Agreement Between Fasting and Postprandial LDL Cholesterol Measured with 3 Methods in Patients with Type 2 Diabetes Mellitus

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BACKGROUND: LDL cholesterol (LDL-C) is a modifiable cardiovascular disease risk factor. We studied the agreement between fasting and postprandial LDL-C in type 2 diabetes (T2DM) patients using 3 LDL-C methods.

METHODS: We served 74 T2DM patients a standardized meal and sampled blood at fasting and 1.5, 3.0, 4.5, and 6.0 h postprandially. We measured LDL-C by use of modified β quantification (MBQ), the Friedewald equation (FE), and a direct homogeneous assay (DA). We evaluated agreement using 95% limits of agreement (LOAs) within ±0.20 mmol/L (±7.7 mg/dL).

RESULTS: LDL-C concentrations at all postprandial times differed from those at fasting for all methods. In 66 patients who had complete measurements with all LDL-C methods, maximum mean differences (95% LOA) in postprandial vs fasting LDL-C were −0.16 mmol/L (−0.51; 0.19) [−6.2 mg/dL (−19.7; 7.3)] with MBQ at 3 h; −0.36 mmol/L (−0.89; 0.17) [−13.9 mg/dL (−34.6; 6.6)] with FE at 4.5 h; and −0.24 mmol/L (−0.62; 0.05) [−9.3 mg/dL (−24; 1.9)] with DA at 6.0 h. In postprandial samples, FE misclassified 38% of patients (two-thirds of statin users) into lower Adult Treatment Panel III (ATP III) risk categories. Greater disagreement between fasting and postprandial LDL-C was observed in individuals with postprandial triglyceride concentrations >2.08 mmol/L (>184 mg/dL) and in women (interactions: $P = 0.038$).

CONCLUSIONS: Differences up to 0.89 mmol/L (34 mg/dL) between fasting and postprandial LDL-C concentrations, with postprandial LDL-C concentrations usually being lower, were found in T2DM by 3 different LDL-C methods. Such differences are potentially relevant clinically and suggest that, irrespective of measurement method, postprandial LDL-C concentrations should not be used to assess cardiovascular disease risk.

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In individuals with or without diabetes, the plasma concentration of LDL cholesterol (LDL-C) is an important and modifiable cardiovascular disease (CVD) risk factor (1–4). Intervention studies, mainly of fasting samples, are the basis of current LDL-C treatment goals (1–3). The choice between fasting and nonfasting samples is debated, however (5–10). For example, in contrast to fasting LDL-C, large-scale epidemiological studies disagree about whether nonfasting LDL-C predicts CVD (5, 6, 10). Also, unlike international recommendations (1, 3, 11–13), the Danish national recommendation is for nonfasting assessment of LDL-C (14).

A recent large-scale epidemiological study (15) resulted in a similar message to the public: “No fasting” for cholesterol test (16).

Previous studies demonstrated that LDL-C concentrations are lower in the nonfasting than fasting state (5, 6, 10, 17–29). These studies focused on the mean fasting-nonfasting difference. For the individual, however, the extent of agreement [i.e., the mean difference with 95% limits of agreement (LOAs)] between fasting and nonfasting samples is probably a more relevant parameter (30).

By definition, ascertainment of LDL-C concentrations requires ultracentrifugation (31), often by the β quantification, and most data for plasma lipid fractions other than LDL cholesterol have been reported (as acknowledged in the text (29)).

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Previous presentation of the manuscript: For all 74 patients, but not for the 66 patients with complete measurements of LDL-C with all methods, data of subject characteristics, parts of the investigative procedure, most data from the modified β quantification, and most data for plasma lipid fractions other than LDL cholesterol have been reported (as acknowledged in the text (29)).

