

# A Standardized Triglyceride and Carbohydrate Challenge

## The oral triglyceride tolerance test

NINA MOHANLAL, MBBS  
RURY R. HOLMAN, FRCP

**OBJECTIVE** — A standardized method of assessing postprandial triglyceride changes is not available. We evaluated an oral triglyceride tolerance test (OTTT) designed for routine clinical and research use.

**RESEARCH DESIGN AND METHODS** — A 200-ml strawberry-flavored test drink (50 g fat, 50 g carbohydrate) was administered twice to 30 diabetic and 20 nondiabetic subjects. Venous plasma triglyceride and glucose levels were measured when fasting and every 2 h for 8 h after the drink. Fingerprick plasma triglyceride levels were measured when fasting and at 6 and 8 h after the drink.

**RESULTS** — The drink was consumed within 3 min and well tolerated by all subjects. The median triglyceride rise at 6 h was similar in diabetic and nondiabetic subjects (0.23 vs. 0.42 mmol/l, NS) and correlated with glucose increase at 2 h ( $r = 0.429$ ,  $P = 0.018$  and  $r = 0.509$ ,  $P = 0.026$ ; respectively). Diabetic subjects had higher 6-h geometric mean (1 SD range) triglyceride levels (1.82 [1.87 to 3.23] vs. 1.11 [0.66 to 1.11 mmol/l],  $P < 0.003$ ) but a similar coefficient of variation (17.5 vs. 17.0%, NS) and a similar median (interquartile range) time to achieve maximal concentration ( $T_{max}$ ) (6.0 [4.0 to 6.0] vs. 5.0 [4.0 to 6.0] h, NS). Capillary triglyceride values were equivalent to simultaneous venous samples but consistently 10% greater.

**CONCLUSIONS** — The OTTT permits simple evaluation of postchallenge triglyceride levels, is acceptable to subjects, and can be performed with capillary sampling. It could be used to monitor triglyceride-lowering therapies and to provide additional information concerning cardiovascular disease risk, particularly in diabetic subjects.

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People with type 2 diabetes have a two- to fourfold increased risk of coronary heart disease (CHD) compared with the general population (1) that is only partly explained by the addition of hyperglycemia to traditional CHD risk factors (2). In diabetes, a mixed fasting dyslipidemia is common, characterized by an increased LDL-to-HDL cholesterol

ratio, an increase in small dense LDL particles, and elevated triglyceride levels (3). Although fasting triglyceride levels may be an independent risk factor for CHD (4), their exact contribution remains uncertain because of displacement in most analyses by HDL cholesterol (2). This has led some authors to suggest that part of the excess CHD risk seen in type 2 diabe-

tes may related more to postprandial rather than fasting triglyceride levels (5).

In the absence of a standardized triglyceride challenge, studies to date have used a variety of different test meals consisting of “everyday” foods such as dairy products, bacon, margarine, and eggs or liquid formulations. These have contained various amounts of fat (33–66%; up to 110 g), carbohydrate (14–43%; up to 75 g), and protein (16–20%; up to 46 g) and, occasionally, have had vitamin A added (6–11). To facilitate routine triglyceride tolerance assessment, we have formulated an easily produced and palatable 200-ml test drink. This oral triglyceride tolerance test (OTTT) has been evaluated in subjects with and without type 2 diabetes. In addition, the possibility of using capillary fingerprick samples as a simple alternative to venepuncture for use in the community or in large-scale studies has been investigated.

