Simple and Rapid Determination of Fecal Fat by Nuclear Magnetic Resonance Spectroscopy.

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Background

Measurement of the fecal fats can be useful in establishing a diagnosis of such pancreatic diseases as cystic fibrosis, chronic pancreatitis, malnutrition, or other obstruction and of such intestinal diseases as Whipple's disease, regional enteritis, tuberculosis enteritis, gluten-induced enteropathy (sprue), and the absorption of malnutrition. Quantification of fecal fat has historically been carried out using the classic van de Kamer extraction and gravimetric determination. Here we describe a simple and rapid analysis of fecal fat utilizing nuclear magnetic resonance (NMR) spectroscopy.

Methods

A method for fat analysis in feces was developed using the CEM SmartTrac System (Matheson, NJ, a bench top analyzer that utilizes nuclear magnetic resonant (NMR) technology. We homogenized stool sample was weighed, dried, and placed into a 75 ml NMR tube. The samples with known fat concentrations (established using the van de Kamer gravimetric methods) were used as calibrators. The NMR produces a signal ( offline baseline decay from protons in the sample. Five fold decay of lipid proton occurs as a slower rate than two fold decay of protons from other substances in the stool. This allows determination of the percent of fat in the measured sample. The percentage is then converted to grams fat/24 hr, based on the total weight of the specimen and the collection duration. Complete analysis time is 1.5-5 minutes per sample.

Results

Serial dilution of two high level fecal samples produced linear regression equations of y = 0.953 – 0.353x, y = 0.964 and y = 0.119x, y = 0.946. Mean recovery of fat from three fecal samples spiked to varying levels with a sample of known fat concentration was 97%. Intra-assay precision (within-run) was performed at three levels: 2.2, 3.1, 5.4 and 7.7 g of fat/24 hr producing CV's of 10.6%, 5.7% and 3.9% respectively. Inter-assay precision at three levels of crude fat were 6.4, 11.7% and 7.3 g of fat/24 hr producing CV's of 15.1%, 16.2% and 3.5% respectively. The SmartTrac method was compared with the gravimetric method in diet samples submitted to our laboratory. The methods correlated strongly, producing a regression equation of y = 1.161 – 1.188, (r² = 0.877). A Bland-Altman plot of the two methods showed a mean difference of 0.7 g of fat/24 hr. Of the 48 samples analyzed, four samples with fat exceeding 5 g/24 hr by gravimetric had values less than 1 g/24 hr by NMR (Values of 7.4, 7.9, 7.4, and 7.9 by gravimetric were 4.8, 5.1, 5.4, and 6.6, respectively, by NMR). The cutoff point of 7 g of fat/24 hr to distinguish fat malabsorption (established using the gravimetric method) the sensitivity, specificity, and positive predictive values of the NMR method were 85%, 100%, and 96% respectively. The samples were stable for 10 days after homogenization when stored at 4ºC.

Conclusions

A rapid accurate method for fecal fat determination was developed using the SmartTrac NMR system. The sensitivity of the NMR system was achieved using samples with known percent of fat determined using a gravimetric method. Complete analysis time is only 5 minutes per sample. The NMR method is rapid, an reliable means to assess fat malabsorption in patients.

References