition Research Center to evaluate near infrared interactance as a possible method to determine per cent body fat in humans.

### 25.2 Materials and methods

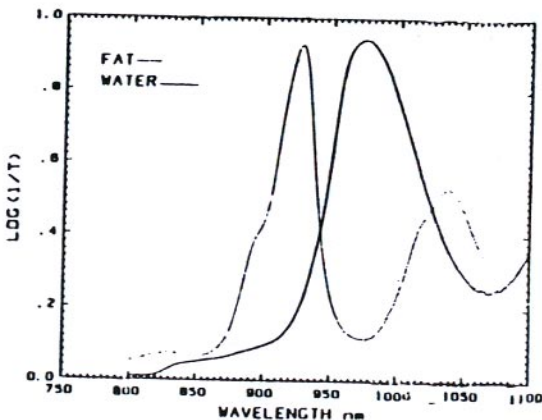
In the first study, 53 men and women, aged 23 to 65 years and ranging in per cent body fat from 12 to 50, had body composition estimated by skinfold thicknesses (SF) and by isotope dilution ( $D_2O$ )<sup>5</sup>. In the second study, to which hydrostatic weighing (UWW) was added, 68 men and women, aged 20 to 61 years and with body fat between 4.5 and 40 per cent, participated. In both studies infrared interactance (IRI) data were collected. All subjects were deemed healthy by physical and biochemical evaluation. Research protocols were approved by the human studies committees of the USDA/ARS, Georgetown University, and the University of Maryland.

Near infrared measurements were made at the standard anatomical locations used in skinfold measurements. These sites included triceps (Tr), biceps (Bi), subscapular (Sc), suprailiac (Si), and mid-thigh (Th). Each site was scanned 30 times in triplicate to yield a total of 90 scans per site. About 15 minutes were required to measure and store the data from all five sites on each subject. An average spectrum was computed for each subject by averaging across the triplicates of all five sites. Optimal linear regressions

between the ratio of two second derivatives of the average spectral data and per cent fat from D<sub>2</sub>O, SF, and UWW, where applicable, were determined.

Spectral measurements were made using a computerized spectrophotometer Model 6250, (Noctec Instruments Division of Pacific Scientific, Silver Spring, MD). The instrument uses a single-beam rapid scanning monochromator and a fibre optic probe. Scans were made over the midrange wavelengths (700 to 1100 nm). The fibre optic probe conducted the radiation from the monochromator to the selected site on the body, collected the interactive radiation (IRI), and conducted it to the detector. Opaque black felt was fitted around the end of the probe to prevent stray radiation from entering each specific site.

All data were processed to  $\log I/I$  ( $I$  = interactance) to be similar to absorption spectra plotted as  $\log I/T$  ( $T$  = transmission). Earlier work in analysis of agricultural foodstuffs has shown that  $\log I/T$  varies linearly with concentration of a specific absorber in a mixture with other materials, and that pure fat absorbs at 930 nm, with water absorbing at 970 nm (*figure 25.1*). The data reduction treatment used the ratio of second derivatives of the  $\log I/I$  data obtained at two different wavelengths. The mathematical transformation to the second derivative is a standard procedure in near infrared spectroscopy to reduce effects of such variables as particle size and temperature<sup>4</sup>.



**Figure 25.1** Near infrared spectra from samples of pure pork fat (---) and distilled water (—).

The ability of the IRI method to predict the per cent fat of an unknown population was tested by developing a calibration (prediction) equation, using average curves for each subject, on one population (Study 1), and predicting another population (Study 2). This prediction was then compared to the actual per cent fat as estimated by deuterium oxide dilution.

Average curves were also generated for each individual site (Tr, Bi, Sc,



Si, Th) for each subject. These site-specific, averaged spectral data were used in regression analyses with data from  $D_2O$ , SF, and UWW to determine the site that gave the most reliable information.

To study the possibility of developing a lower cost, portable instrument, a computer simulation of a wide-slit spectrophotometer was done by the multiple regression of deuterium oxide dilution data as the dependent variable against a selection of independent variables including highly smoothed spectral values (equivalent to 50 nm), weight (wt), height (ht), sex, age, and race.