### RESEARCH DESIGN AND METHODS

The study was approved by the Central Oxford Research Ethics Committee (COREC). A total of 23 nondiabetic university staff and members of the general public came forward after seeing advertisements, and 180 diabetic patients were approached through general practice and specialist diabetes clinics. Subjects were excluded if they had a plasma triglyceride level  $>5$  mmol/l or a major lipid abnormality, poorly controlled diabetes ( $HbA_{1c} >10\%$ ), gastrointestinal problems (e.g., delayed gastric emptying), chronic inflammatory bowel disease, a serious medical condition, a history of alcohol abuse, or were pregnant or lactating. A total of 20 nondiabetic subjects and 30 subjects with type 2 diabetes were recruited and gave written informed consent. They were Caucasian, aged 35–65 years, not taking lipid lowering therapy, and had no history of established cardiovascular disease. Those with type 2 diabetes had been diagnosed according to World Health Organization criteria (12) for at least 1 year. Of these subjects, 10

From the Churchill Hospital, Diabetes Trials Unit, Oxford Centre for Diabetes, Endocrinology & Metabolism (OCeM), Headington, U.K.

Address correspondence and reprint requests to Prof. Rury Holman, Diabetes Trials Unit, Oxford Centre for Diabetes, Endocrinology & Metabolism, Churchill Hospital, Headington, Oxford OX3 7LJ. E-mail: rury.holman@dtu.ox.ac.uk.

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**Abbreviations:** apo, apolipoprotein; AUC, area under the curve; CHD, coronary heart disease; IAUC, incremental AUC; IQR, interquartile range; OTTT, oral triglyceride tolerance test.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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**Table 1—Test drink nutrient composition**

Component	Per 200 ml
Fat	50 g
Saturated	19.0%
Monosaturated	59.5%
Polyunsaturated	21.5%
Fatty acid profile	g/50 g fatty acid
C16:0	5.0
C18:0	1.5
C18:1	29.0
C18:2	10.0
C18:3	<0.5
C20:0	0.5
C20:1	0.5
C22:0	1.5
C24:0	0.5
Carbohydrate	50 g
Glucose	1.6 g
Maltose	2.7 g
Polysaccharides	45.7 g
Protein	0 g
Fiber	0 g
Water	92.5 g
Sodium	<10 mg
Energy	2,715 kJ 656 kcal

The 200-ml test drink was formulated by adding 50 g of carbohydrate (spray-dried maltodextrin gluten-free powder Polycal, Nutricia, Cuijk, Holland) to 100 ml of a commercially produced strawberry-flavored fat emulsion (Calogen, SHS, Cheshire, U.K.) containing 50 g long-chain triglyceride.

were treated by diet alone, 10 with a sulfonylurea, and 10 with metformin.

### Study design

At entry, subjects were weighed, blood pressure was recorded as the mean of three automatic sphygmometer readings (Takeda UA-751; A&D, Burgess Hill, U.K.), waist circumference was measured (13), and subcutaneous fat was estimated by measuring tricep skinfold thickness (mean of three readings) using Harpenden calipers (John Bull; British Indicator, U.K.). All subjects were asked to complete a 3-day food diary. The 200-ml test drink was formulated by adding 50 g of carbohydrate (spray-dried maltodextrin gluten-free powder Polycal; Nutricia, Cuijk, Holland) to 100 ml of a commercially produced strawberry-flavored fat emulsion containing 50 g long-chain triglyceride (Calogen; SHS, Cheshire, U.K.). Nutritive constituents of the test drink, which had an energy content of 2715 kJ (656 kcal), are listed in Table 1.

To evaluate reproducibility all subjects underwent the OTTT on two occasions, at least 1 week apart. For 24 h before each test, subjects were asked not to consume alcohol or undertake any undue exercise. Routine medication was continued as prescribed except for subjects taking a midday dose of an oral hypoglycemic agent, which was omitted on test days. Subjects were admitted on the morning of the test after a 10-h overnight fast (water permitted). A 20-G venous cannula was inserted into a forearm vein, and blood samples were collected for measurement of fasting plasma lipid fractions and glucose levels, with an additional sample for apolipoprotein (apo) E genotyping on the first occasion. The test drink was served chilled for ingestion within 10 min. During the test, subjects were allowed water to drink and could move around freely but were not allowed to eat food or smoke. Venous blood samples were drawn at 2, 4, 6, and 8 h for determination of lipid and glucose levels. Plasma glucose levels were monitored at each time point using a glucose meter (One Touch II blood glucose monitoring system; Lifescan, Johnson and Johnson, High Wycombe, U.K.). Capillary blood samples for triglyceride measurement were collected at 0, 6, and 8 h using an Autolancet (Medisense; Abbot Laboratories, Maidenhead, U.K.) and placed in microvette capillary tubes.

