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0.DC1.html

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Study of agreement between LDL size as measured by nuclear magnetic resonance and gradient gel electrophoresis

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Abstract LDL particle size can be measured by gradient gel electrophoresis (GGE) and NMR. The agreement between the two methods has not been extensively evaluated. Therefore, we measured LDL size by NMR and GGE in 324 individuals (152 with type 1 diabetes and 172 controls). The Spearman correlation between both methods was 0.39 [95% confidence interval (CI) = 0.29, 0.48]. The average difference was 5.38 nm (NMR being smaller), but it increased with increasing LDL size. Less than 50% of people classified as pattern B on GGE were classified as pattern B on NMR (κ = 0.31; 95% CI = 0.17, 0.45). Agreement was lower for diabetic subjects compared with controls, for women compared with men, and for subjects with triglycerides less than 1.30 mmol/l compared with subjects with triglycerides greater than 1.30 mmol/l. External validation showed that cholesteryl ester transfer rate was related to LDL size on GGE in all subgroups and to LDL size on NMR only in men and nondiabetic subjects. Our findings show that agreement between NMR- and GGE-based LDL size is far from perfect and is not consistent across subgroups of patients. In particular, the two methods should not be assumed to be interchangeable in women and diabetic subjects. Whether NMR or GGE predicts cardiovascular disease risk better has not yet been evaluated.—Witte, D. R., M. R. Taskinen, H. Perttunen-Nio, A. van Tol, S. Livingstone, and H. M. Colhoun. **Study of agreement between LDL size as measured by nuclear magnetic resonance and gradient gel electrophoresis. *J. Lipid Res.* 2004.** 45: **1069–1076.**

Supplementary key words low density lipoprotein particle size • low density lipoprotein subspecies • method comparison

One of the main current objectives in cardiovascular risk management is to improve risk prediction. It has become clear that information on lipoprotein subclass in addition to total lipid concentration may be of value in determining the level of risk (1–7).

LDL and HDL particle size and subclass distribution are determined by a number of metabolic steps, including the exchange of cholesteryl esters from LDL and HDL with triglycerides from VLDL under the influence of cholesteryl ester transfer protein (CETP) (8). It has previously been shown that small, dense LDLs are rich in triglycerides (9). Triglyceride-rich lipoproteins are remodeled by hepatic lipase, which hydrolyzes triglycerides, resulting in the specific LDL subspecies (10, 11). Although LDL particle size is known to be highly associated with the level of triglycerides (2, 12, 13), there is evidence that the association between LDL particle size and cardiovascular risk is independent of the baseline level of triglycerides (3–5, 12). Therefore, estimation of LDL size has been advocated as a component of cardiovascular disease risk prediction. A small LDL size is considered particularly indicative of risk when the absolute number of LDL particles is high (14).

A number of methods for the assessment of lipoprotein size exist, the most widely used being gradient gel electrophoresis (GGE) (15) and proton NMR spectroscopy (16, 17). GGE separates LDL particles based on the principle that particles migrate through the gradient gel until their further penetration into the gradient is restricted by their size and to a lesser extent by their charge (18). NMR spectroscopy measures the signal emitted by plasma lipid methyl groups during magnetic resonance scanning (16). Based on empirically measured signal amplitudes of purified VLDL, LDL, and HDL subclasses, this method distin-

Abbreviations: CET, cholesteryl ester transfer rate; CETP, cholesteryl ester transfer protein; GGE, gradient gel electrophoresis.

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The online version of this article (available at http://www.jlr.org) contains an additional two tables.

Manuscript received 15 September 2003, in revised form 2 February 2004, and in re-revised form 20 February 2004.

Published, JLR Papers in Press, March 1, 2004.
DOI 10.1194/jlr.M300395-JLR200

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guishes the component peaks associated with lipoprotein subclasses. These subclass signal amplitudes are then regarded as a direct measure of the levels of subclass particles (17).

Despite the broad application of both methods, very few data on the agreement between the two for LDL size measurement have been published. The only published information is based on a small group of healthy middle-aged men (6). Better insight into the level of agreement between the two methods is of particular importance in view of the increasing application of LDL particle size in both research and clinical practice. The data derived from LDL size measurements by NMR and GGE are currently assumed to be interchangeable (14). However, evidence that this is the case and that this is generally true is lacking. Therefore, we set out to evaluate the agreement between LDL particle size measured by NMR and peak LDL size measured by GGE in patients with type 1 diabetes mellitus and healthy controls.

