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Reproducibility of postprandial lipemia tests and validity of an abbreviated 4-hour test

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Abstract

Postprandial lipemia test (PPLT) results are predictive of cardiovascular disease risk. However, their reproducibility must be established before they can be clinically useful. Therefore, we investigated PPLT reproducibility by testing 9 men and women (body mass index, 20-41 kg/m^2; age, 21-40 years) on 4 separate occasions (n = 36 PPLTs total) separated by 1 week. Furthermore, because PPLTs are time consuming, we assessed the validity of an abbreviated PPLT. During the PPLT, venous blood was obtained before and every hour for 8 hours after a high-fat meal, which consisted of ice cream and heavy cream (~800 kcal, 71% fat calories). Total and triglyceride-rich lipoprotein (TRL) triglyceride concentrations were measured in plasma. Total area under the curve (AUC) for total triglycerides was highly reproducible (within-subject coefficient of variation, 8%; intraclass correlation coefficient, 0.82); however, reproducibility was low for total triglyceride incremental AUC and both total and incremental TRL triglyceride AUCs (within-subject coefficients of variation, 20%-31%; intraclass correlation coefficients, 0.28-0.54). Four-hour lipemic responses were highly predictive of 8-hour responses (R^2 = 0.89-0.96, $P \leq .0001$). In conclusion, PPLTs are highly reproducible when lipemic responses are determined as the total AUC for total triglycerides. However, large variability in incremental AUC and TRL triglyceride responses may preclude their clinical utility. Furthermore, abbreviated 4-hour PPLTs are a valid surrogate for longer tests and may make PPLTs more feasible in a clinical setting.

1. Introduction

Exaggerated postprandial lipemia after a high-fat meal is associated with increased intima media thickness in the carotid arteries [1], impaired endothelial function [2], and insulin resistance [3,4] and is greater in individuals with cardiovascular disease (CVD) than it is in individuals without CVD [5,6]. Postprandial lipemia is reduced by interventions that decrease insulin resistance and CVD risk including exercise [7], diets low in saturated fat [8], statin therapy [9], and metformin therapy [10]. Although the mechanistic relationship between postprandial lipemia and CVD is not clear, chylomicrons and their remnants, which are mainly present in circulation postprandially, can penetrate the endothelium and be retained in the subendothelial space [11] where they may trigger the inflammatory reactions involved in atherogenesis. Taken together, these findings suggest that exaggerated postprandial lipemia may promote the development of CVD and insulin resistance.

As additional evidence accumulates to suggest that excessive postprandial lipemia may be a cardiometabolic risk factor, postprandial lipemia tests (PPLTs) may become clinically useful for assessing disease risk and monitoring responses to risk reduction interventions. However, to be of clinical utility, these tests must be reproducible. Therefore, the primary purpose of the present study was to assess the reproducibility of PPLTs in lean and obese individuals. In addition, because PPLTs typically require 8 hours or more to perform, they may not be practical in a clinical setting. Therefore, a secondary purpose of our study was to assess the validity of an abbreviated 4-hour PPLT.

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2. Methods

2.1. Subjects

Five lean and 4 obese subjects participated in the study. A medical history, physical examination, resting electrocardiogram, and standard blood chemistries and lipids were used to identify and exclude volunteers with clinical evidence of cardiovascular, pulmonary, metabolic, or other chronic diseases. A 75-g glucose tolerance test [12] was used to identify and exclude any of the obese subjects who may have had occult diabetes. Subjects were required to be sedentary (≤1 exercise session per week and ≤30 minutes per session according to self-report) and not taking medications known to affect lipid metabolism. Smoking and pregnancy were also exclusionary. The study protocol was approved by the Human Subjects Committee and the General Clinical Research Center (GCRC) Scientific Advisory Committee of Washington University School of Medicine. Written informed consent was obtained from each subject before participation in the study.

2.2. Experimental protocol

Each subject completed a PPLT on 4 occasions (n = 36 PPLTs total), with approximately 1 week between consecutive tests.

2.3. Prestudy dietary intake and physical activity

The subjects were instructed by a registered dietitian to ingest a weight-maintaining diet that included at least 250 g/d of carbohydrate and approximately 12% of total energy intake as protein, 55% as carbohydrate, and 33% as fat for 3 days before each PPLT. Subjects were advised to refrain from exercise and not consume caffeine or alcohol for 24 hours before each study.