Blood samples were centrifuged at 3,000 rpm for 10 min at 4°C, and the plasma was separated. Capillary plasma volume was determined by weighing the sample on a three-place Sartorius digital balance. Lipids were measured in EDTA plasma using a Beckman Synchron CX4 Delta analyzer (Beckman Instruments, High Wycombe, U.K.), with no correction for free glycerol (~0.11 mmol/l) for triglyceride measurements. HbA<sub>1c</sub> was measured in EDTA whole blood by high-performance liquid chromatography (Variant hemoglobin testing system; Bio-rad Laboratories, Hemel Hempstead, U.K.). Plasma glucose was measured in plasma (fluoride/oxalate) by a hexokinase method for a Beckman Synchron CX4 Delta analyzer. The intra-assay coefficient of variation (CV) for the mid-level control was 2% at 1.4 mmol/l for triglyceride, <2% for HbA<sub>1c</sub>, and 2% for glucose at 6.7 mmol/l.

DNA was extracted from EDTA whole blood using the Nucleon II DNA extrac-

tion kit (Scotlab, Coatbridge, Strathclyde, U.K.). DNA yield and purity was quantified spectrophotometrically at 260 and 280 nm, and deionized water was used for suspension of DNA. Genotyping for apo E was carried out by PCR amplification of a 246–base pair fragment, followed by mapping of restriction fragments obtained by digestion with *CfoI*, on 4% metaphore agarose gels (14).

### Statistical analysis

Data are presented as means ± SD or median and interquartile range (IQR) as appropriate for their distribution, with geometric means (1 SD range) for triglyceride values. Food diaries were coded by a specialist dietitian and nutrient content determined using Dietplan 5 (15).

Subject characteristics, biochemistry data, and food diary results were compared between the nondiabetic and diabetic groups using the two-sample *t* test or  $\chi^2$  test. Diabetic subgroups were compared using ANOVA or Fisher's exact test. Reproducibility of triglyceride measurements was assessed at each time point by plotting graphs of the differences between the two tests against the mean for each subject, and by calculating the median and IQR within the coefficient of variation (CV) in each group. Biochemistry profiles presented in this paper are data from the first test. Triglyceride maximum concentration ( $C_{max}$ ) and time to achieve maximal concentration ( $T_{max}$ ) values were determined for each test, and the area under the curve (AUC) was calculated by trapezoidal integration. Simultaneous venous and capillary triglyceride values were compared using mean difference plots.

**RESULTS**— A total of 50 subjects completed both tests. One subject who withdrew because of a migraine was replaced. Diabetic and nondiabetic groups had similar baseline characteristics (Table 2), except that the diabetic group had a higher mean BMI, waist circumference, systolic blood pressure, fasting triglyceride (geometric mean [1 SD range]) levels (1.55 [0.91–2.62] vs. 0.82 [0.55–1.22] mmol/l,  $P < 0.0001$ ), and lower HDL cholesterol (mean ± 1 SD) levels (1.21 ± 0.31 vs. 1.45 ± 0.31 mmol/l,  $P = 0.01$ ).