METHODS

Subjects

The study population has previously been described in detail (19–21). It consisted of a random sample of 199 men and women with type 1 diabetes mellitus, aged 30–55 years, and a random sample of 201 control subjects from the general population stratified to have a similar age and gender distribution to the patients with diabetes. Ethics Committee approval was obtained, and all participants gave fully informed written consent before participation, having received full details of the study procedures.

Individuals with unavailable data on LDL size from NMR ($n = 7$) or GGE ($n = 59$), and those with nonfasting triglyceride samples ($n = 9$) or fasting triglyceride levels > 6.0 mmol/l ($n = 3$), were excluded from analyses, yielding a study population of 324 individuals (152 with type 1 diabetes mellitus and 172 controls). Medication use was low in the study population, with 91% of participants using no medication (excluding insulin use in all diabetic participants). A higher proportion of diabetic patients compared with controls used antihypertensive medication (11% and 3%, respectively; $P < 0.01$), whereas differences in other drugs were nonsignificant. The use of antihypertensive medication was not related to either GGE- or NMR-based LDL size, and the exclusion of patients using medication did not materially alter the findings.

Laboratory methods

Blood samples were collected between March 1998 and March 1999. After an overnight fast, blood samples were taken from patients and total cholesterol, HDL cholesterol, and triglycerides were measured using standard enzymatic colorimetric methods. Lipoprotein subclass levels were measured on freshly thawed frozen specimens (0.5 ml) that had been frozen at −70°C immediately after collection and had never been thawed before. Several separate tubes of plasma were frozen from each patient, and a separate tube was unfrozen for NMR and another for GGE. Samples were shipped on dry ice and analyzed immediately after thawing.

NMR measurements were performed after an average freezing time of 9 months using a 400 megahertz proton NMR analyzer at LipoScience, Inc. (Raleigh, NC). The detailed methods of these analyses have been previously described (22, 23). In short, spectra for each plasma sample were acquired and deconvoluted to give the amplitudes of the contributing signals. Using empirically measured relations between lipid contents and signal amplitudes of purified LDL subclass standards, conversion factors were derived to transform NMR subclass signal amplitudes into subclass particle levels (units of nanomoles per liter). Three LDL subclasses can thus be distinguished: L3 (21.3–23 nm; mean, 22 nm), L2 (19.8–21.2 nm; mean, 20.5 nm), and L1 (18.3–19.7 nm; mean, 19 nm). LDL size is expressed as the lipid mass-weighted average particle diameter in nanometers. The corporate documentation provided by LipoScience (14) states that LDL subclass diameters are uniformly ∼5 nm smaller than those estimated by GGE and that an average LDL particle size of ≤20.5 nm on NMR is equivalent to pattern B on GGE.

GGE measurements were executed in two batches according to an identical laboratory protocol. The first batch ($n = 70$) was measured on average 14 months after collection, and the second batch ($n = 254$) was measured on average 38 months after collection. The distribution according to sex and diabetes status was similar in both batches: 52% male and 52% with diabetes in the first batch, 47% male and 45% with diabetes in the second batch. The average LDL peak size from both batches and the respective standard deviations did not differ: 26.49 nm, SD 0.92 nm and 26.41 nm, SD 0.96 nm for the first and second batches, respectively. A comparison of the main results by GGE batch is given in the online supplemental data.

LDL peak particle size on GGE was determined using 1 mm thick 2–10% nondenaturing polyacrylamide linear gradient gels. Reagents (acrylamide-bisacrylamide, Tris, glycine, sucrose, ammonium persulfate, and tetramethylenediamine were obtained from Bio-Rad (Hercules, CA). The vertical slab gels were run in the Bio-Rad Mini-Protean II Electrophoresis Cell. After electrophoresis, gels were stained with newly prepared Sudan Black B lipid stain (Merck, Whitehouse Station, NJ), destained, and dried. Dried gels were photographed with a Kodak Digital Science DC120 camera and analyzed with Amersham Pharmacia Biotech's ImageMaster 1D software. Two isolated LDL samples were used as the size standard on each gel. The sample LDL major peak diameter (LDL size) was determined by comparing the mobility of the sample with the mobility of the two standard LDL preparations run on each gel. Details of the GGE method used have been described elsewhere (24).