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6 Nonstandard abbreviations: LDL-C, LDL cholesterol; CVD, cardiovascular disease; LOA, limit of agreement; BQ, β quantification; FE, Friedewald equation; DA, direct assay; T2DM, type 2 diabetes mellitus; MBQ, modified BQ; BMI, body mass index; TC, total cholesterol; ATP III, Adult Treatment Panel III; TRL, triglyceride-rich lipoprotein; NCEP: National Cholesterol Education Program.
quantification (BQ) method (13, 32). Ultracentrifugation is cumbersome, however, and usually assessment of LDL-C is made by calculation, e.g., use of the Friedewald equation (FE). The FE was constructed on fasting samples and, moreover, triglyceride concentrations <4.52 mmol/L (<400 mg/dL) (19, 33). Most intervention trials have used FE as recommended (34). Contrary to recommendations, however, many outpatient clinics and epidemiological studies use FE in nonfasting samples (5, 10, 15). The widespread use of FE makes it the most important method for further evaluation. There is a need to examine its LOAs under nonfasting conditions.

Direct measurement of LDL-C by use of homogeneous assays (DA) without ultracentrifugation has been possible since the 1990s. These assays are potentially less affected by fasting/nonfasting conditions, at least in nondiabetic individuals (35). However, triglycerides are present at higher concentrations postprandially in type 2 diabetes mellitus (T2DM) patients than in nondiabetic individuals (36–38). Such changes could influence DA performance (22, 26, 34, 39–41).

Hence, for assessing LDL-C, the use of fasting or nonfasting samples and various methodological issues remain controversial. We investigated the agreement of LDL-C concentrations in paired fasting and postprandial samples from 74 T2DM patients with a modified BQ (MBQ), the FE, and a DA method.

Materials and Methods

Our work was a nested study within an ongoing trial at Steno Diabetes Center (42, 43). We investigated 74 consecutive patients; among these, 66 had complete measurements with all LDL-C methods. In 8 patients at 1 or more times, we could not use FE because of missing underlying measurements or because the concentration of triglycerides exceeded 4.52 mmol/L (400 mg/dL), whereas MBQ and DA had complete measurements (see Supplemental Data, which accompanies the online version of this article at www.clinchem.org/content/vol57/issue2). We previously reported part of the LDL-C results measured by the MBQ and part of the results for non-LDL lipids for all 74 patients (29). Unless otherwise noted, here we report data for the 66 patients in whom LDL-C could be determined with all 3 methods. Where relevant, however, we also report data for all 74 patients, since this population included those patients who had the highest triglyceride concentrations. The Ethics Committee of Copenhagen County, Denmark, approved the study, and we conducted it in accordance with the Declaration of Helsinki.

PATIENT CHARACTERISTICS

Participants were insulin-naive white T2DM patients with a body mass index (BMI) ≤27 kg/m² (29, 42). At the time of investigation, median age was approximately 60 years; median duration of diabetes was approximately 5 years; approximately 20% were women; median BMI was approximately 25 kg/m²; median HbA₁c was 7.7%. Median concentrations of fasting total cholesterol (TC), HDL-C, triglycerides, and LDL-C were 4.70 mmol/L (182 mg/dL), 1.14 mmol/L (44 mg/dL), 1.27 mmol/L (113 mg/dL), and 2.85 mmol/L (110 mg/dL), respectively. Approximately 20% had previous CVD; they received either diet-only or oral agents as blood glucose–lowering treatment; and approximately 25% used statins. Women had lower body weight than men, and approximately 60% were receiving hormone replacement therapy (see online Supplemental Table 1). All patients had serum creatinine and thyroid-stimulating hormone concentrations within normal reference intervals.

Most of the study procedures have been published (29, 43). They are also described in detail in the online Supplemental Data and summarized below.

Time is in hours since initiating the meal, fasting refers to measurements taken at time 0; and postprandial refers to measurements taken between 1.5 and 6.0 h, without reference to any specific time unless stated.