The test drink was consumed in a median (IQR) of 2.0 (1.0–2.8) min, with all subjects reporting it to be agreeable in flavor, of palatable texture, and in an

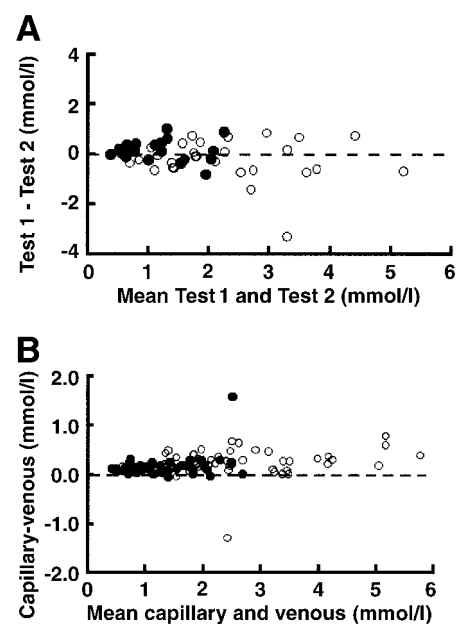
Table 2—Subject characteristics and triglyceride profiles

	No diabetes	Diabetes	P
n	20	30	
Sex (M/F)*	10/10	15/15	NS
Age (years)	52.1 ± 9.0	55.5 ± 7.4	0.15
Duration of diabetes (years)†	N/A	4.5 ± 2.0–6.0	N/A
BMI (kg/m <sup>2</sup> )	27.9 ± 5.6	32.7 ± 6.2	0.0078
Waist (cm)	92.9 ± 17.0	103.1 ± 14.5	0.026
Triceps skinfold thickness (mm)	15.9 ± 6.3	18.8 ± 10.4	0.22
Blood pressure (mmHg)			
Systolic	129 ± 13.3	143 ± 20.8	0.0049
Diastolic	81 ± 8.9	84 ± 8.4	0.23
Smoking (%)			0.77
Nonsmoker (n = 25)	45	54	
Ex-smoker (n = 20)	50	33	
Smoker (n = 5)	5	13	
Hormone use (%) (n = 6)	10	33	0.42
Menopausal (%) (n = 12)	40	53	0.84
Nutrient Intake (%)			
Fat	36	36	0.64
Carbohydrate	43	44	0.56
Alcohol	5.4	3.2	0.21
Apo E genotype*			0.63
3/3	6	22	
4/3	1	5	
2/3	2	2	
2/4	1	1	
HbA <sub>1c</sub> (%)	5.7 ± 0.4	7.6 ± 1.2	N/A
Fasting plasma glucose (mmol/l)	5.4 ± 0.59	9.0 ± 2.5	N/A
Fasting total cholesterol (mmol/l)	5.0 ± 1.00	5.0 ± 0.98	0.87
Fasting LDL cholesterol (mmol/l)	3.11 ± 0.880	3.25 ± 0.824	0.004
Fasting HDL cholesterol (mmol/l)	1.51 ± 0.399	1.20 ± 0.309	0.57
Fasting VLDL cholesterol (mmol/l)	0.38 ± 0.221	0.50 ± 0.221	0.05
Triglyceride (mmol/l)‡			
0 h	0.77 (0.457–1.291)	1.35 (0.858–2.138)	0.0002
2 h	0.90 (0.557–1.462)	1.63 (1.026–2.598)	—
4 h	1.06 (0.589–1.897)	1.70 (1.117–2.590)	—
6 h	1.11 (0.655–1.106)	1.82 (1.027–3.225)	0.003
8 h	0.78 (0.468–1.290)	1.40 (0.787–2.493)	—
AUC (mmol/l h)	1.2 (0.750–1.873)	1.8 (0.718–2.528)	0.0003
I <sub>AUC</sub> (mmol/l h)†	1.3 (0.963–2.230)	2.1 (1.323–3.828)	0.31
C <sub>max</sub>	1.34 (0.823–2.180)	2.10 (1.343–3.293)	0.0015
T <sub>max</sub> (h)†	5 (4–6)	6 (4–6)	0.29
CV triglyceride (%)†			
0 h	14.5 (9.5–23.0)	12.0 (5.0–24.0)	NS
2 h	14.5 (6.0–21.5)	12.0 (8.0–27)	
4 h	15.5 (8.5–25.5)	15.5 (8.0–31.0)	
6 h	17.5 (7.0–29.0)	17.0 (10.0–28.0)	NS
8 h	15.5 (10.0–26.0)	10.5 (6.0–25.0)	
C <sub>max</sub>	16.0 (9.5–26.5)	13.0 (6.0–20.0)	NS