Plasma cholesteryl ester transfer rate (CET) was measured as the rate of cholesteryl ester transfer out of HDL into VLDL plus LDL during incubation of plasma in vitro, that is, measured with the endogenous lipoprotein substrates using the method of Channon et al. (25) modified as described by Dullaart et al. (26). The rate of CET is expressed in nanomoles of cholesteryl ester transferred per milliliter of plasma per hour and is constant during 3 h of incubation. CET is strongly related to the level of plasma VLDL triglycerides (27).

Reproducibility

Within-laboratory reproducibility was good for both NMR and GGE. For GGE, the within-subject coefficients of variation in those with low, normal, and high triglycerides were 0.99, 1.30, and 1.92%, respectively (based on 26 repeated measurements in three individuals), and an overall between-gel coefficient of variation of 1.4% has been previously reported (24). For NMR the Spearman correlation for duplicate measurements in 22 individuals in our study population was 0.92. Previously, a coefficient of variation of 0.5% was reported for 20 replicate measurements of two plasma pools (23). Currently, the method for gradient gel electrophoresis is not standardized. Some gradient gels are commercially available but vary in quality. Therefore, many laboratories develop their own, potentially leading to unsatisfactory inter-

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laboratory agreement in the GGE measurement. Interlaboratory agreement is not an issue in the case of NMR, because LipoScience is the only facility currently performing these measurements.

Statistical analysis

Analyses were carried out with the Stata statistical package, version 7. We examined the relation between the average LDL size measurement on NMR and the peak LDL size on GGE, calculating Spearman's rho for the group as a whole and for subgroups according to gender, the presence of diabetes mellitus, and tertiles of triglycerides. Analyses in subgroups of gender and diabetes were repeated in the highest tertile of triglycerides to exclude the effect of low triglyceride values.

The individual differences between the two methods were calculated and plotted against the mean of both measurements, as described by Bland and Altman (28). This method enables the assessment of the presence of bias when the difference between two methods is not equal across increasing mean levels of the parameter under study. To further clarify the observed differences between LDL size measured by NMR and GGE, we modeled the relation between the difference and the mean of both measurements using linear regression.

The categorical classification of individuals as having pattern B (LDL size of <20.5 nm on NMR and <25.5 on GGE) was compared in a two-by-two table. The κ statistic, 95% confidence interval (CI), and percentage agreement were calculated using the DAG Stat diagnostic and agreement statistics spreadsheet (29) for the group as a whole and for the same subgroups as mentioned above. Analyses of the classification as pattern B were repeated in subjects with LDL particle numbers greater than 1,400 to assess agreement in the subset of patients for whom LDL size measurements were clinically most relevant according to the definition by LipoScience (14).

The external validity of both measurements was evaluated by assessing the relation of LDL size by both methods with CET. The CET measurement assesses the rate of transfer of cholesteryl ester from HDL to apolipoprotein B-containing lipoproteins, a process catalyzed by CETP (8). Plasma CETP activity yields triglyceride-rich LDL particles, which are subsequently remodeled by hepatic lipase, yielding small, dense LDL particles. Observed LDL size can thus be considered to depend partly on CET (30), and an inverse relation between CET and LDL (24, 31) size is to be expected. Therefore, comparison of the strength of the relation between CET and LDL size according to NMR and GGE can be considered a measure of external validity. The relation between CET and LDL size was assessed using Spearman correlations.

RESULTS

The mean difference between LDL size on NMR and peak LDL size on GGE was 5.38 nm (with NMR being smaller). The 95% limits of agreement at this point were 3.97 and 6.79, indicating that 95% of the differences between the two methods can be expected to fall within this range. The Bland-Altman plot shown in **Fig. 1** indicates that the absolute difference between the two methods increases with increasing average LDL size. The linear regression line shown in the figure deviated significantly from a horizontal line ($P < 0.001$). Log transformation as proposed by Bland and Altman (28) did not alter these findings.

