Recent publications in the *Journal of the American Medical Association* (1, 2) report the superiority of the measurement of nonfasting over conventional fasting triglycerides in predicting risk for cardiovascular events, observations consistent with previous studies (3, 4). For decades the usual practice has been to measure triglycerides in blood samples obtained after patients have fasted 8–12 h, a procedure consistent with population studies that specified fasting blood collections to decrease variability and achieve consistency of metabolic state in patients at the time of sample collection (5). Triglyceride values fluctuate widely over time, with a CV of biological variability averaging about 23% and ranging up to 40%, and this variability can confound estimation of the associated cardiovascular disease (CVD) risk (6). Fasting blood collection is thought to decrease this variability (7), a theory supported by the observed correlation between fasting and nonfasting values (8). Nevertheless, because postprandial nonfasting values are more representative of the usual metabolic state, the observed improved prediction of the associated CVD risk is not unexpected. A change in practice to nonfasting collections, however, might affect other applications of the triglyceride determination or other measurements made on the specimens.

Triglycerides are measured in clinical practice not only to assess CVD risk, but also to detect the extreme increases that can contribute to pancreatitis. For this purpose the question of fasting vs nonfasting is largely irrelevant; in fact such high levels could be detected by visual observation of the extent of turbidity and/or a floating fat layer associated with the larger triglyceride-rich lipoprotein (TRL) particles. Triglyceride-associated turbidity, likely increased in nonfasting specimens, can interfere with other laboratory determinations, necessitating thorough studies with various instrument/reagent systems.

Triglyceride values are also used to calculate LDL cholesterol (LDL-C) by difference using the Friedewald equation, which was derived using fasting specimens. Hence, the equation might require revision and validation for specimens collected under nonfasting conditions. On the other hand, the calculation has been shown to be less reliable with increasing triglycerides even with fasting (9) and there are alternative measures of atherogenic particles that do not require fasting collections, such as non-HDL-C as recommended by current guidelines (10), total-C to HDL-C ratio as supported by recent studies (11), and apoB, the major protein constituent of VLDL and LDL, shown in many studies to be superior to LDL-C (12).

Before considering alternatives to the current practice it is instructive to consider the complex heterogeneous nature of triglyceride as an analyte. Triglycerides, as the name implies, consist of 3 fatty acids linked through ester bonds to a glycerol backbone (13). Fatty acids, the body’s major fuel source, are heterogeneous, typically ranging in size from the 12-carbon saturated lauric acid through the 18-carbon monounsaturated oleic acid to the 22-carbon polyunsaturated (omega 3) docosahexaenoic acid. Triglycerides of dietary origin enter the circulation as chylomicrons, the largest of lipoprotein particles (80 –1000 nm) packaged together with the truncated form of apolipoprotein B, apoB-48, whereas fatty acids originating in the liver are secreted as VLDL (25–80 nm) with apoB-100. In the circulation these nascent TRL particles are usually rapidly remodeled through lipolysis by various lipases (lipoprotein, hepatic, and endothelial lipases), transitioning through remnant lipoprotein particles (22–24 nm), resulting eventually in LDL particles ranging in size from 19 to 23 nm, which are relatively depleted in triglycerides but carry about 70% of circulating cholesterol. The other major lipoprotein class, the HDLs, provides proteins that facilitate and regulate the processing of the TRL particles and also accept triglycerides into their core in exchange with remnants for cholesteryl esters through the action of cholesteryl ester transfer protein.

Most of the common triglyceride assays employed today measure triglycerides in terms of their glycerol content, employing lipase enzymes to hydrolyze the triglycerides and any di- and monoglycerides; the latter 2 forms typically present in serum at levels representing 5% to 10% of total glycerides. The tri- and diglycerides are readily hydrolyzed by general lipases and the monoglycerides less so; newer assays may add
a specific monoglyceride lipase to achieve complete hydrolysis. The common colorimetric assays include glycerol kinase and glycerol triphosphate oxidase, producing hydrogen peroxide, which in the presence of peroxidase results in a measurable color. Any free glycerol is included by most assays, typically amounting to 5–20 mg/DL triglyceride equivalent; but substantially higher in some instances, for example in serum from diabetic patients and from patients stressed or on glycerol-containing infusions. Assays are available that remove or correct for any free glycerol, e.g., by depleting glycerol in a preliminary reaction by adding glycerol kinase and catalase, but are less common. Thus, a measurement of serum triglycerides reflects all glycerides in all the lipoprotein particles in the circulation and may include any free glycerol as well. There may be differential effects of nonfasting conditions on these analyte-related interactions with the triglyceride assays; e.g., effects on proportion of free glycerol, which has apparently not been studied. In this regard, the recent Women’s Health Study paper (2) indicates use of a triglyceride assay with correction for free glycerol, inferring that the observed improved CVD risk prediction is attributable to glycerides and not an artifact of an indirect association of diabetic patients at higher risk and with increased free glycerol.

Both of the recently reported nonfasting studies (1, 2) observed peak triglyceride values at approximately 4 hours postprandial. The Copenhagen study (1) concluded the increased peak triglycerides were a result of remnant particles not yet cleared, using a relatively crude approximation, total serum cholesterol minus LDL-C and HDL-C. Impaired clearance of remnant particles is the likely cause of the increased CVD risk, a conclusion consistent with current perceptions that remnants are more atherogenic than the larger nascent triglyceride-rich particles. In support of the increased atherogenicity of remnants, another recent retrospective analysis of Copenhagen study samples matched for HDL concentrations observed that the primary distinguishing characteristic of patients with CVD was increased remnants, determined by an electrophoretic method (14). Direct determination of TRL remnants has been challenging owing to their low concentrations and their overlapping size and other properties (15); therefore a postprandial triglyceride measurement might prove to be a more convenient indicator of their impaired clearance. Triglycerides are also associated inversely with HDL concentrations and positively with atherogenic small, dense LDL particles, which might be affected by nonfasting collections, suggesting further study (16). Of interest, clearance of TRLs is a function of the apoE genotype; patients with the E4 and E2 alleles, demonstrate slower clearance and increased concentrations of remnants as well as increased CVD risk (17), so there might be a differential association with apoE genotypes.

In summary, replacing the current 8–12 h fasting convention with a nonfasting blood collection protocol might improve CVD risk prediction. The most convenient way to implement this procedure would be to collect specimens at a specified postprandial time interval range, e.g., 2–4 h after a representative meal.

Collection after a defined fat load, as described in the companion clinical commentary (18) could be more definitive but certainly would be more tedious. The difference in measured triglycerides between the current fasting protocol and a defined nonfasting collection after a specified diet and time interval would be logistically practical and could be informative.

These observations suggest questions for further study: 1. Compare predictive associations of conventional fasting to nonfasting collections at various time intervals after defined fat loads and after representative meals. 2. Determine the effect of nonfasting collections on other laboratory measurements, especially important for lipoprotein parameters including the LDL-C calculation, HDL-C, and the lipoprotein subclasses. 3. Characterize postprandial effects on contributions of mono and diglycerides and free glycerol to triglyceride measurement and the CVD risk associations.

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