PROTOCOL

We served patients a standardized fat-rich breakfast meal [3515 kJ; 54% fat (50.4 g total fat), 13% protein, 33% carbohydrates] and sampled blood at fasting and at 1.5, 3.0, 4.5, and 6.0 h postprandially. Only drinking water was allowed postprandially (see online Supplemental Data).

MEASUREMENTS

We measured TC and triglycerides using enzymatic methods (Roche) in freshly drawn lithium-heparin plasma samples. We measured HDL-C and LDL-C by use of homogeneous assays (HDL-C Plus and LDL-C Plus, Roche) in these samples (see online Supplemental Data). We also calculated LDL-C using the FE \[LDL-C = TC - HDL-C - (triglycerides/2.2)\] in samples with triglyceride concentrations <4.52 mmol/L (400 mg/dL) (divide triglycerides by 5 if measured in mg/dL) (33). Measurement of LDL-C by MBQ was as follows. We ultracentrifuged EDTA plasma samples for 18 h at 105,000g. We isolated the fraction of density >1.006 g/mL (infranatant) and measured TC and HDL-C as described above. We calculated the LDL-C concentration as the difference in the infranatant between TC and HDL-C (see online Supplemental Data).
RESULTS

LIPID PROFILES
Concentrations of TC and HDL-C each decreased by approximately 0.1 mmol/L (3.9 mg/dL) in the postprandial vs fasting state, whereas geometric mean triglyceride concentrations increased by 0.7–0.8 mmol/L (62–71 mg/dL) (Table 1; see online Supplemental Figs. 3 and 4). The maximum triglyceride concentration was 4.4 mmol/L (390 mg/dL) in the group of 66 patients, and 6.07 mmol/L (538 mg/dL) in all 74 patients.

COMPARISON OF LDL-C IN PAIRED FASTING AND POSTPRANDIAL SAMPLES
With all methods and at all postprandial times, mean LDL-C concentrations were significantly lower than at time 0 (P < 0.005; Fig. 1). The lowest mean LDL-C concentrations occurred at 3.0, 4.5, and 6.0 h with MBQ, FE, and DA, respectively. The corresponding mean changes from fasting concentrations were −0.16, −0.36, and −0.24 mmol/L (−6.2, −13.9, and −9.3 mg/dL), respectively (Fig. 1; Table 1). Inspection of LDL-C trajectories revealed an approximately linear change with time after the meal with each method for most patients (see online Supplemental Fig. 4).

At none of the postprandial times did MBQ, FE, or DA meet the criteria for acceptable agreement with fasting concentrations. With all methods, the lowest of the lower 95% LOAs (estimated maximum disagreement) occurred at 4.5 or 6.0 h and were −0.52, −0.89, and −0.62 mmol/L (−20, −34, and −24 mg/dL), for MBQ, FE, and DA, respectively (Table 1; Fig. 2; online Supplemental Fig. 5). Likewise, when we evaluated agreement using 95% CIs for the 95% LOAs (30), agreement was not met with any of the LDL-C methods (Fig. 2; online Supplemental Fig. 5).

In those patients with fasting LDL-C ≥2.6 mmol/L (≥100 mg/dL), use of postprandial LDL-C misclassified 10%–38% of patients into a lower CVD risk group, most pronounced for FE (Table 2). Similarly, in those patients with a postprandial LDL-C <2.6 mmol/L, 25%–50% had fasting LDL-C ≥2.6 mmol/L. In statin users, the corresponding percentages were 13%–63% and 10%–36%, respectively. With a LDL-C cut point of 1.8 mmol/L (70 mg/dL), the corresponding percentages were 3%–6%; 38%–100%, 6%–21%, and 40%–100%, respectively (Table 2).

One patient showed an extreme difference with much lower fasting than postprandial LDL-C as measured with DA (Fig. 2; online Supplemental Figs. 4 and 5). The above conclusions did not change if this patient was excluded (data not shown) or if all 74 patients (with or without excluding the outlier) were analyzed (see online Supplemental Tables 2 and 3 and Supple-

STATISTICS
We describe patient characteristics using median (interquartile range; range) for continuous variables and numbers (percentage) for categorical variables. Group comparisons for these 2 types of data were carried out by use of nonparametric tests (Mann–Whitney U) and χ² tests, respectively.