\*Number of subjects, †median (IQR), ‡geometric mean (SD range).

amount that was not excessive. It was well tolerated, with no episodes of nausea, vomiting, or abdominal discomfort after consumption. A single nondiabetic subject suffered an episode of loose stools 4 h

after the end of the first test. One diabetic subject on sulfonylurea therapy had mild hypoglycemic symptoms at the 4-h time point, but returned to complete two further tests.



**Figure 1**—A: Mean difference plot comparing triglyceride concentrations at the 6-h time point for patients with no diabetes (○) and diabetes (●) from test 1 and test 2. The average value for the two tests is plotted on the x-axis, and the difference between the two values is plotted on the y-axis. B: Mean difference plot comparing triglyceride concentrations at the 6-h time point for patients with no diabetes (○) and diabetes (●) for capillary and venous samples. The average value for the capillary and venous samples is plotted on the x-axis, and the difference between the two values is plotted on the y-axis.

### Reproducibility of triglyceride levels

The median (IQR) triglyceride CV was similar in the nondiabetic and diabetic groups at baseline (14.5 [9.5–23.0%] vs. 12.0 [5.0–24.0%], respectively) and at 6 h (17.5 [7.0–29.0%] vs. 17.0 [10.0–28.0%]) (Table 2, Fig. 1A).

### Comparison of diabetic with nondiabetic subjects

The geometric mean (1 SD range) triglyceride level differed between diabetic and nondiabetic groups at the 6-h time point (1.82 [1.03–3.23] mmol/l and 1.11 [0.66–1.11] mmol/l, respectively,  $P = 0.003$ ) (Table 2). AUC triglyceride was significantly greater in the diabetic group compared with the nondiabetic group ( $P = 0.0003$ ), with median  $T_{max}$  at 6 and 5 h, respectively ( $P = 0.29$ ). In the nondiabetic group, 60% of subjects attained  $T_{max}$  triglyceride at  $\leq 4$  h postchallenge, whereas 60% of diabetic subjects had maximal triglyceride levels at 6 or 8 h

postchallenge. There were no significant differences in postchallenge triglyceride levels between diet-, metformin-, and sulfonylurea-treated diabetic subgroups. Mean total LDL cholesterol, HDL cholesterol, and VLDL cholesterol levels did not change significantly in any group over the time points.

**Comparison of simultaneous venous and capillary triglyceride values**

Capillary triglyceride values were similar to venous triglyceride values at all time points evaluated, but they were consistently higher by 10% (Fig. 1B).

**Glucose profiles**

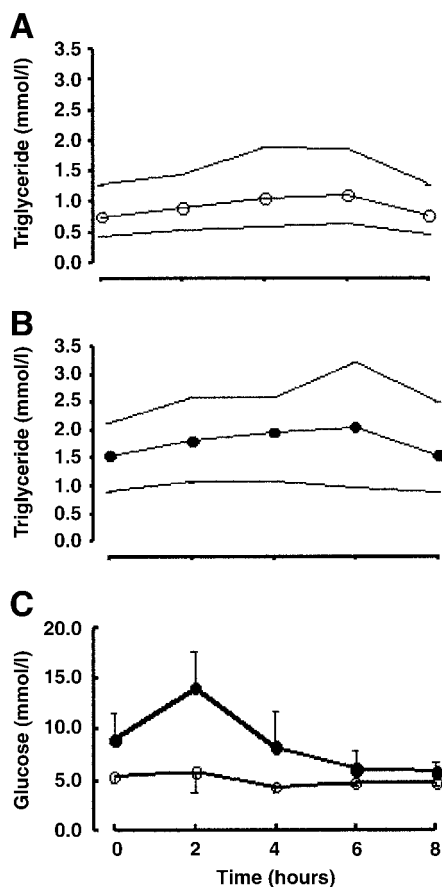
The CV (mean ± SD) for glucose at 2-h postchallenge was 8.3 ± 9.3% and 15.3 ± 12.7% in diabetic and nondiabetic groups, respectively. Glucose levels (mean ± SD) peaked at 14.1 ± 3.5 mmol/l at the 2-h time point and were lowest (5.8 ± 1.0 mmol/l) at the 8 h time point for diabetic subjects (Fig. 2C).