2.4. Postprandial lipemia test

Subjects were admitted to the GCRC in the evening before the test. At 7:00 PM, a standardized meal was consumed that provided approximately 55% of total energy as carbohydrates, 30% as fat, and 15% as protein. The energy content of the meal for lean subjects was 12 kcal/kg screening body weight. To account for different energy requirements per unit body mass in obese subjects, the energy content of the meal was 12 kcal/kg adjusted body weight, where adjusted body weight = ideal body weight + 0.25 × (screening body weight − ideal body weight); and ideal body weight was determined from the Metropolitan Life Insurance [13] tables for individuals with a medium frame. After the evening meal, the subjects fasted, except for water, until the PPLT the following day.

At 5:30 AM on the day of the PPLT, an intravenous catheter was inserted into a dorsal hand vein for blood sampling. To obtain arterialized blood, the hand was placed in a heating box set at 55°C for 20 minutes before each blood sample was acquired [14]. The catheter was kept patent by infusing 0.9% NaCl at 25 mL/h. At 6:30 AM, subjects received the high-fat meal and consumed it within 20 minutes. The high-fat meal was prepared by mixing heavy whipping cream (Pevely Dairy, St Louis, MO) and vanilla ice cream (Häagen-Dazs; Ice Cream Partners, San Ramon, CA) in a mass ratio of 1 part heavy cream to 4 parts ice cream. Total energy content was 2.84 kcal/g of meal. Fat, carbohydrate, and protein provided 71%, 23%, and 6% of the energy in the meal, respectively. The meal dose was 162 g of meal per square meter of body surface area (equivalent to 35 g of fat per square meter), where body surface area was calculated according to DuBois and DuBois (body surface area = $0.20247 \times \text{height [in meters]}^{0.725} \times \text{weight [in kilograms]}^{0.425}$). For the duration of the test, the subjects remained recumbent in bed or seated in a chair until the completion of the study at 2:30 PM. Blood samples for the determination of plasma triglyceride (TG) concentrations were obtained immediately before ingestion of the fat meal and every hour for 8 hours after the initiation of meal ingestion. Data from the first 4 hours of the tests were interpreted as the abbreviated PPLT results. Four hours was chosen as the abbreviated PPLT duration because peak lipemia typically occurs 4 hours after oral fat loads that are of similar magnitude to the one used in the present study and because 4-hour tests have been used previously [4,15].

2.5. Blood sampling

Blood samples were collected in prechilled tubes containing EDTA and kept on ice. Plasma was isolated within 30 minutes by using standard procedures. A portion of each sample (~2 mL) was kept in the refrigerator for the isolation of TG-rich lipoprotein (TRL); the remainder of the plasma was stored at −70°C for later quantification of total TGs.

2.6. Isolation of TRL

Immediately after completion of each PPLT, 2 mL plasma was transferred into Optiseal tubes (Beckman, Palo Alto, CA), overlayed with an EDTA/NaCl solution (D = 1.006 kg/mL), and centrifuged for 16 hours at 100 000g and 10°C in a 50.4-Ti rotor (Beckman Instruments) [16]. The TRL fraction was recovered by tube slicing (Beckman Instruments), and the exact amounts recovered (~1.5 mL) were recorded for later calculation of concentrations. The TRL fractions were stored at 4°C, and TG concentrations were measured within 48 hours of separation from plasma.

2.7. Triglyceride analysis

Total and TRL TG concentrations were determined by performing an enzymatic colorimetric assay (Sigma-Aldrich, St Louis, MO) with a Du Series 500 spectrophotometer (Beckman, Fullerton, CA). The intraassay variability for the determination of TG concentration in our laboratory is less

than 6.5%; the variability for the determination of TRL TG concentration in plasma is less than 10%.

2.8. Calculations

The trapezoidal rule [17] was used to calculate total area under the curve (AUC) and AUC above baseline (incremental AUC). For the incremental AUCs, the difference between fasting and postprandial TG values was used in the calculations. Furthermore, for the incremental AUCs, postprandial values that were less than fasting values or values that occurred after a value that was less than fasting were excluded. The AUCs for the 4-hour tests were calculated using the same approaches that were used for the full-length test except that data after the 4-hour time point were excluded.