We analyzed the meal test data for triglycerides using natural log–transformed values and report them as geometric mean (range) or relative changes owing to the skewed distribution. Other variables (and their changes) showed an approximately normal distribution, and we report them as untransformed values, as mean (SD), mean (SE), or absolute changes.

We evaluated absolute agreement between fasting and postprandial measurements for each LDL-C method according to the Bland–Altman method, calculating the mean LDL-C differences and 95% LOA (mean difference ±1.96 SDs of the differences) (30, 44). We defined acceptable agreement (the window of acceptable agreement) as the mean LDL-C difference and 95% LOA being within ±0.20 mmol/L (±7.7 mg/dL) (see online Supplemental Data). We chose the ±0.20 mmol/L window because differences this large between fasting and nonfasting LDL-C are thought to influence CVD prediction (6, 7, 10) (see online Supplemental Data).

Note that 95% LOAs are not equivalent to 95% CIs for the mean differences. This is because 95% LOAs represent the mean difference ±1.96 × the standard deviation of the individual differences, whereas 95% CIs (for the mean difference) represent the mean difference ±1.96 × the standard error of the mean difference. Hence, 95% LOAs that include zero do not exclude the possibility that the mean difference (e.g., between postprandial and fasting lipid concentrations) differs from zero at a statistically significant level.

Furthermore, we analyzed mean differences and patient group effects by use of a repeated-measures mixed model with a general (unstructured) covariance structure. We included fixed effects (group and time) and, where relevant, group–time interactions (45). We investigated the proportion of patients misclassified by using postprandial instead of fasting samples according to the Adult Treatment Panel III (ATP III) criterion for diabetes patients’ desired fasting concentration of LDL-C <2.6 mmol/L (<100 mg/dL) (1). Hence, we investigated the proportion of patients having fasting LDL-C ≥2.6 mmol/L and at least 1 postprandial LDL-C <2.6 mmol/L, and vice versa. We made a similar analysis using the optional ATP III cut point of 1.8 mmol/L (70 mg/dL) (2).

Data are shown as crude (unadjusted) values. Statistical significance was evaluated at the 5% level. We performed all analyses with SPSS version 14.0.
creases in LDL-C than the group whose postprandial measurement of postprandial triglycerides showed greater postprandial decrease in LDL-C than women 

**PATIENT GROUPS**

At most times, the absolute LDL-C concentrations were significantly lower in statin users vs nonusers ($P < 0.05$). For other patient groups classified by sex, CVD status, or a median postprandial triglyceride concentration threshold of 2.08 mmol/L (184 mg/dL), we observed no significant differences between groups ($P > 0.05$) (Fig. 3; online Supplemental Fig. 7).

The shapes of the LDL-C–time curves of the patient groups classified by statin use or CVD status were similar (group–time interactions $P > 0.05$) (Fig. 3; online Supplemental Fig. 7).

Conversely, the group of patients with at least 1 measurement of postprandial triglycerides >2.08 mmol/L (184 mg/dL) showed greater postprandial decreases in LDL-C than the group whose postprandial triglyceride concentrations all remained <2.08 mmol/L (184 mg/dL) (group–time interactions $P = 0.038, P < 0.001$, and $P = 0.019$ for MBQ, FE, and DA, respectively) (Fig. 3).

Likewise, for sex, MBQ and DA measurements showed significant heterogeneity between groups (group–time interactions $P = 0.008$ and $P = 0.017$, respectively) (see online Supplemental Fig. 7). Thus, the postprandial decreases in LDL-C were greater in women than in men [albeit, for DA measurements, men showed a significantly greater postprandial decrease in LDL-C at 1.5 h compared with women ($P = 0.024$) (see online Supplemental Fig. 7)]; the greater postprandial decrease in LDL-C in women than in men ($P = 0.016$ at 4.5 h) became apparent when data from all 74 patients were analyzed (see online Supplemental Data and Supplemental Fig. 8). However, FE did not capture the postprandial heterogeneity between sexes (interaction $P = 0.987$) (online Supplemental Fig. 7).

