Trial of Near-Infrared Reflectance Spectroscopy in a Human Fiber Digestibility Study†

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A trial of near-infrared reflectance spectroscopy (near-IRS) to predict fecal concentration of dietary fiber (DF) in humans was performed. Fecal samples from 34 persons consuming 6 diets of known DF content were scanned by near-IRS. Diets consisted of a liquid nutrition supplement and quick breads containing either 0 g of fiber, 10 g of wheat bran, 30 g of wheat bran, 10 g of vegetable fiber mix, 30 g of vegetable fiber mix, or 30 g of sugar beet fiber. Neutral detergent fiber (NDF) determinations were made on all fecal samples. An equation to predict NDF concentration was derived from 109 fecal samples using modified partial least-squares analysis. Neutral detergent fiber concentrations predicted from near-IRS data were compared to observed NDF concentrations of 75 other samples to validate the equation. Comparison of observed vs predicted NDF revealed nearly perfect correlation between the two. Calibration and validation of a near-IRS equation generally requires that tradiational chemical analysis be done on only two subsets of samples, thereby reducing the time and effort needed to analyze DF concentrations. This study demonstrates that near-IRS is a viable tool for studying DF digestibility *in vivo* in humans.

Near-infrared reflectance spectroscopy (near-IRS) is a highly sensitive analytical method that has been used to predict variation in chemical composition of biological samples (Marten et al., 1985). Simply, the method involves mathematical and statistical analysis of the reflectance spectra in the near-infrared region due to hydrogen bonds in the sample. This analysis is compared to traditional chemical analysis of the component of interest to generate a multiple-regression equation. Prediction of the concentration of the component in similar samples that have been scanned by near-IRS can then be made using the regression equation. Chemical analysis need only be done on one subset of samples to obtain the near-IRS prediction equation, also known as the calibration equation, and on another subset to validate the equation. Therefore, labor, time, and materials are conserved in large-scale studies.

Development and use of near-IRS have largely occurred in the field of agriculture. This technique has been used for many years for determining forage quality (Marten et al., 1985) and in forecasting feed intake and digestibility in animals (Allison, 1983; Eckman et al., 1983; Lippke and Barton, 1988). Estimates of animal intake and potential weight gain can be made from the content of nutrients of feeds, such as fiber and crude protein, and from *in vitro* and *in vivo* digestibility data, all predicted by near-IRS. Quality of foods and pharmaceuticals for humans has been studied by this method (Lanza, 1983; Osborne et al., 1984; Williams and Sobering, 1986; Williams et al., 1991; Corti and Dreassi, 1993), and it has had limited applications in clinical practice (Koumantakis and Radcliff, 1987; van Toorenenbergen et al., 1988; Benini et al., 1992).

Consumption of dietary fiber (DF) is known to result in a myriad of physiologic effects, some of which are the result of fermentation of the DF by colonic bacteria (Pilch, 1987). While *in vitro* fermentation using human fecal innoculum can be studied, the *in vivo* fermentation process is affected by the transit time through the colon, the nature of the bacterial flora of the host, and the viscosity and pH of the digesta (Robertson, 1988). Thus, predicting the *in vivo* digestibility of a given DF is difficult, but important in ascertaining the possible consequences of its consumption. Traditional chemical methods of studying digestibility, where diet and fecal fiber content must be determined for all subjects, are tedious and expensive for large study groups. A more rapid but cost-effective and reliable method would allow more comprehensive studies of DF digestibility *in vivo* in humans.

Given the success of near-IRS analysis in animal nutrition, it seems that the method may have utility in human studies. To study this possibility, fecal samples were examined from a study of human subjects consuming diets of known DF content with the objective of testing the ability of near-IRS to accurately predict concentrations of DF in feces. Near-infrared reflectance spectroscopy was used to predict neutral detergent fiber (NDF) concentrations in these samples, on the basis of a calibration equation derived from analysis of a selected subset of samples. Predicted NDF concentrations were compared to NDF determinations made in the usual fashion on the remaining samples to validate the equation.