### 25.3 Results

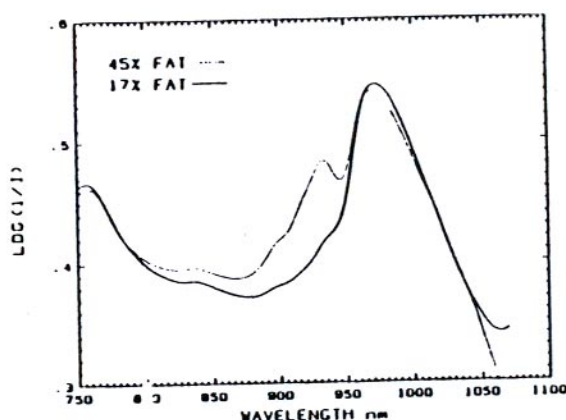


Figure 25.2 Averaged near infrared spectra (from Tr, Bi, Sci, Si, Th) from one subject with 17% body fat (—) and from another subject with 45% body fat (....).

Figure 25.2 shows the average spectrum for a subject who was found to have 45 per cent fat by deuterium oxide dilution. There is significant absorption in the 930 nm region, the fat band. It also shows the spectrum for a subject with 17 per cent body fat which exhibits less absorption in this region. Here the relative magnitudes of the fat and water bands indicate a much lower fat level for this subject compared to the preceding one.

Table 25.1 Correlation coefficients  $r$ (see) between near infrared interactance and isotope dilution ( $D_2O$ ), skinfold (SF), and hydrostatic weighing (UWW).

	$D_2O$	SF	UWW
IRP <sup>a</sup>	0.94 (3.0)	0.86 (4.4)	—
IRB <sup>b</sup>	0.90 (3.2)	0.82 (3.9)	0.85 (4.3)

<sup>a</sup>Study 1,  $d^2/d^2$  916 nm/1026 nm,  $n = 53$

<sup>b</sup>Study 2,  $d^2/d^2$  870 nm/945 nm,  $n = 68$

The correlation coefficients between values derived from the D<sub>2</sub>O, SF, UWW and average IRI data were generally high, ie, 0.82 to 0.94 (table 25.1). The standard errors of the estimate ranged from 3.0 to 4.4 per cent body fat. These correlations were determined from optimal regressions with the first study using the second derivative ratio of 916 nm/1026 nm. The optimal regression for the second study was found to use the second derivative ratio of 870 nm/945 nm.

Optimum regression results with D<sub>2</sub>O combining both studies were found at the second derivative ratio of 867 nm/914.5 nm, using spectral data averaged from all sites. The calibration equation was

$$\% \text{ body fat} = -4.2 + 64.2 \frac{\text{second derivative (867nm)}}{\text{second derivative (914.5nm)}};$$

$r = 0.91$  ( $p < 0.001$ ), SE of estimate = 3.7 per cent body fat. The relationship between IRI-predicted fat and the per cent body fat as determined by deuterium oxide dilution is illustrated in the regression plot (figure 25.3). The SE of prediction was 4.0 per cent body fat, with a bias of + 1.7 per cent.

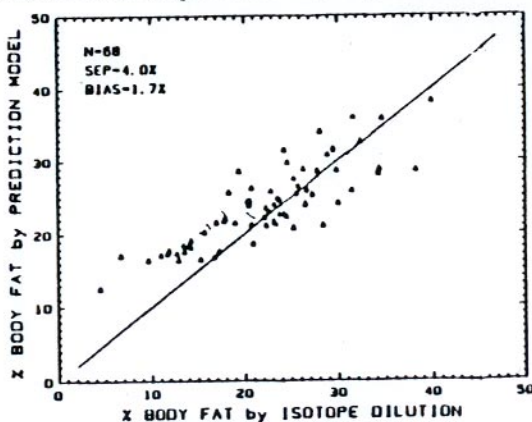


Figure 25.3 Relationships in 68 subjects (Study 2) between per cent body fat as predicted by the IRI second derivative model and per cent body fat as estimated by the D<sub>2</sub>O dilution technique. The IRI prediction equation was based on data (IRI and D<sub>2</sub>O) obtained on 52 subjects (Study 1). The line shown is the line of identity.