Figure 2 shows the univariate relation between LDL size as determined by NMR and GGE. The Spearman rank correlation between the two methods was 0.39 (95% CI = 0.29, 0.48). The diagonal line of identity indicates where the points would be expected in the case of perfect agreement, assuming that values on NMR are uniformly 5 nm smaller than values on GGE, as indicated by LipoScience (14). Regression analysis showed that a model including a quadratic term ($R^2 = 0.25$) was significantly better ($P <$

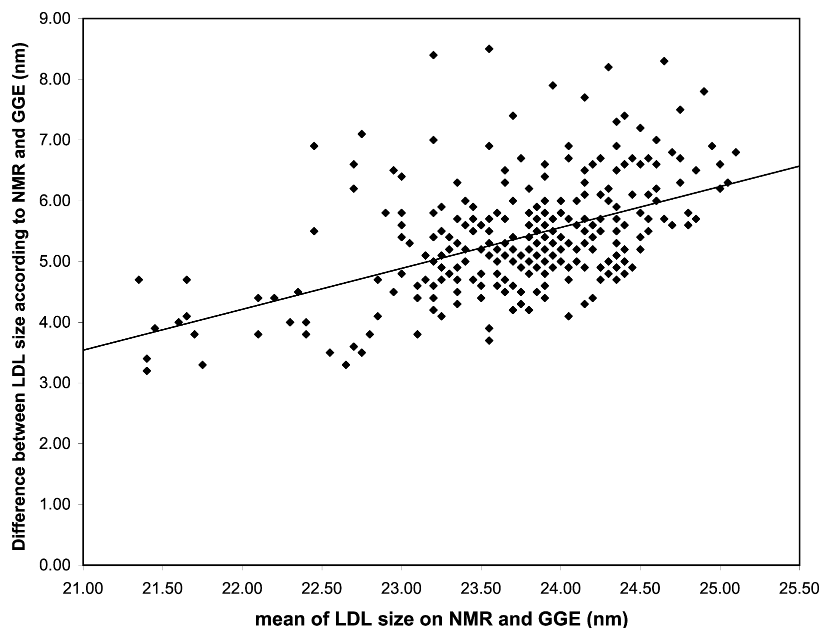

Fig. 1. Bland-Altman plot with a regression-based estimate for the difference in LDL size according to NMR and gradient gel electrophoresis (GGE).

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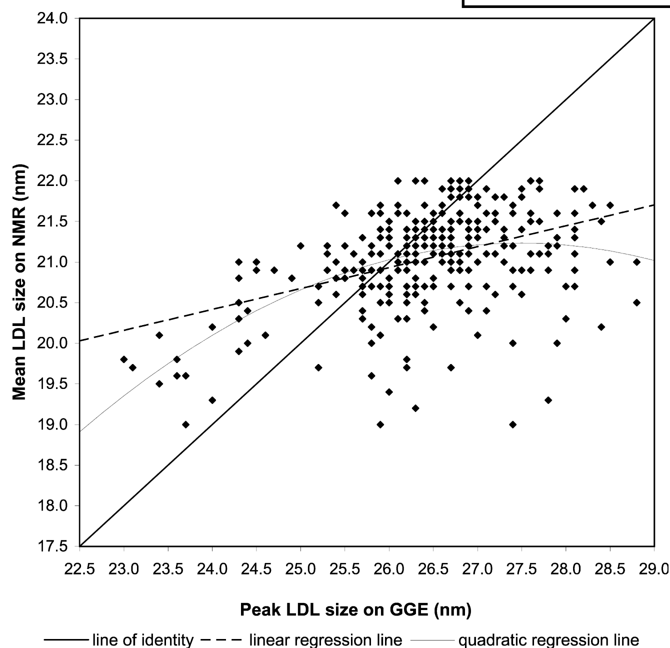

Fig. 2. Scatterplot showing the relation between LDL particle size measured with GGE and NMR. The line of identity is based on the assumption of values on NMR being uniformly 5 nm smaller than those on GGE. Model equations are as follows: linear, NMR = 14.23 + (0.26 × GGE); quadratic, NMR = −49.43 + (5.14 × GGE) + (−0.094 × GGE^2).

0.0001) than a linear model ($R^2 = 0.18$) in describing the relation between the two methods. The quadratic model line demonstrates that the relation between the two methods is mostly confined to small LDL sizes and is almost absent for large LDL sizes.