MATERIALS AND METHODS

Thirty-four healthy volunteers participated in a fiber-feeding study (16 women, 18 men, ages 19–50 years). The design of the study was approved by the Committee on Use of Human Subjects

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Table 1. Dietary Fiber Content of Diets, as Total Dietary Fiber (TDF) and Neutral Detergent Fiber (NDF), and NDF Content of Fecal Material from Human Subjects

diet	fiber level (g/day)	fiber content (g/day) TDF	NDF	n	NDF concn of feces (%) mean	SD	min	max
0 g of fiber		2.1	3.1	34	6.6	±3.5	1.6	17.1
WB	10	11.0	11.4	34	27.1	±4.1	20.3	35.3
	30	29.9	29.7	34	42.0	±4.2	32.8	51.6
VF	10	14.3	9.9	33	29.2	±6.4	8.5	41.5
	30	36.5	26.4	34	44.9	±10.4	3.4	55.8
SBF	30	30.5	19.5	15	21.8	±10.9	10.9	43.3

in Research at the University of Minnesota. The fiber-feeding experimental protocol and diets were previously described (Lampe et al., 1991). Briefly, subjects consumed five diets in assigned random order, each consisting of a liquid nutrition supplement (Resource, Sandoz Nutrition Inc., Minneapolis, MN) and quick breads containing 0 g of added fiber, 10 g of dietary fiber as wheat bran (10 g of WB), 30 g of dietary fiber as wheat bran (30 g of WB), 10 g of dietary fiber as mixed vegetable fiber (62% pea fiber, 33% soy polysaccharide, and 5% orange pectin) (10 g of VF) or 30 g of dietary fiber as mixed vegetable fiber (30 g of VF). During the study, a sixth feeding period was added in which 15 subjects participated. The quick bread in this period contained 30 g of dietary fiber as sugar beet fiber (SBF). (Fiber suppliers were American Association of Cereal Chemists, St. Paul, MN, for WB; Food Products, Inc., Minneapolis, MN, for Fiberich pea fiber; Protein Technologies International, St. Louis, MO, for Fibrim 1450 soy polysaccharide; Hercules, Inc., Wilmington, DE, for pectin; and American Crystal Sugar Co., Moorhead, MN, for SBF.) Fiber content of breads, as determined in our laboratory using the Association of Official Analytical Chemists method for total dietary fiber (TDF) analysis (Baglien, 1991) and using the NDF method (Robertson and Van Soest, 1977), is summarized in Table 1. Subject characteristics and macronutrient content of breads have been previously described (Lampe et al., 1991).

Feeding periods were 23 days each, separated by wash-out periods of 2–4 weeks during which subjects consumed their habitual diets. All stools were individually collected and frozen. Feces collected from days 14–19 were used for fiber analysis. Feces were composited and lyophilized. Well-mixed portions of lyophilized samples were ground to pass a 0.5-mm screen (Tecator Cyclotech mill, Model 1093, Tec-tron, Inc., Herndon, VA). Samples of each quick bread were also lyophilized, composited, and ground, as were the raw starch and fiber sources. The raw ingredients included rice flour (the major component of all quick breads), wheat bran, pea fiber, pectin, soy polysaccharide, and sugar beet fiber.

All samples were analyzed for NDF using the method of Robertson and Van Soest (1977). Briefly, 0.5-g samples were refluxed with 100 mL of neutral detergent, 0.5 g of sodium sulfite (Aldrich Chemical Co., Milwaukee, WI), and 0.1 mL of heat stable amylase (Sigma Chemical Co., St. Louis MO) for 1 h. The solution was then filtered through Gooch crucibles using glass filters (Whatman International Ltd., Maidstone, England, GF/D grade) as a prefilter. Residues were washed twice with hot distilled water, followed by two washes of acetone, and dried for 16–18 h at 100 °C. Analyses were done in duplicate. When differences between NDF concentrations in the two replicates were greater than two percentage units, the procedure was repeated. Dry matter of samples was determined by drying at 100 °C for 16–24 h. Neutral detergent fiber was expressed as percent of sample dry matter. The average NDF concentration of each sample and replicate was used as the input for the calibration equation.

All fecal samples were placed in plastic bottles and tumbled in a rotating plastic drum for approximately 15 min to ensure a uniform subsample. A total of 184 samples were scanned by near-IRS (Pacific Scientific Instrument Div., Model 6250, Silver Spring, MD), and the signal was recorded as log(1/reflectance). Analysis of the spectra and development of the prediction equation were done according to the methods of Shenk and Westerhaus (1991a,b) using InfraSoft International (Port Matilda, PA) analytical software. Spectra from all samples were analyzed by the CENTER program, and no outliers were found.

