clear as for LDL subfractions, larger size HDL in most studies appears to be more strongly inversely related to CVD risk (10).

**NMR SPECTROSCOPY**

Laboratories also use NMR spectroscopy to perform lipoprotein subfraction analysis. NMR spectroscopy quantitatively measures the spectral signals generated by the terminal methyl groups on lipids within lipoprotein particles. Unlike most other methods, NMR spectroscopy does not require physical separation of lipoproteins, and aside from separating plasma from blood cells, no pre-analytic sample processing is necessary. At least one reference laboratory offers the method, and others are developing it. The NMR Lipoprofile test is currently the only NMR assay for measuring LDL-particle number (LDL-P), triglycerides, and HDL-C that has been approved by FDA. The other lipid and lipoprotein parameters that this method measures are shown in Table 3. This test also reports a lipoprotein insulin resistance score based on the lipoprotein profile that is associated with insulin resistance and diabetes risk. The position of the resonance in the NMR spectra of the terminal methyl groups on lipids is affected by the size of the lipoprotein particle, which after a deconvolution algorithm enables the laboratory to calculate the number of particles within each lipoprotein size subfraction. LDL-P is simply calculated as the sum of all the individual numbers of LDL size subfractions (11).

As illustrated in Figure 2, two individuals with similar LDL-C levels can have quite a substantial variance in LDL-P, because larger size LDL particles can carry more cholesterol (12). Researchers have found that CVD risk tracks more closely with LDL-P when there is discordance between LDL-C and LDL-P—as often occurs in patients with metabolic syndrome and diabetes (13). In the future, laboratories might obtain even more discrimination by specifically measuring the different size subfractions within VLDL, LDL, and HDL, but this is still an active area of investigation.

**ION MOBILITY ANALYSIS**

Both the size and concentrations of lipoprotein particle subfractions can also be measured by mass spectrometry, using gas-phase differential electrical mobility (also known as ion mobility). This method depends on the principle that particles of a given size and charge behave differently when put in a laminar flow of air and subjected to an electric field. Quest Diagnostics has adapted this method for directly measuring the size distribution of lipoprotein particles in a range from 7 nm to about 120 nm. The analysis part of the method is automated and generates profiles of particle number and particle mass versus particle diameter. However, the plasma/serum sample requires extensive pre-analytical preparation to isolate the lipoproteins.

In this step, the laboratory first adds an albumin removal reagent to the sample; then ultracentrifuges the sample for about 2 hours to isolate the lipoprotein fraction. The laboratory then dilutes the sample in a volatile buffer and electrosprays it in a differential mobility analyser. This process yields particle number distributions within 2 minutes for HDL, LDL, IDL, and VLDL converted into particle mass distributions. Other conventional lipid and apolipoprotein tests are also included in the panel (Table 4) (14).

**CONCLUSION**

Numerous methods now exist for measuring lipoprotein subfractions and there is growing evidence for how such tests improve CVD risk prediction and cost-effectiveness (15). At this time, however, they are not widely used and are not recommended by national and international guidelines.