The difference and the strength of the relation between LDL size according to NMR and GGE were also different across different subgroups of the study population. As shown in **Table 1**, the mean difference was larger for patients with diabetes, women, and those with lower triglyceride levels. Agreement, expressed as the Spearman rho, was considerably higher in nondiabetic subjects, in men, and in those with high triglycerides, with estimates for all groups lying outside the confidence interval of their respective comparison group. In subjects with triglyceride levels of less than 0.87 mmol/l (the lowest tertile), the two methods were unrelated. To assess whether the observed differences in agreement could be explained simply by the lack of agreement at low triglyceride levels, we repeated the analyses in the highest tertile (>1.30 mmol/l; n = 107). Spearman correlations were 0.47 (0.18, 0.69) for women, 0.67 (0.52, 0.79) for men, 0.52 (0.26, 0.71) for subjects with diabetes, and 0.68 (0.51, 0.79) for controls.

Table 2 displays the number of subjects who were classified as having pattern B on NMR and GGE. Forty-eight percent of subjects classified as having pattern B on GGE were classified in the same category by NMR. Inversely, 36% of subjects classified as having pattern B on NMR were classified as pattern B on GGE. **Table 3** shows how observed agreement and the κ statistic differ across subgroups of patients. Agreement was considerably higher in subjects without diabetes, men, and subjects in the highest tertile of triglycerides. Restriction of analyses to the clinically most relevant group of patients [LDL particle number > 1,400 (14)] showed slightly higher agreement in all groups, but agreement continued to be lower in women compared with men and in those with diabetes compared with controls.

Table 4 shows the relation between LDL size according to both methods and CET for the total group and subgroups. In the case of GGE, the relation for all subgroups is similar to the overall relation, whereas in the case of NMR, a relation between LDL size and CET is present in men and in nondiabetic subjects but is absent in women and in subjects with diabetes.

DISCUSSION

This study found that agreement between LDL size measured by NMR and GGE is moderate, with considerable differences across subgroups of patients. Agreement in women, patients with diabetes, and patients with low triglyceride levels is considerably lower than agreement in men, controls without diabetes, and patients with high triglyceride levels, respectively. Differences in triglyceride

TABLE 1. Comparison of LDL peak size from GGE and LDL size from NMR

Difference between NMR and GGE	N	Mean Difference	95% Limits of Agreement	Spearman's Rho	95% CI
		nm			
Complete group	324	5.38	(3.63, 7.13)	0.39	(0.29, 0.48)
By diabetes					
Diabetes	152	5.49	(3.68, 7.31)	0.27	(0.11, 0.41)
No diabetes	172	5.27	(3.60, 6.96)	0.51	(0.39, 0.61)
By gender					
Men	156	5.20	(3.53, 6.86)	0.52	(0.40, 0.63)
Women	168	5.55	(3.68, 7.41)	0.23	(0.08, 0.36)
By triglyceride tertile					
Low (<0.87)	108	5.73	(3.92, 7.54)	−0.03	(−0.21, 0.17)
Medium (0.87–1.30)	109	5.41	(3.89, 6.94)	0.29	(0.11, 0.45)
High (>1.30)	107	4.99	(3.37, 6.61)	0.60	(0.47, 0.71)

CI, confidence interval; GGE, gradient gel electrophoresis.

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TABLE 2. Two-by-two table of the classification of individuals as having pattern B according to GGE and NMR with their respective cutoff levels

	Pattern B on GGE (peak particle size ≤25.5 nm)		
	No	Yes	Total
Pattern B on NMR (average particle size ≤20.5 nm)			
No	**250**	21	271
Yes	34	**19**	53
Total	284	40	324

Boldface values indicate cases in which both methods agree.

TABLE 4. Spearman correlations for the relation between cholesteryl ester transfer rate and LDL size according to NMR and GGE across subgroups

Variable	LDL Peak Size on GGE		LDL Average Size on NMR	
	Rho	95% CI	Rho	95% CI
Total group	−0.48	−0.55, −0.39	−0.06	−0.16, 0.04
By gender				
Men	−0.51	−0.62, −0.38	−0.20	−0.35, −0.04
Women	−0.41	−0.53, −0.27	0.02	−0.13, 0.18
By diabetes				
Diabetes	−0.46	−0.58, −0.33	0.05	−0.11, 0.21
No diabetes	−0.47	−0.58, −0.35	−0.26	−0.39, −0.11

distributions do not seem to explain the difference in agreement between subjects with and without diabetes and account for only a limited degree of the difference in agreement between men and women.