**Correlations**

Postchallenge triglyceride levels correlated with fasting triglyceride values (6 h:  $r = 0.909, P < 0.0001$  and  $r = 0.717, P = 0.0004$  for diabetic and nondiabetic subjects, respectively). In diabetic subjects the Δ 6-h triglyceride level correlated with the Δ 2-h glucose level ( $r = 0.429, P = 0.018$ ). In nondiabetic subjects, the Δ 6-h triglyceride concentration correlated with the Δ 2-h postchallenge glucose levels ( $r = 0.509, P = 0.026$ ) and incremental AUC (IAUC) glucose over 8 h ( $r = 0.471, P = 0.042$ ). The  $T_{max}$  triglyceride correlated with IAUC glucose ( $r = 0.395, P = 0.031$ ; respectively) in diabetic subjects. Neither fasting nor postchallenge triglyceride levels correlated with baseline characteristics.

**CONCLUSIONS**— The OTTT studied here has been shown to be palatable and acceptable to subjects, and it provides reproducible evaluation of postchallenge triglyceride and glucose profiles. The results obtained confirm that in type 2 diabetes, there is relative fasting hypertriglyceridemia, with prolonged postchallenge elevation of triglyceride levels most evident at 6 h.

Because postmeal metabolic changes may last up to 10 h and most individuals have at least three meals a day, a major portion of a day is spent in a postprandial



**Figure 2**—Geometric mean (1 SD range) triglyceride levels for nondiabetic (A) and diabetic (B) subjects and glucose profiles (C) over an 8-h time period in patients with no diabetes (○) and diabetes (●).

state. The development of a standardized triglyceride tolerance test provides a reliable method for assessing fasting and postchallenge lipid levels in clinical trials and in routine practice. A relationship between postprandial lipemia and CHD was suggested as early as the 1950s (16), yet there remains a lack of studies providing evidence for reproducible postchallenge triglyceride profiles. A similar CV was seen in diabetic and nondiabetic subjects in this study, with median values of ~14 and ~17% for the fasting and 6-h values, respectively. Other studies (17) have reported fasting triglyceride CVs of 22% in nondiabetic subjects. Because the intraindividual variation of triglyceride levels can be influenced by smoking, alcohol, and exercise (18,19), these were prohibited during and the day before the OTTT. Also, we found 2-h postchallenge glucose CVs of ~15 and ~8% in diabetic and nondiabetic subjects, respectively. In

comparison, the well-established oral glucose tolerance test has a reported 2-h CV of 15–46% (20). Our findings may relate to our test challenge combination of fat and carbohydrate affecting the rate of gastric emptying and glucose absorption (21).