In the second study, a comparison was made of IRI data collected from each of the five sites using the optimal calibration for that study. The data from the individual sites were compared to data from the average spectra (table 25.2). Using information from the biceps site alone or an average of the biceps and triceps spectral data gave correlations, with D<sub>2</sub>O, SF, UWW, which were as good as IRI data resulting from an average of all five sites. These correlations,  $r$  (SEE), ranged from 0.81 (4.0) to 0.90 (3.2).

Table 25.2 Comparison of correlation coefficients  $r$ ( $se$ ) from selected anatomical locations.

	Sites	D <sub>2</sub> O	UWW	SF
IRI*	Average	0.90 (3.2)	0.85 (4.3)	0.82 (3.9)
IRI*	Tr&Bi	0.90 (3.2)	0.87 (4.2)	0.82 (3.9)
IRI*	Tr	0.88 (3.5)	0.84 (4.5)	0.84 (3.7)
IRI*	Bi	0.90 (3.2)	0.89 (3.8)	0.81 (4.0)

\*Optimal regression  $d^2/d^2$  870 nm/945 nm

A comparison between spectra generated by the computerized spectrophotometer and spectra generated by computer simulation of a wide-slit instrument is shown in figure 25.4. The bottom half has spectra from the computerized instrument, which has an equivalent bandpass of 6 nm. The spectra in the top portion were generated by smoothing to an equivalent of 50 nm. The spectrum drawn with a dotted line is higher in fat and lower in water than the spectrum drawn with a solid line. In both sets of spectra the absorption in the 930 nm region is greater for the individual with higher per cent body fat.

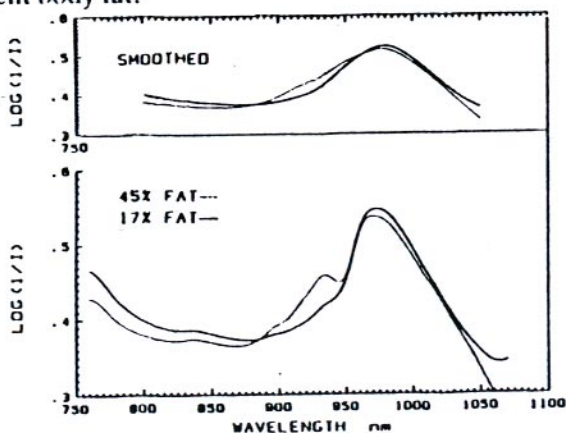


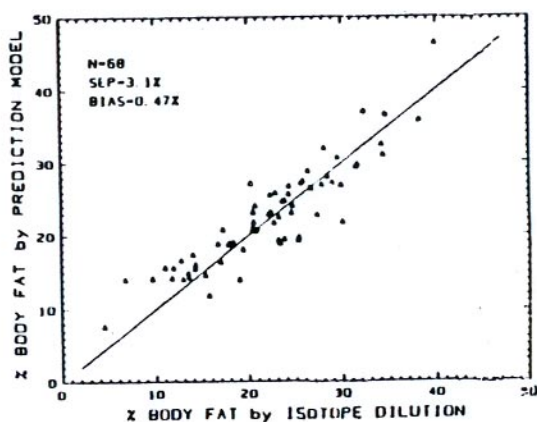
Figure 25.4 Near infrared spectra from two subjects, one 17% fat (—), the other 45% body fat (· · ·). The lower panel shows spectra from a narrow-slit, computerized spectrophotometer. The upper panel shows spectra generated by a 50 nm smoothing, which represents a computer simulation of a wide-slit spectrophotometer.

The smoothed data ( $\log I/I$ ) from points at 930 nm/950 nm and 965 nm/950 nm were used in the regression analysis with D<sub>2</sub>O. The correlation between IRI (averaged from all sites) and D<sub>2</sub>O for the spectral data alone was  $r$  (SE): 0.72 (6.3). This increased to 0.82 (5.2) when weight was added, to 0.88 (4.5) when sex was added, and to 0.90 (4.1) when height was added (table 25.3). Calibrating again on the first study and predicting the second gave a regression plot, shown in figure 25.5. The SE of prediction was 3.1 per cent body fat with a bias of + 0.47.