### Table 1. Comparison of postprandial vs fasting LDL-C concentrations assessed with 3 methods in 66 T2DM patients.

<table>
<thead>
<tr>
<th></th>
<th>Fasting (0 h), mean (SD)</th>
<th>1.5 h</th>
<th>3.0 h</th>
<th>4.5 h</th>
<th>6.0 h</th>
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<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>95% LOA</td>
<td>Mean</td>
<td>95% LOA</td>
</tr>
<tr>
<td>LDL-C by MBQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mmol/L</td>
<td>3.13 (0.71)</td>
<td>$-0.06$</td>
<td>$-0.35$; $0.22$</td>
<td>$-0.16$</td>
<td>$-0.51$; $0.19$</td>
</tr>
<tr>
<td>mg/dL</td>
<td>121 (27)</td>
<td>$-2.3$</td>
<td>$-13.5$; $8.5$</td>
<td>$-6.2$</td>
<td>$-19.7$; $7.3$</td>
</tr>
<tr>
<td>LDL-C by FE</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>mmol/L</td>
<td>3.00 (0.74)</td>
<td>$-0.15$</td>
<td>$-0.54$; $0.23$</td>
<td>$-0.35$</td>
<td>$-0.82$; $0.11$</td>
</tr>
<tr>
<td>mg/dL</td>
<td>116 (29)</td>
<td>$-5.8$</td>
<td>$-21.8$; $8.9$</td>
<td>$-13.5$</td>
<td>$-32.4$; $43$</td>
</tr>
<tr>
<td>LDL-C by DA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>mmol/L</td>
<td>2.85 (0.74)</td>
<td>$-0.06$</td>
<td>$-0.36$; $0.23$</td>
<td>$-0.18$</td>
<td>$-0.53$; $0.17$</td>
</tr>
<tr>
<td>mg/dL</td>
<td>110 (29)</td>
<td>$-2.3$</td>
<td>$-13.9$; $8.9$</td>
<td>$-7.0$</td>
<td>$-20.6$; $6.6$</td>
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<tr>
<td>TC</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>mmol/L</td>
<td>4.82 (0.85)</td>
<td>$-0.06$</td>
<td>$-0.43$; $0.30$</td>
<td>$-0.09$</td>
<td>$-0.48$; $0.30$</td>
</tr>
<tr>
<td>mg/dL</td>
<td>186 (33)</td>
<td>$-2.3$</td>
<td>$-16.6$; $11.6$</td>
<td>$-3.5$</td>
<td>$-18.6$; $11.6$</td>
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<tr>
<td>HDL-C</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>mmol/L</td>
<td>1.18 (0.33)</td>
<td>$-0.03$</td>
<td>$-0.13$; $0.06$</td>
<td>$-0.07$</td>
<td>$-0.19$; $0.05$</td>
</tr>
<tr>
<td>mg/dL</td>
<td>46 (13)</td>
<td>$-1.2$</td>
<td>$-5.0$; $2.3$</td>
<td>$-2.7$</td>
<td>$-7.3$; $1.9$</td>
</tr>
<tr>
<td>Triglycerides</td>
<td></td>
<td></td>
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<tr>
<td>mmol/L</td>
<td>1.24 (0.4; 3.4)$^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/dL</td>
<td>110 (35; 301)$^b$</td>
<td>$22$</td>
<td>$-10$; $65$</td>
<td>$54$</td>
<td>$-6$; $152$</td>
</tr>
</tbody>
</table>

95% CIs are not shown; however, data marked with $^a$ indicate that the 95% confidence intervals (for the mean difference) did not include zero (i.e., statistical significance at the 5% level). For the 95% CIs, see Fig. 2 and online Supplemental Fig. 5. For the corresponding table for all 74 patients, see online Supplemental Table 2.