Consistent with this, we found that the agreement of NMR and GGE in distinguishing pattern A from pattern B was limited in women and diabetic subjects both in the complete study population and in the subset with large numbers of LDL particles, in whom the clinical utility of LDL size measurements is greatest. External validation showed that the expected inverse relation with CET was more consistent across subgroups for GGE than for NMR.

Importance of these findings

It would be naïve to expect perfect agreement between LDL size according to GGE and NMR given the different physical features of LDL particles each method is based on. Whereas GGE results depend directly on the particle dimensions and charge, NMR results are calculated from a measurement of the contents of the particle. Furthermore, comparing average particle size on NMR with peak particle size on GGE is akin to comparing a mean and a mode. Nevertheless, both methods are currently assumed to be more or less interchangeable and are widely used both in research and in clinical practice on this assumption. The cutoff point for the distinction between pattern A (low risk) and pattern B (high risk) (32) and most of the prospective evidence for a relation between LDL size and cardiovascular risk stem from studies based on the GGE method (2–5). Recently, a relation between LDL size and the angiographic progression of coronary artery disease has been found based on GGE measurements from the same facilities used in the present study (33). One prospective study has confirmed a relation with cardiovascular risk with the NMR method (6). The only currently available data on agreement between average LDL size measured by NMR and peak LDL size on GGE were in a report on LDL size in the Women's Health Study (6). The study population for the agreement section consisted of 21 healthy middle-aged men. A correlation coefficient of 0.86 was reported, but no information was given on the linearity of the relation, the distribution of the differences, the distinction between pattern A and pattern B, or on agreement in women. In conjunction with our results, this indicates that although the assumption of interchangeability may be broadly reasonable in hypertriglyceridemic men, it is not reasonable in women or diabetic subjects. It might be argued that this disagreement is more important for research than for clinical uses.

TABLE 3. Agreement and κ statistics for the agreement in classification of patients as pattern B across subgroups according to diabetes mellitus, gender, and triglyceride levels

Variable	Observed Agreement (95% CI)	κ (95%CI)
Total group	0.83 (0.78, 0.87)	0.31 (0.17, 0.45)
By diabetes		
Diabetes	0.78 (0.71, 0.85)	0.16 (−0.02, 0.34)
No diabetes	0.87 (0.81, 0.92)	0.47 (0.28, 0.66)
By gender		
Men	0.79 (0.72, 0.85)	0.36 (0.18, 0.54)
Women	0.87 (0.81, 0.92)	0.15 (−0.06, 0.36)
By triglyceride level		
Low (<0.87)	0.86 (0.78, 0.92)	0.05 (−0.17, 0.27)
Medium (0.87–1.30)	0.86 (0.78, 0.92)	0.06 (−0.16, 0.27)
High (>1.30)	0.77 (0.67, 0.84)	0.42 (0.22, 0.61)

Potential limitations

Frozen storage has the potential to cause alterations to lipoproteins (34). The effect on the results of our study, however, can be expected to be small. There is evidence that a single freeze-thaw cycle and storage at −80°C does not affect LDL peak particle diameter on GGE after 3 (35) to 6 (36) months of storage. Furthermore, any changes attributable to freezing can be assumed to be randomly distributed across individuals and subgroups. Consequently, freezing may lead to effect dilution but it cannot lead to the introduction of spurious relations. We found that the duration of sample freezing did not affect average LDL size: the average LDL size on GGE and its standard deviation were close to identical for the samples measured in the first and second GGE batches, which differed in freezing time by 2 years on average.

The principal finding of this report pertains to the marked differences in agreement across subgroups. Because differences in freezing duration were equally present in all subgroups, freezing cannot explain the observed subgroup differences in agreement. To substantiate this, we repeated the statistical analyses by duration of sample freezing (i.e., GGE batch). Both batches showed the same pattern of agreement differences: agreement was better in men compared with women and better in nondiabetic

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controls compared with diabetic subjects. Furthermore, the relation between GGE and CET was stronger than the relation between NMR and CET in both batches (data presented in the online supplemental data). Thus, we would have arrived at the same conclusions had we limited our study to the 70 individuals with the shortest freezing time. The confirmation of our findings in those with longer freezing time allowed us to regard both batches jointly and to present the results for the full study population.