**Table 25.3** Correlations between isotope dilution  $D_2O$  data and smoothed spectral data, weight, height and sex.

	Model	$D_2O$
IRI (n = 68)	9.30/950, 9.30/965	0.72 (6.3)
	+ Wt	0.82 (5.2)
	+ Wt + Sex	0.88 (4.5)
	+ Wt + Sex + Ht	0.90 (4.1)



**Figure 25.5** Relationship in 68 subjects (Study 2) between per cent body fat as predicted by the IRI 50 nm smoothed model and per cent body fat as estimated by the  $D_2O$  dilution technique. The IRI prediction equation was based on data (IRI and  $D_2O$ ) obtained in 52 subjects (Study 1). The line shown is the line of identity.

## 25.4 Discussion

The shape of an interactance spectrum is a function of the amount of fat, water and protein present in the sample being analysed. Visual examination of figures 25.1 and 25.2 reveals the possibility of using near infrared spectroscopy to quantitate body composition.

When IRI was regressed against other standard methods of estimating body composition, the SE of estimate, for Study 1 and Study 2 respectively, was 3.0 and 3.2 for IRI vs  $D_2O$ , 4.4 and 3.9 for IRI vs SF, and 4.3 for IRI vs UWW. The standard errors of the estimate probably reflect a combination of inherent errors in the IRI method as well as in the other methods used for comparison. Lohman has reported a range in errors due to biological variation alone of 2.5 to 3.8 per cent for hydrostatic weighing, and of 3.3 per

cent or higher for skinfold thicknesses<sup>6</sup>. Jackson and Pollock have suggested that the size of the error in skinfold measurements increases with the per cent body fat of the subjects<sup>7</sup>. The error for the total body water method was calculated to be 2 per cent by Culebras *et al*, based on tritium dilution and desiccation studies<sup>8</sup>. At present the inherent error of the IRI method is not known.

When second derivative IRI data vs D<sub>2</sub>O data from Study 1 were used to develop a calibration (prediction) equation for Study 2 (*figure 25.3*), the SE of prediction was 4.0 per cent body fat, with a bias of + 1.7 body fat. This indicates overprediction of an average of + 1.7 per cent body fat. Since the inherent error of the IRI method has not yet been established, it is difficult to compare the prediction of per cent body fat by IRI to prediction from other methods such as skinfold thicknesses.

IRI data from the subscapular, suprailiac, and thigh were not as highly correlated with per cent fat measured by (D<sub>2</sub>O) as was the average of all sites, the triceps site, the biceps site, or the Tr and Bi combined (*table 25.2*). Biceps was the single best site. The reason for this is not known, but it is thought that the combination of skin thickness and subcutaneous fat thickness at the biceps site is such that this site allows sufficient penetration of the low-level near-infrared radiation to afford the best measurement. We postulate that the composition of subcutaneous fat at the biceps site is highly correlated with the composition of total body fat.

IRI could be very suitable for field work. Therefore, before this method could be widely used, once validated, the cost of the instrument would have to be made affordable and the size would have to be reduced to increase portability. The computer simulation was performed to predict the accuracy of a low cost portable instrument. The original computerized instrument uses a scanning monochromator and a narrow slit, which makes the optical system quite costly. However, an instrument with a cheaper diffraction grating or optical filters, or a wider slit of approximately 50 nm, could be designed. This wider slit was simulated by a 50 nm smoothing transformation of the spectral data. The effects on sample spectra are seen in *figure 25.3*. The correlations obtained between the IRI data and the deuterium oxide dilution data are as good with the smoothed data (*table 25.3*), 0.90, as those obtained with the second derivative transform... (*table 25.1*), 0.90. The SE of prediction, however, is less for the wide-slit model, 3.1 vs 4.0 per cent for the second derivative model, and the bias is only + 0.47 per cent body fat vs + 1.7 per cent body fat for the second derivative method.

The potential of IRI as a portable method for measuring body composition remains an exciting possibility. Further validation is necessary on other populations before it can be widely applied.



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