* $P < 0.05$ for comparisons of means vs fasting concentrations. $^b$ Geometric mean (range).
These interactions for sex were unchanged after adjustment for diabetes duration, body weight, BMI, use of concomitant medications, or current triglyceride concentrations.

In all 74 patients, statin users showed significantly lower LDL-C concentrations at all times ($P < 0.05$) (see online Supplemental Data and Supplemental Fig. 8). Otherwise, the above observations were similar between populations (Fig. 3; Supplemental Data; Supplemental Figs. 7 and 8).

**Discussion**

We investigated the agreement of LDL-C concentrations in fasting and postprandial samples as measured with commonly used LDL-C methods, including a MBQ method, in 74 T2DM patients.

**LDL-C CHANGES AND CVD RISK**

The 0.2–0.4 mmol/L (7.7–15.5 mg/dL) lower mean LDL-C concentration in postprandial vs fasting samples observed in our study is consistent with studies from the general population or in T2DM patients using unpaired ($5, 6, 10$) and paired ($17–25 28$) samples, and also with studies using BQ ($19, 25$).

Two recent large-scale studies analyzed unpaired (and nonrandomized) samples and claimed that mean LDL-C differences of approximately 0.2 mmol/L (7.7 mg/dL) (measured by FE or a DA) between fasting and nonfasting individuals were “clinically insignificant” or “clinically unimportant” ($5, 6$). A metaanalysis of cholesterol-lowering studies showed a 21% decrease in CVD risk per 0.5 mmol/L (19.3 mg/dL) drop in LDL-C [range 0.2–0.7 (7.7–27 mg/dL)] ($46$). Moreover, in an observational study, a approximately 0.2 mmol/L (~7.7 mg/dL) difference in LDL-C was associated with a approximately 20% difference in CVD risk ($47$). Hence, the 0.2–0.4 mmol/L mean lower postprandial than fasting LDL-C may represent a meaningful increment in CVD risk.

**MEASURES OF AGREEMENT**

A study of 31 T2DM patients suggested poor agreement with FE, but good agreement with a DA in fasting vs nonfasting LDL-C (unspecified assay) ($24$). However, relative agreement was evaluated (the number of nonfasting measurements within 10% of the fasting concentration). Thus, since the mean difference (bias) between fasting and nonfasting LDL-C is relatively constant over a wide range of values (Fig. 2), using relative agreement will yield more disagreements at low than at high LDL-C values. This can give a misleading impression, since CVD risk increases log-linearly with increasing LDL-C, i.e., a given absolute LDL-C change results in a greater absolute risk change at high than at low LDL-C concentrations ($1, 2$). Hence, the higher the LDL-C concentration, the more relative agreement gives the impression of a closer agreement between fasting and nonfasting samples than found when the absolute agreement is evaluated.

In other studies, fasting/nonfasting agreement of LDL-C has been evaluated by the proportion of patients meeting targets ($17, 22–24, 48–51$), including T2DM patients ($23, 24, 51$). Despite the importance of this method of assessment (see below), it also is influenced by the underlying LDL-C concentrations (as well as by targets and accuracy of methods).

Conversely, assessment of the absolute fasting/nonfasting agreement by the Bland–Altman method, as in our study, provides a measure of the extent of agreement that is largely independent of underlying LDL-C concentrations. In addition to providing the mean changes (bias) at the population level, it also produces an estimate of such agreement at the individual level (the LOAs) (see online Supplemental Data).
Fig. 2. Bland–Altman plots of the difference between postprandial and fasting concentrations of LDL-C measured by 3 methods in 66 T2DM patients.