Previous lipid test meals often required specialist dietitian preparation, were not necessarily suitable to individuals with dietary restrictions, and could not easily be reproduced in different countries. It is important also to have a sufficient quantity of fat while maintaining palatability. Although the OTTT fat content was not adjusted for body weight, to standardize and simplify preparation, it was sufficient to elicit adequate postchallenge responses in the subjects tested. Previous studies have demonstrated an increase in triglyceride-rich lipoproteins postprandially upon the ingestion of various amounts of fat, even as low as 0.2 g/kg (22). The addition of carbohydrate, making the test challenge more akin with everyday meals, can accentuate the degree of postprandial lipemia (18), yet the type and amount is important when designing a test meal for diabetic subjects because of hyperglycemia and the risk of hypoglycemia with periods of fasting. Only one sulfonylurea-treated diabetic subject had hypoglycemic symptoms, but the same subject returned to complete two uneventful tests. A small-volume liquid formulation was chosen to ensure similar gastric delivery and digestive handling in all groups because delayed gastric emptying may be present in up to 30–50% of people with type 1 or type 2 diabetes (23). Previous studies have added vitamin A to test meals to assess postprandial chylomicronemia; however, this may be an inaccurate marker because retinyl palmitate is transferred from chylomicrons to other lipoproteins, and larger chylomicrons carry more than smaller particles (24).

Patsch et al. (25) showed that single postprandial triglyceride levels at 6-h postchallenge may predict the presence of CHD, and this may be attributable to the transfer of cholesterol ester from LDL and HDL cholesterol and the uptake of triglyceride, making these particles smaller, denser, and more atherogenic or (through enhanced oxidant stress) causing endothelial function (24). Thus, AUC and IAUC may be particularly helpful in quantifying an individual's triglyceride



"exposure." In our study, diabetic subjects appeared to have greater triglyceride levels at fasting and during the postprandial period compared with nondiabetic subjects, consistent with previous studies (6–11). Although 60% of nondiabetic subjects attained  $T_{\max}$  triglyceride at  $\leq 4$  h postchallenge, 60% of diabetic subjects had maximal triglyceride levels at 6 or 8 h postchallenge. Further illustration of the differences between the diabetic and nondiabetic groups may have been evident if the fat content was increased or adjusted for body surface area or in subjects with fasting hypertriglyceridemia. We found significant relationships between postchallenge but not fasting triglyceride levels and glucose responses, despite postchallenge triglyceride levels being highly correlated with fasting values. This may indicate a possible mechanistic interaction between hyperglycemia and postprandial triglyceridemia.

Studies of postprandial lipemia have limitations regarding the interpretation of results because triglyceride levels may be affected by factors such as age, BMI, and ethnic background (26–28). Menopause has been shown to aggravate postprandial lipemia (29), with estrogen improving chylomicron remnant clearance (18). Apo E2 homozygosity is associated with hyperlipidemia and premature coronary and peripheral atherosclerosis, and individuals with apo E genotypes E2/3 and E3/4 show slower clearance than those with E3/3 (11). Other pitfalls in the understanding of lipid measurements are related to diet. In our study the nutrient intake, assessed in food diaries, was comparable to that of a typical northern European diet (30). However, the demonstration of chylomicrons appearing in the bloodstream after a meal resembling those produced by the previous meal (18) has led to some investigators giving a standard meal before the actual test challenge. All of these factors must be considered when conducting clinical tests of postprandial lipemia.

To facilitate postprandial testing in a community or large-scale setting, fingerprick sampling as an alternative to venepuncture would be advantageous. The consistent 10% higher triglyceride values seen with capillary samples may reflect greater extraction of triglyceride as blood circulates from the capillary to venous vasculature. Relatively few studies have compared capillary and venous triglycer-

ide levels in diabetic patients; however, a study by Stewart et al. (31) demonstrated that self-monitored capillary triglyceride measurements using the Reflotron analyzer were similar to laboratory-measured venous samples.

In summary, this newly developed OTTT provides a standardized, acceptable, and reproducible method for evaluating postchallenge triglyceride levels. For convenience, triglyceride measurements need only be made on fasting and 6-h capillary blood samples because venous and capillary values are similar. Prospectively, the OTTT should allow the relationship of postchallenge triglyceride levels and CHD risk to be determined.

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