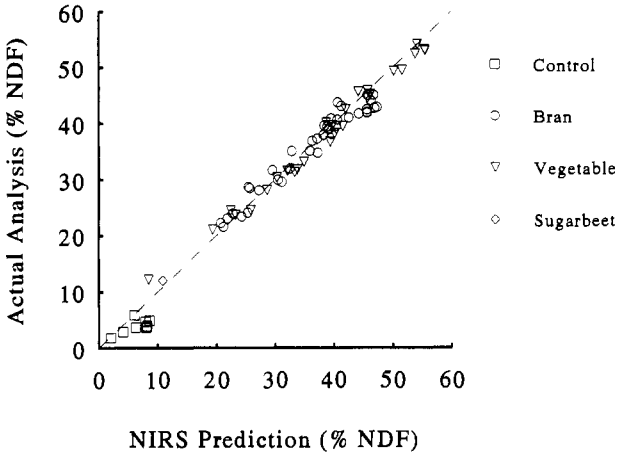

Figure 1. Plot of near-IRS predicted and observed NDF concentrations for fecal samples of subjects consuming various fiber sources. These samples were not included in the calibration set for developments of the near-IRS prediction equation. The diagonal line represents unity.

Table 2. Linear Regression Relationships for Predicted Near-IRS NDF Concentrations vs Observed Chemical Analysis of Validation Set Fecal Samples

diet	sample no.	slope	intercept	R^2	probability
0 g of fiber	8	1.23 ± 0.55	1.58 ± 2.25	0.46	<0.07
WB	39	1.11 ± 0.04	−3.43 ± 1.51	0.95	<0.0001
VF	27	1.07 ± 0.03	−1.97 ± 1.00	0.97	<0.0001
combined[a]	75	1.00 ± 0.02	0.63 ± 0.59	0.98	<0.0001

[a] Combined data set includes one sugar beet fiber sample.

The SELECT program developed a calibration set of 109 samples, being representative of all of the spectra obtained. Modified partial least squares (MPLS) and the math treatment, 1,4,4,1, were used to find the best multiple-regression equation fit. In math treatment terminology, the first number represents the first derivative, the second is the number of data points within each derivative segment of equation, and the third and fourth numbers are the number data points averaged to smooth the curve. To validate the near-IRS prediction equation, predicted NDF concentration was compared to that observed in the 75 other samples.

Simple linear regression of observed and predicted NDF concentrations was done using the SAS statistical package on a personal computer.

RESULTS AND DISCUSSION

Three of the fecal samples were identified as outliers in the calibration set in two passes by MPLS. All of these samples had very low concentrations of NDF; two of the samples were from the 0 g of fiber quick bread and the other from the 30 g of VF diet in a subject with an extremely long mean transit time on that diet. No outliers were found in the validation set. Twenty-six percent of samples in the calibration set were taken during consumption of the 0 g of fiber diet, 20% during the 10 g of WB diet, 7% with the 30 g of WB diet, 17% with the 10 g of VF diet, 18% with the 30 g of VF diet, and 13% with the SBF diet.

Statistics of the calibration equation were as follows: standard error of calibration (SEC) = 1.27, standard error of cross validation (SECV) = 1.83, and R^2 = 0.99. Plots of observed vs predicted concentrations of the full validation set (Figure 1) and of the distinct fiber types revealed excellent correlation. Linear regression relationships of these plots are given in Table 2. For the full validation set, with all fiber types combined, the slope was not statistically different from one ($p > 0.05$), and the intercept was not different from zero. Regressions of the WB and

VF diets combined the two doses of DF intake. The slopes of these lines were not significantly different from one, but the intercepts of the regression lines for WB and for VF were less than zero ($p < 0.05$). Therefore, the equation appears to be biased to overestimate NDF slightly when only WB or only VF is considered. Too few samples from consumption of the SBF bread remained in the validation set for meaningful comparison.

The fibers used in the study represent a range of dietary fibers that would be expected to be excreted to a significant degree in feces. Differences in the fibers' chemical composition are apparent in the percent of DF determined by the NDF method compared to a TDF analysis (Table 1). About 25% of TDF in the VF mix was lost by NDF, as was about 34% of SBF, whereas none of the DF in WB was lost in the method. Soluble fibers are lost during NDF analysis, and while it is assumed that soluble fibers are completely fermented in the colon, this is unknown. The NDF method of analysis was chosen for this project because it is effective in removing bacterial mass from fecal samples (Robertson and Van Soest, 1981).

The SEC for the developed prediction equation, 1.27, was comparable to those calculated in studies of NDF content of feeds and lower than one reported in another fecal study. Various studies of forages have reported SECs of 1.12–2.23 when NDF content of feeds and forages has been analyzed (Marten et al., 1985). A more comparable study, the Koumantakis study of fat in human feces (Koumantakis and Radcliff, 1987), used an equation with a SEC of 3.8. A stepwise multiple-regression method was used to develop these equations. The current method, MPLS, has been shown to result in a lower SEC (Shenk and Westerhaus, 1991a), although not always (Williams et al., 1991). The paper reporting fecal nitrogen concentrations in human stools (Benini et al., 1992) did not include the SEC or SECV of the equation and, further, used repeated determinations on unhomogenized samples which yielded a within-run CV of 5.5%.

