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Concerns Regarding Lipoprotein Particle Measurement by Ion Mobility Analysis

To the Editor:

We read with interest the report by Caulfield et al. (1) describing a new method for measuring lipoprotein particle concentrations by ion mobility (IM)1 analysis. There is growing evidence that the LDL particle concentration, as assessed by apolipoprotein B (apo B) or nuclear magnetic resonance (NMR) measurements, is a better discriminator of cardiovascular risk than the LDL cholesterol concentration, and measures of VLDL and HDL particles have also been suggested to have clinical advantages over analyses of their lipid counterparts. For these reasons, methods capable of quantifying lipoprotein particle concentrations accurately and efficiently are of considerable interest, and we congratulate the authors on their efforts.

As part of the validation of the IM method, Caulfield and colleagues present several plots that compare IM-measured particle concentrations with corresponding lipid or apolipoprotein measurements. The results of some of these comparisons do not agree with expectations and raise concerns in our minds about the accuracy of the measured particle concentrations. As the authors point out, only a moderate association is expected between VLDL, LDL, and HDL particle concentrations and the corresponding lipid measurements, given that the cholesterol and triglyceride contents of these lipoproteins are known to vary considerably from person to person; however, because there is one apo B molecule on every non-HDL particle (LDL, intermediate-density lipoprotein, VLDL), very good agreement is expected between plasma apo B concentrations and IM-measured non-HDL particle concentrations. The comparison data in Fig. 4C of the report by Caulfield et al. do indeed show a strong correlation \( r = 0.92 \) between the 2 particle assays. What is not clear is why particle concentrations obtained with the IM method are much lower than concentrations of apo B particles (30%–40% at median concentrations) and why the relationship appears to be nonlinear. The authors attribute the discrepancies to the apo B assay overestimating particle concentrations rather than to the IM method underestimating them and suggest that limitations of the apo B immunoassay are the source of the problem. The implicit assumption is that the IM method correctly quantifies lipoprotein particles because it “directly counts the particles present.”

The accuracy claim is called into more serious question by data in the study of Caulfield et al. that indicate that HDL particle concentrations measured by IM are strikingly lower than expected. The median total HDL particle concentration reported by Caulfield et al. for healthy adults was approximately 3000 nmol/L, only about 3-fold higher than LDL particle concentrations. In contrast, the median HDL particle concentration measured by NMR spectroscopy in healthy adults in the Multi-Ethnic Study of Atherosclerosis was about 10 times greater, approximately 30 000 nmol/L (2). Are HDL particle concentrations measured by IM much too low, or are NMR values much too high? The answer is provided by data provided by Caulfield et al. that relate the concentrations of apolipoprotein A-I (apo A-I) to total HDL particles (see Fig. 3B in these authors’ online Data Supplement at http://www.clinchem.org/content/vol54/issue8). Apo A-I does not have a fixed stoichiometric relationship with HDL particles, unlike apo B with LDL particles. Rather, compositional studies have indicated that small, medium, and large HDL particles contain 2, 3, and 4 molecules of apo A-I per particle, respectively (3–5). Because the concentrations of small HDL particles are much greater than those of large HDL particles, an individual on average has approximately 2.5 apo A-I molecules per HDL particle. In Fig. 3B of their online Data Supplement, Caulfield et al. plot apo A-I concentration against the IM-measured total HDL particle concentration. An examination of these data indicates that an HDL particle concentration of 3000 nmol/L corresponds to an

1 Nonstandard abbreviations: IM, ion mobility; apo B, apolipoprotein B; NMR, nuclear magnetic resonance; apo A-I, apolipoprotein A-I.
Letters to the Editor

To the Editor:

I read with great interest the recent editorial in Clinical Chemistry on protein microarrays (1). “The Elephant in the Room” in its title refers to a long-ignored issue, namely the problems involved in standardization and normalization of data from immunoaassays on protein microarrays. I would like to take this opportunity to review approaches to those issues in the first publications on protein arrays, hence my title. Kattah et al. (1) referred to the wide use of normalization via total immunoglobulin for antigen arrays, and the inability to correct for differences in the spotting of individual features. This imperfection notwithstanding, a 1982 publication on protein arrays (2) already grappled with this issue and showed a calibration curve of immunoglobulin concentration against a densitometric scan intensity. This was further elaborated in a subsequent publication (3), in which empirical equations were used to fit the calibration curves and a computer program was used in extraction of the data.

Indeed, there is a body of literature from that period which foreshadowed current approaches, and which is now largely forgotten or ignored. Reasons for this include the fact that the current terminology was not in use then, so key term searches would not find these publications, and work in the patent literature is not widely read by the scientific community. I therefore consider it of interest to look back at this literature from a current perspective.

Protein Microarrays: Before the Elephant Got in the Room

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