It is furthermore important to be aware that the evidence for LDL size as a cardiovascular risk predictor, and for the cutoff level for pattern B itself, stems from samples with considerably longer freezing time than in our study [ranging from 3 to 15 years (2–4, 6)]. Therefore, our findings are applicable and relevant to all situations in which the evidence of these studies is used in research and clinical practice.

It is important to ascertain whether our findings of differences in agreement between men and women, and between subjects with and without diabetes, are solely attributable to poor agreement in subjects with low triglyceride levels. We found that generally in patients with triglyceride levels greater than 1.30 mmol/l, agreement was better than in the total group. Importantly, however, the differences in agreement between men and women, and between those with and without diabetes, remained. This means that overrepresentation of individuals with low triglyceride levels among women and diabetic patients cannot explain the differences in agreement between men and women and between subjects with and without diabetes.

Furthermore, we examined whether our analyses in subgroups could have led to selection of groups with very narrow distributions of LDL size, which could affect the correlations within those groups. Comparison of the LDL size distributions, according to both methods, showed that the LDL size distribution in women was somewhat narrower than that in men. However, the distributions did not differ between subjects with and without diabetes within each gender. This means that a narrower distribution might partially account for the difference in agreement between men and women but not for the difference in agreement between diabetic subjects and controls.

Agreement between NMR and GGE

Our study confirmed that on average NMR yields values 5 nm smaller (5.38 in our data) than GGE, as indicated by LipoScience (14). However, this average difference cannot be applied uniformly in practice, because it becomes considerably larger with increasing LDL size. The 95% limits of agreement, defining the range within which most differences are expected to lie, show a band of ~3 nm width. The range for expected differences thus covers approximately half the range of the LDL size measurement itself. The sources of this difference remain largely unclear, because both methods have initially been calibrated against cryo-electron microscopy (18, 37). A potential explanation might be found in differences in LDL particle charge. Particle charge increases with increasing LDL size (38), possibly influencing the migration of particles through the gradient gel. It has recently been proposed that LDL particles have a discoid rather than a spherical shape and that particle height is primarily determined by core lipids, whereas diameter is determined by surface free cholesterol (39). This observation may mean that the assumption of a spherical shape used in the derivation of LDL particle size from NMR signals is a potential source of the agreement differences reported in our study.

Methods can only be considered interchangeable if the differences between them are small (i.e., the methods are strongly related) and distributed randomly (i.e., there is no bias present). This study has shown that for LDL size according to NMR and GGE, neither condition is met. We found an overall Spearman rank correlation of 0.39, which can be considered poor for two methods assessing the same entity. The best-fitting model for the relation between both methods was a quadratic one. In this model, 25% of the variation in NMR LDL size was explained by GGE LDL peak size, which again is too low for two measures to be considered interchangeable. Furthermore, the model curve showed that the relation between the two methods is confined to small LDL sizes and is almost absent when LDL size is large.

Distinction between pattern A and pattern B

An important clinical cutoff point is the distinction between pattern A (large LDL) and pattern B (small LDL). Two methods exist for making this classification. A patient can be classified as having pattern B if the peak particle size (GGE) or average particle size (NMR) is below the threshold of 25.5 or 20.5 nm, respectively. Alternatively, a patient can be classified as having pattern B if the majority of LDL particles are below the threshold. In other words, the first method uses a summary measure for the distribution (peak size or average size), whereas the second method uses the area under the distribution curve. In this article, we have used the former method, which is most common in clinical practice. Our observation that the average difference between the two methods increases with increasing LDL size makes it clear that the assumption that LDL size of $\leq$20.5 nm on NMR is equivalent to LDL size of $\leq$25.5 nm on GGE as a determinant of pattern B is not valid for all patients.

When examining agreement, it is important to differentiate between observed agreement and chance-adjusted agreement (κ). The observed agreement depends heavily on the underlying frequencies. Because pattern A is much more common than pattern B (~85% and 15%, respectively, in our data), in ~75% of patients both methods would be expected to agree by chance alone. Therefore, a method should be evaluated by its ability to do better than chance. The κ statistic focuses on the possible scope for distinguishing groups beyond chance, indicating the attained proportion of this range (40). Arbitrarily, agreement is considered very good when κ is greater than 0.8, good when κ is between 0.8 and 0.6, moderate when κ is between 0.6 and 0.4, fair when κ is between 0.4 and 0.2, and poor when κ is less than 0.2 (40). Our finding that κ for the group as a whole was 0.31 further strengthens our

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observation that the classifications of pattern B according to GGE and NMR are not equivalent.