Difference represents the postprandial concentration of LDL-C minus that of the fasting concentration, whereas Average is the mean of the postprandial and fasting LDL-C. The solid line represents the line of identity. The dashed lines in black represent mean differences with associated 95% LOA (95% LOA is the mean difference ± 1.96 SDs of the differences), whereas, for each of these, the corresponding lines in gray represent their 95% CIs. The area in gray represents the window of acceptable agreement of ±0.20 mmol/L (±7.7 mg/dL). For the corresponding plots at 3.0 and 6.0 h postprandially, see online Supplemental Fig. 5. For the corresponding plots for all 74 patients, see online Supplemental Fig. 6. To convert mmol/L to mg/dL, multiply by 38.67.
Our evaluation observed a disagreement of up to −0.89 mmol/L (34 mg/dL) between paired fasting and postprandial LDL-C, which is a clinically relevant difference. Thus, given that the fasting/postprandial LDL-C differences are approximately normally distributed, in about half of T2DM patients, postprandial and fasting LDL-C measured by FE, for example, may differ by −0.4 mmol/L (the bias) to −0.9 mmol/L (the LOA) (∼16 to −35 mg/dL) (Fig. 2; online Supplemental Figs. 5 and 6).

Accordingly, we demonstrated that with a target of (fasting) LDL-C <2.6 mmol/L (100 mg/dL), use of postprandial LDL-C values would typically misclassify 10%–40% of T2DM patients and up to two-thirds of statin users into lower CVD risk categories. This was most pronounced for FE. With the lower target of <1.8 mmol/L (70 mg/dL), 10%–20% of statin users would still be misclassified. Despite lower on-treatment LDL-C concentrations, statin users might be high-risk patients for whom such misclassification could incorrectly lead to less intensive lipid-lowering therapy.

**SPECIFICITY, INTERFERENCES, AND PATIENT GROUPS**

We found different shapes of the postprandial LDL-C–time curves among methods. Thus, nonspecificity and/or physiological changes could be of importance and could differ by fasting status and/or between patient groups. Statin use or known CVD did not appear to be influential in this respect. Conversely, we demonstrated greater postprandial decreases in LDL-C with higher postprandial triglyceride concentrations and female sex. FE did not show the sex difference, however. Irrespective of the LDL-C method, in nonfasting samples, a triglyceride concentration >2.08 mmol/L (>184 mg/dL) had a larger influence on the discrepancy vs fasting samples. Notably, this triglyceride concentration is far below that recommended, <4.52 mmol/L (<400 mg/dL), for using FE in fasting samples (33), although it has been reported (also with fasting samples) that FE was unreliable with triglyceride concentrations >2.26 mmol/L (>200 mg/dL) (52). Thus, our findings suggest a significant influence of postprandial triglycerides on the measurement of LDL-C with all 3 methods.

FE calculates LDL-C and assumes that, among triglyceride-rich lipoproteins (TRL) (i.e., chylomicrons and VLDL), only VLDLs are present and have a constant ratio of total triglycerides to VLDL-C (33). Nonfasting samples do not meet these assumptions (19, 29). Chylomicrons are present, and they are more triglyceride-rich than VLDLs, resulting in a rising ratio of total triglyceride to TRL cholesterol. Hence, FE cannot adjust for the postprandial rise in triglycerides, which exaggerates (confounds) the physiological postprandial decrease in LDL-C. BQ removes TRL before measurement, and DA attempts to inhibit the cholesterol reactivity in non-LDLs (see online Supplemental Data). Thus, technically, MBQ is less sensitive to TRL, but the postprandial physiologic reduction in LDL-C remains a problem in interpreting results. DAs have been shown to have limitations when abnormal lipoproteins and increased triglycerides are present (41).

