Comparison of Ultracentrifugation and Nuclear Magnetic Resonance Spectroscopy in the Quantification of Triglyceride-Rich Lipoproteins after an Oral Fat Load

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Background: The measurement of triglyceride (TG)-rich particles after an oral fat challenge has been used to provide a measure of risk for coronary artery disease independent of the fasting plasma triglyceride concentration. The analytical “gold standard” for measuring TG-rich lipoproteins uses density gradient ultracentrifugation; however, this technique is labor-intensive. Because of our need to perform numerous postprandial analyses of TG-rich lipoproteins for a large interventional study (Genetics of Lipid Lowering Drugs and Diet Network), we evaluated the use of nuclear magnetic resonance (NMR) spectroscopy for measuring TG-rich particles.

Methods: EDTA-blood samples were obtained 0, 3.5, 6, and 8 h after ingestion of an oral fat meal (89% of calories from fat) in 20 apparently healthy individuals. The plasma TG concentrations of chylomicron and chylomicron remnant/VLDL fractions were analyzed by ultracentrifugation and NMR spectroscopy.

Results: Comparison of all values (n = 78) by ultracentrifugation (x) and NMR (y) produced a linear regression equation of $y = 0.979x - 0.035$ mmol/L ($R^2 = 0.90$) for chylomicrons and $y = 1.398x + 0.067$ mmol/L ($R^2 = 0.96$) for the fraction containing chylomicron remnants and VLDL. Postprandial response of chylomicrons and chylomicron remnant/VLDL was similar, with maximum response occurring between 3.5 to 6 h regardless of method of measurement.

Conclusion: Chylomicron and chylomicron remnant/VLDL fraction measurements obtained by NMR have a high degree of correlation with results produced by ultracentrifugation. NMR may therefore be suitable as an alternative method for the measurement of postprandial TG-rich lipoproteins in individuals consuming a high-fat meal.

Several experimental and observational studies have demonstrated that concentrations of triglyceride (TG)-rich lipoproteins, such as remnants of chylomicrons and VLDL, are associated with the progression of atherosclerosis (1–3). To determine an individual’s ability to clear postprandial lipids, TG-rich particles can be measured after an oral fat loading. The resulting concentration of TG-rich lipoproteins is thought to correlate with an individual’s risk of coronary artery disease that is independent of the fasting plasma TG concentration.

The analytical “gold standard” for measuring TG-rich lipoproteins uses density gradient ultracentrifugation (4, 5). This technique is labor-intensive and involves a 24-h analysis to fractionate the plasma lipoproteins. Moreover, only a few samples can be processed at the same time in each ultracentrifuge, precluding use of this method in studies with large numbers of samples. An immunochemical method (Japan Immunoresearch Laboratories) has also been used to isolate remnant-like particles (6, 7).

Recently, nuclear magnetic resonance (NMR) spectroscopy has been used to quantify subspecies of VLDL, LDL, and HDL (8, 9). The NMR method uses characteristic
signal amplitudes of the lipoprotein subclasses of different sizes as its basis of quantification. Although the data produced by NMR analysis have been primarily of interest in determining the proportion of large vs small LDL, NMR analysis can simultaneously provide data for the quantification of all lipoprotein classes, potentially including chylomicron and chylomicron remnant/VLDL fractions in postprandial samples. To date, there has not been a published report comparing the measurement of TG-rich particles by NMR with results obtained by classic ultracentrifugation.

In the Genetics of Lipid Lowering Drugs and Diet Network study, we have proposed determining the genetic basis of the variable response to fenofibrate, a drug used primarily for the lowering of serum TG concentrations. One of the objectives of the Genetics of Lipid Lowering Drugs and Diet Network study is to assess postprandial response to a high-fat meal by quantifying TG-rich lipoproteins at different time points after a fat challenge. We intend to recruit 1200 individuals and assess the postprandial response both before and after fenofibrate treatment. Thus, it is desirable to have a postprandial analysis technique that is less labor-intensive and has a faster turnaround time than density gradient ultracentrifugation. In the current study, we present the results of a pilot study in which we compared values for TG-rich particles obtained by NMR with those obtained by density gradient ultracentrifugation on 20 individuals who had blood samples drawn after a 10-h fast and at 3.5, 6, and 8 h after an oral fat challenge meal.