The other estimate of equation performance, SECV, determined by validating the calibration equation with each quarter of the calibration set, was not reported in the above papers. Because it is generally higher than the SEC (Shenk and Westerhaus, 1991b), it may be a stronger predictor of equation performance. Both the SEC and SECV of this study were lower than the error allowed by the laboratory, ±2 percentage units.

Error in the equation can also result from variables in samples such as particle size (Wetzel, 1977), replicate, time in storage, and method of storage (Marten et al., 1985; Buxton and Mertens, 1991). These variables can lead to bias by the near-IRS equation. In a forage-quality study of five types of forages, these factors, plus year of cultivation, entry × year interaction, and cultivars within species, resulted in significant bias in NDF concentration equations (Buxton and Mertens, 1991). For our study, particle size and method of storage were controlled. Time in storage may be a factor, however, as the study continued over 10 months. Plots of residuals of actual vs predicted values have been used to check for bias in near-IR calibrations equations of forages (Buxton and Mertens, 1991). Residual plots of the current data revealed only the tendency to underreport NDF of WB and VF.

Others who have looked at the use of near-IRS in human studies have found good correlation between near-IRS predictions and traditional methods of analysis. Recently, Benini et al. (1993) used near-IR and the Kjeldahl technique to determine protein content of feces of patients with gastroenterological diseases who were orally and enterally fed. In unhomogenized samples, protein excretion could be accurately predicted when three readings of the same sample were averaged. Koumantakis and Radcliff (1987) found near-IRS predictions of fat in human feces to be as accurate as the laboratory method, and that accuracy was not affected by the addition of water to samples. Van Toorenenbergen et al. (1988) determined that near-IRS could predict total serum protein accurately and precisely. They detected no interference from urea or creatinine, although high bilirubin may result in low predicted values.

Near-infrared reflectance can be used to predict hemicellulose, cellulose, and lignin fractions of NDF and is used this way in forage-quality determinations (Marten et al., 1985). Since the current study demonstrates the ability of near-IRS to accurately estimate NDF concentrations, testing for prediction of these fiber fractions seems to be a logical next step in developing the methodology for human studies.

Quick bread samples were also scanned, but the number done was too small to have developed a calibration for NDF content. Breads were prepared in large batches and given to subjects randomly; that is, breads from a single batch may have been fed over several feeding periods. The raw fibers were purchased at the beginning of the study and used throughout the study, so it was felt that the fibers were consistent throughout the project. If more bread samples had been scanned by near-IRS, a calibration equation for bread NDF content could have been developed. However, prediction of NDF content of a broader range of amounts and sources of DF would be a more practical application of the method. Then, intake as well as excretion of NDF can be predicted by near-IRS and, from the estimates, apparent digestibility could be calculated. Assuming absence of bias in both intake and excretion estimates, calculation of digestibility by this route could be expected to be accurate. Using near-IRS to predict digestibility in various research projects would add to the knowledge of DF fermentation in general populations and under specific conditions.

Very few large-scale human studies of DF digestibility have been done. Given the variability in human fiber digestibility (Slavin et al., 1981) and response to DF (Lampe et al., 1993), more studies of this type are needed to determine more precisely the physiologic effects of a given fiber under a variety of conditions. The methodologies involved in digestibility studies are deterrents, however, to this type of work. Near-infrared reflectance spectroscopy is a viable tool for these needed studies involving large numbers of samples. The technique can decrease the time needed to perform traditional fiber analysis because only a portion of the samples collected needs to be actually analyzed to calibrate and validate the equation used for prediction of DF concentration in foods and feces. The equations generated in this experiment were shown to give accurate predictions of NDF content in human fecal material. Whether the equation generated by this experiment is valid for other studies is unknown. Samples from other experiments involving various fiber sources in feeding trials would need to be scanned and analyzed for NDF and the actual values compared to those predicted by the equation.

ABBREVIATIONS USED

Near-IRS, near-infrared reflectance spectroscopy; DF, dietary fiber; NDF, neutral detergent fiber; WB, wheat

bran; VF, vegetable fiber; SBF, sugar beet fiber; TDF, total dietary fiber; MPLS, modified partial least squares; SEC, standard error of calibration; SECV, standard error of cross validation.

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