2.9. Statistical analyses

Between-group comparisons (lean vs obese) were performed by using independent *t* tests. For comparisons of the lipemic responses in the lean and obese groups, the mean of all 4 tests was used for each subject. Reproducibility of postprandial lipemia was assessed by calculating within-subject coefficient of variation (WCV) and intraclass correlation coefficient (ICC). The WCV was calculated by using the logarithmic method [18] and has an advantage over ICC in that it does not depend on the between-subject variance and is therefore considered population independent [19]. The WCV values can be 0% or greater, with lower values representing higher reproducibility. Measures with WCV values of 10% to 20% or less have been described as having sufficient reproducibility for use in monitoring treatment effects [19]; we chose the more strict end of this range (ie, ≤10%) as the criterion for high reproducibility in the present study. The ICC was calculated by using a 1-way random α model for measurement consistency. The ICC has an advantage over WCV in that it does not depend on the mean values [19]. The ICC values range from 0.00 to 1.00, with higher values reflecting better reproducibility. Based on guidelines used by others, ICC values of at least 0.75 were interpreted as high reproducibility [20,21]. To assess the validity of 4-hour tests for predicting results from the full-length tests, linear regression analyses were performed that included data from each subject's first test only. Significance was accepted at $P \leq .05$. All data are reported as means ± SEM unless otherwise noted. Analyses were performed with SAS for Windows XP Pro (version 9.1; SAS, Cary, NC), SPSS for Windows (version 13.0; SPSS, Chicago, IL), and Excel (Microsoft, Redmond, WA).

3. Results

3.1. Subjects

For the group as a whole, age ranged from 21 to 40 years and body mass index (BMI) was from 20 to 41 kg/m^2. Mean age, body weight, and BMI were greater in the obese group than in the lean group (Table 1). Fasting plasma glucose and lipid concentrations were within reference ranges for all subjects. Plasma insulin and low-density lipoprotein cholesterol concentrations were higher and high-density lipoprotein cholesterol was lower in obese than in lean subjects.

Table 1
Subject characteristics

	Lean	Obese	All subjects	Lean vs obese
	n = 5	n = 4	n = 9	*P* value
Male/female	1/4	1/3	2/7	1.00
Age, y	23 ± 1	36 ± 4	29 ± 5	.01
Body weight, kg	58 ± 4	114 ± 8	83 ± 4	.0003
BMI, kg/m^2	21 ± 1	40 ± 1	29 ± 3	<.0001
Fasting glucose, mg/dL	84 ± 1	99 ± 3	91 ± 2	.06
2-h glucose, mg/dL[a]	–	133 ± 4	–	–
Insulin (μU/mL)	2 ± 0	15 ± 3	8 ± 1	.02
Total TGs (mg/dL)	64 ± 15	114 ± 23	87 ± 15	.10
Total cholesterol (mg/dL)	162 ± 11	182 ± 12	171 ± 8	.26
LDL cholesterol (mg/dL)	85 ± 5	119 ± 9	100 ± 5	.01
HDL cholesterol (mg/dL)	64 ± 5	42 ± 6	54 ± 4	.03

Values are means ± SEM except for male/female data, which are counts. All measures were made after an overnight fast except for 2-hour glucose, which was assessed 2 hours after a 75-g oral glucose load. LDL indicates low-density lipoprotein; HDL, high-density lipoprotein.

[a] Two-hour glucose was only assessed in obese subjects (to screen for occult diabetes).

3.2. Average lipemic responses

For both lean and obese groups, total plasma TG concentrations doubled from fasting to 3 or 4 hours after the meal and returned to baseline by 8 hours postprandially (Fig. 1). Although there was a tendency for obese subjects to have higher fasting and postprandial total TG concentrations, neither the fasting values nor the AUCs were significantly different between groups (Table 2). Furthermore, neither the fasting values nor the AUCs for TRL TGs were different between lean and obese participants.

3.3. Reproducibility based on WCV

The WCV for fasting total TGs was low (Table 3), as would be expected for this commonly used clinical measure, with values for lean, obese, and all subjects combined meeting the ≤10% criterion for high reproducibility [19]. Total AUC for total TG also had low WCV values, which were comparable with those for fasting TG. In contrast, WCVs for total TG incremental AUC was 2- to 3-fold greater, indicating relatively poor reproducibility. For total TG from the abbreviated 4-hour tests, the total AUC was