Materials and Methods

STUDY POPULATION

We studied 20 apparently healthy individuals [7 males and 13 females; age range, 25–70 years, mean (SD) age, 44.8 (13.3) years]. All participants were instructed to fast for at least 10 h and to abstain from strenuous exercise and alcohol consumption for at least 3 days before participation in this study. This study was approved by the Human Studies Committee of the University of Minnesota Institutional Review Board, and all participants gave informed consent.

EXPERIMENTAL DESIGN

Fasting blood samples for lipoprotein analysis were collected in EDTA-containing tubes. Immediately after the fasting sample was collected, participants were fed a high-fat test meal according to the protocol of Patsch et al. (10). The typical test meal consisted of 175 mL of heavy whipping cream (39.5% fat), 1 tablespoon of chocolate-flavored syrup, and 0.5 tablespoon instant nonfat dry milk. Participants were instructed to consume the “shake” within 15 min. The meal contained 700 calories/m² of body surface area (3% derived from protein, 14% from carbohydrate, and 83% from fat) with a cholesterol content of 240 mg and a ratio of polyunsaturated to saturated fat of 0.06. Participants were allowed to return to work but were instructed to take nothing by mouth except water or unsweetened black coffee or tea and to abstain from strenuous physical work or exercise until the postprandial lipoprotein blood samples were obtained. Postprandial lipoprotein samples were collected in EDTA-containing tubes 3.5, 6, and 8 h after ingestion of the high-fat meal. An 8 h specimen was unavailable on one participant, and a 0 h specimen was unavailable for NMR on a second participant; therefore, the final number of values available for comparison by ultracentrifugation and NMR was 78.

ASSAY PROCEDURES

The fasting and 3.5, 6, and 8 h postprandial lipoprotein samples were centrifuged within 20 min of collection at 2000g for 15 min at 4 °C. Plasma samples were stored at 4 °C, and all analyses were performed within 4 days of collection.

TGs were measured by a glycerol-blanked enzymatic method (Triglyceride GB reagent; Roche Diagnostics Corporation) on the Roche COBAS FARA centrifugal analyzer (Roche Diagnostics Corporation).

Chylomicrons, as well as remnants of chylomicrons and VLDL, were isolated by salt density gradient ultracentrifugation according to the method of Lindgren et al. (4) as modified by Redgrave and Carlson (5). We used ultracentrifugation with a SW 41 rotor to separate two fractions: S₁ >400 (4.5 × 10⁶ g · min), containing chylomicrons, and S₁ 20–400 (147.5 × 10⁶ g · min), containing chylomicron remnants and VLDL. Because 90% of the chylomicron fraction by weight consisted of TGs, the concentrations of chylomicrons and chylomicron remnants/VLDL were measured in each fraction in terms of its TG concentration. The TG concentration was adjusted for the amount of plasma initially used and the amount of supernatant collected during ultracentrifugation.

The TG concentrations in the chylomicron and chylomicron remnant/VLDL fractions were measured by proton NMR spectroscopy on nonfractionated plasma (Lipscience). This method quantifies particle concentration by converting the characteristic signal amplitude generated by the methyl group NMR signal of each lipoprotein subclass particle (9).

STATISTICAL ANALYSIS

Least-squares linear regression analysis was used to compare concentrations of chylomicron and chylomicron remnant/VLDL fractions obtained by ultracentrifugation and NMR spectroscopy methods (Pearson, Microsoft Excel 2000). Ultracentrifugation was used as the independent variable; NMR was the dependent variable. The paired Student t-test was used to compare the mean TG concentration as measured by each method and to determine significant differences (SPSS, Ver. 10.0). Significance was defined as P < 0.05.
Results

Shown in Fig. 1 is a comparison of the concentrations of chylomicron and chylomicron remnant/VLDL fractions obtained by ultracentrifugation (x) with values obtained by NMR spectroscopy (y) on 78 samples.

Dashed lines indicate lines of perfect correlation. Results of linear regression analysis: chylomicrons (top panel), \( y = 0.979x - 0.035 \text{ mmol/L} \) (\( R^2 = 0.8994; S_{XY} = 0.16 \text{ mmol/L} \)); chylomicron remnant/VLDL, \( y = 1.397x + 0.0674 \text{ mmol/L} \) (\( R^2 = 0.9579; S_{XY} = 0.17 \text{ mmol/L} \)).