According to the manufacturer, triglyceride concentrations up to 1200 mg/dL [13 mmol/L; i.e., far higher than the maximum 6.07 mmol/L (538 mg/dL) in our study] do not significantly interfere with LDL-C Plus (53). Using a single nonfasting sample (3.5 h), LDL-C Plus appeared to be valid in the general population (22), but increasing triglyceride concentrations negatively biased it (and other DAs as well) (22, 40, 41). This is concordant with the increasing...
negative bias of LDL-C Plus observed postprandially in our study. In a single postprandial sample (2–3 h), DAs were not recommended in nonfasting samples owing to increased fasting–nonfasting variability. Also, larger-scale studies, some of which used LDL-C Plus (10, 26, 49, 54) (Dr. S. Mora, pers. comm. for (10)), support our findings (10, 21, 26, 49, 54).

Whether use of nonfasting samples compromises patient management is a medical matter, and the decision to use them also involves practical considerations.

**LIMITATIONS**

First, we used a standardized high-fat meal (approximately 50% fat) (online Supplemental Data). We can-
not therefore make conclusions regarding such changes with other diets. Individuals from a general population who consumed typical self-selected foods (40% fat), showed a 0.5–0.6 mmol/L (44–53 mg/dL) postprandial increase in triglycerides, i.e., close to the approximately 0.7 mmol/L (approximately 62 mg/dL) increase we observed (17). Our meal ingredients (bread, butter, cheese, milk, jam, and sausage) are part of a normal Western diet, and T2DM patients are often more dyslipidemic than nondiabetic individuals (38). Hence, we do not consider our experimental conditions unrealistic or unrepresentative, since not all patients are likely to adhere to prescribed low-fat diets.

Second, we did not use or standardize to the CDC’s BQ [the BQ recommended by the National Cholesterol Education Program (NCEP) (13)]. However, an accredited and certified laboratory approved our BQ procedure, and our MBQ met acceptable agreement compared with a more traditional BQ procedure using ultracentrifugation-precipitation (see online Supplemental Data).

Third, as recommended by NCEP, for MBQ analysis we used plasma samples collected into liquid EDTA (13, 32). This could have diluted the MBQ samples slightly (approximately 3%) compared with either FE or DA (using lithium-heparin plasma) (13, 32).

Fourth, as in most outpatient clinics, we measured triglycerides without glycerol blanking (55). This probably influenced conclusions negligibly (online Supplemental Data).

Fifth, we tested a single DA. Other DAs (using other analytical principles) might perform differently (24, 48). However, studies investigating other DAs support our findings (21, 26, 27, 54).

Sixth, 8 patients had some missing values with FE, mainly because of triglyceride concentrations >4.52 mmol/L (400 mg/dL) (MBQ and DA had no missing values). Inclusion of these patients in the analysis corroborated the findings for sex (for the DA) and statin use, and they did not impact on the overall conclusions (see online Supplemental Data).

STRENGTHS
The strengths of our study in T2DM patients include using absolute concentration differences to evaluate the influence of nonfasting samples. Furthermore, we used 4 paired and highly standardized postprandial samples, whereas previous studies, in other populations, typically used, for example, a single nonfasting sample, nonstandardized samples, and/or unpaired (and nonrandomized) samples (5, 6, 10, 21–27, 48–50). We also addressed the influence of statin use, CVD, sex, and triglycerides.

SUMMARY
For 3 LDL-C methods, the mean LDL-C decreased by 0.2–0.4 mmol/L (7.7–15.5 mg/dL) postprandially in T2DM patients. The agreement between postprandial and fasting samples was poor for all LDL-C methods, with the 95% LOA ranging from −0.52 to −0.89 mmol/L (−20 to −34 mg/dL). Using postprandial LDL-C, FE misclassified 38% of patients and two-thirds of statin users into lower ATP-III risk categories. Female sex or postprandial triglyceride concentrations >2.08 mmol/L (>184 mg/dL) had greater disagree-
ment between fasting and postprandial LDL-C. Thus, in T2DM patients, postprandial LDL-C concentrations might differ substantially from fasting concentrations, with postprandial LDL-C concentrations usually being lower. Our findings support that, irrespective of the method, postprandial LDL-C should not be used for assessing CVD risk.

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