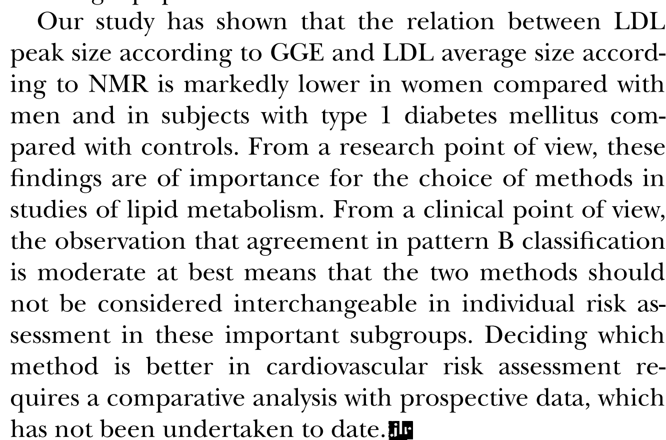
Care has to be taken when comparing κ between subgroups with different underlying distributions (41). However, because the differences in κ across subgroups in our study follow the same pattern as differences in Spearman's rho for the continuous variables, we feel it is important to highlight them. We found that agreement differed considerably in subgroups of patients, being highest in nondiabetic control subjects. Even in this group, which is a sample of the general population, κ was only 0.47. Our finding of poor agreement in diabetic subjects, women, and individuals in the lowest two tertiles of triglycerides (<1.30 mmol/l) indicates that the assumption of interchangeability can lead to considerable misclassification of individuals in these groups. In clinical practice, LDL size will mostly be measured in individuals with high triglycerides, limiting the practical problems according to this subdivision. Lower agreement in women and patients with diabetes, however, remained even when analyses were repeated in the highest tertile of triglycerides, making our findings relevant to clinical practice in these subgroups.

External validation

External validation revealed that LDL size according to GGE was inversely related to CETP-catalyzed CET at a statistically significant level both for the group as a whole and in subgroups according to gender and the presence of diabetes. In the case of NMR, relations with CET were only present in men and in subjects without diabetes. For the group as a whole, no relation was found between CET and NMR-based LDL size. Based on the metabolic functions of CETP, a strong relation with LDL size was expected a priori. Previously, a correlation of −0.79 was reported between CETP activity and LDL size on GGE (31). The absence of a relation between LDL size on NMR and CET in the same subgroups in which agreement between NMR and GGE is lower confirms the findings of the agreement analyses and indicates that mechanisms of cholesteryl ester transfer, or other pathways determining lipoprotein particle composition, might differ specifically in these subgroups. As we hypothesized earlier (22), it is possible that potentially important compositional differences in diabetic subjects and in women are not captured by the current method of NMR-derived size. This might explain why we previously found that NMR-defined particle size is associated with coronary artery calcification in nondiabetic subjects but not in type 1 diabetes (22). In the case of GGE, LDL size was also related to coronary artery calcification in nondiabetic subjects but not in diabetic subjects. Spearman correlations were −0.31 (95% CI = −0.44, −0.17) and −0.10 (95% CI = −0.25, 0.06), respectively. These relations were stronger for GGE than those previously reported for NMR (22).

Further exploration

Despite the differences in agreement between NMR and GGE, the question remains regarding which measure best predicts cardiovascular risk. An association between small LDL and the occurrence of future cardiovascular events has been found with both NMR (6, 7) and GGE (2–5). However, in one study using GGE (2) and in both studies using NMR (6, 7), the relation was abolished by adjustment for cardiovascular risk factors. No study to date has compared the predictive value of both methods in a single population.



Our study has shown that the relation between LDL peak size according to GGE and LDL average size according to NMR is markedly lower in women compared with men and in subjects with type 1 diabetes mellitus compared with controls. From a research point of view, these findings are of importance for the choice of methods in studies of lipid metabolism. From a clinical point of view, the observation that agreement in pattern B classification is moderate at best means that the two methods should not be considered interchangeable in individual risk assessment in these important subgroups. Deciding which method is better in cardiovascular risk assessment requires a comparative analysis with prospective data, which has not been undertaken to date.

This study was supported by a project grant from the British Heart Foundation (PG97/160).

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