As shown in Fig. 2, the postprandial response of chylomicrons, expressed as the mean difference from 0 h, was similar when measured by ultracentrifugation and NMR, with the peak response between 3.5 and 6 h. No

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**Fig. 1.** Comparison of TG concentration in chylomicron (top panel) and chylomicron remnant/VLDL (bottom panel) fractions by ultracentrifugation (x) vs NMR spectroscopy (y) on 78 samples. Dashed lines indicate lines of perfect correlation. Results of linear regression analysis: chylomicrons (top panel), \( y = 0.979x - 0.035 \text{ mmol/L} \) (\( R^2 = 0.8994; S_{XY} = 0.16 \text{ mmol/L} \)); chylomicron remnant/VLDL, \( y = 1.397x + 0.0674 \text{ mmol/L} \) (\( R^2 = 0.9579; S_{XY} = 0.17 \text{ mmol/L} \)).

**Results**

Conversely, mean values for the chylomicron remnant/VLDL fraction measured by NMR were significantly \( P < 0.001 \) higher [1.07 (0.94) mmol/L TGs] compared with mean values obtained by ultracentrifugation [0.72 (0.66) mmol/L TGs]. Comparison of chylomicron remnants/VLDL by ultracentrifugation and NMR spectroscopy produced the following linear regression equation: \( y = 1.398x + 0.067 \text{ mmol/L} \), with excellent correlation between the two methods (\( R^2 = 0.96 \)).

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**Fig. 2.** Postprandial response of TG concentration of chylomicron (top panel) and chylomicron remnant/VLDL (bottom panel) fractions, expressed as the mean difference from 0 h, by ultracentrifugation (A) and NMR (■). *P < 0.05 for the difference between ultracentrifugation and NMR methods in the chylomicron remnant/VLDL fraction. Error bars represent the SE of each data point. The number of individuals at each time point were as follows: 0 h, \( n = 19 \); 3.5 h, \( n = 20 \); 6 h, \( n = 20 \); 8 h, \( n = 19 \).
significant differences in postprandial response were observed at any time interval. With regard to chylomicron remnants/VLDL, the peak response was at 3.5 h when measured by either ultracentrifugation or NMR. No differences in response were observed except at 6 h ($P < 0.05$).

**Discussion**

The interest in NMR as a method for the analysis of lipoproteins has to date been largely restricted to its usefulness in determining the size of lipoprotein subparticles. In particular, the major focus has been on LDL particles (11–13). In fact, when a sample is subjected to NMR analysis, information on all lipoprotein particles, including quantification of the chylomicron and chylomicron remnant/VLDL fractions, becomes available simultaneously. However, although NMR can theoretically provide quantification of TG-rich lipoproteins, to our knowledge no study has validated whether NMR provides reliable results for the quantification of TG-rich lipoproteins, especially after a fat-loading meal.

In the current study, we showed that, based on TG concentration, the results from NMR analysis and ultracentrifugation separation had a high degree of correlation with respect to either the chylomicron fraction or the chylomicron remnant/VLDL fraction. NMR gave lower values for chylomicrons and higher values for chylomicron remnants/VLDL compared with ultracentrifugation. The exact reason for this difference is not known. However, it should be noted that chylomicrons defined by the two methods may not comprise exactly the same population of particles because one (ultracentrifugation) is defined by flotation rate and the other (NMR) by particle diameter. Nevertheless, the high correlation between these two methods suggests that NMR can be considered as an alternative method for the quantification of TG-rich lipoproteins in nonfasting states. Compared with ultracentrifugation, NMR is a more rapid procedure and uses substantially less sample volume than traditional ultracentrifugation. Moreover, it provides information not only on TG-rich lipoproteins but also on the quantities and the relative sizes of both LDL and HDL particles. In addition, because NMR is a physical rather than a chemical procedure, plasma samples subjected to NMR analysis can be preserved for biochemical assays of stable analytes. However, NMR is a relatively new method and is not widely available at present.

In summary, our study comparing NMR with density gradient ultracentrifugation shows that the results obtained by NMR have a high degree of correlation with results produced by ultracentrifugation. Therefore, NMR can be considered as an alternative method for the measurement of postprandial TG-rich lipoproteins in individuals, particularly in studies involving large numbers of participants.

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**References**