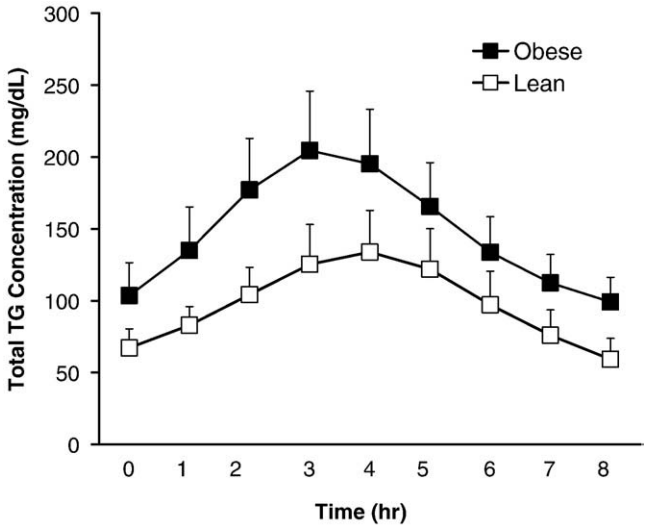

Fig. 1. Time courses for total plasma TG concentrations during the PPLT in lean and obese subjects. Values are means + SEM and were calculated by using the mean of 4 tests for each subject.

highly reproducible and comparable with fasting TG, whereas the incremental AUC had 2-fold greater WCV values, indicating substantially lower reproducibility.

None of the TRL TG outcomes met the ≤10% WCV criterion for high reproducibility [19]. The reproducibility for fasting TRL TGs was poor in comparison with fasting total TGs as reflected by 2- to 4-fold greater WCV values (Table 3). Similarly, WCVs for AUCs calculated from TRL TGs were greater than those for the corresponding total TG AUCs; this was true for the 4-hour tests as well. Total AUCs for TRL TGs had lower WCVs than the corresponding incremental AUCs, indicating greater reproducibility for total AUCs.

Table 2
Postprandial lipemia test results

	Lean	Obese	All subjects	Lean vs obese
				P value
Total TGs				
Fasting, mg/dL	65 ± 12	104 ± 23	82 ± 13	.19
Total AUC × 10^3, mg/(dL min)	46 ± 9	73 ± 14	58 ± 9	.16
Incremental × 10^3, mg/(dL min)	16 ± 5	24 ± 4	19 ± 3	.17
4-h total AUC × 10^3, mg/(dL min)	24 ± 4	40 ± 8	31 ± 5	.13
4-h incremental AUC × 10^3, mg/(dL min)	8 ± 2	15 ± 3	11 ± 2	.08
TRL TGs				
Fasting, mg/dL	32 ± 8	50 ± 19	40 ± 10	.59
Total AUC × 10^3, mg/(dL min)	24 ± 7	39 ± 11	31 ± 7	.32
Incremental AUC × 10^3, mg/(dL min)	11 ± 4	16 ± 3	13 ± 3	.22
4-h total AUC × 10^3, mg/(dL min)	14 ± 4	23 ± 7	18 ± 4	.32
4-h incremental AUC × 10^3, mg/(dL min)	6 ± 2	10 ± 2	8 ± 2	.16

Data are means ± SEM. The means of all 4 tests for each subject were used for statistical analyses. AUC, area under the curve.

Table 3
Within-subject coefficient of variation for the lipemic response to a high-fat meal conducted on 4 different days

	Lean	Obese	All subjects
Total TGs			
Fasting	6.3 (4.3, 8.4)	6.2 (4.0, 8.4)	6.3 (4.8, 7.8)
Total AUC	9.4 (6.4, 12.6)	6.0 (3.9, 8.2)	8.1 (6.1, 10.0)
Incremental AUC	23.5 (15.6, 31.9)	16.9 (10.6, 23.4)	20.7 (15.6, 26.1)
4-h total AUC	7.7 (5.2, 10.2)	5.0 (3.2, 6.8)	6.6 (5.0, 8.2)
4-h incremental AUC	18.9 (12.6, 25.5)	11.6 (7.4, 16.0)	16.0 (12.0, 20.0)
TRL TGs			
Fasting	11.6 (7.8, 15.5)	32.4 (19.9, 46.1)	22.6 (17.0, 28.6)
Total AUC	14.0 (9.4, 18.8)	25.4 (15.8, 35.8)	19.7 (14.8, 24.8)
Incremental AUC	29.0 (19.1, 39.7)	32.5 (20.0, 46.3)	30.6 (22.7, 39.0)
4-h total AUC	11.0 (7.5, 14.8)	23.3 (14.5, 32.7)	17.3 (13.1, 21.8)
4-h incremental AUC	24.9 (16.5, 33.9)	28.1 (17.4, 39.7)	26.4 (19.7, 33.4)

Data are WCVs reported as percentages, with 95% confidence intervals in parentheses. Lower WCV values indicate better reproducibility, with values not exceeding 10% being reflective of high reproducibility [19].

3.4. Reproducibility based on ICCs

As expected for a common clinical measure, ICCs for fasting total TGs were well above the 0.75 cut point for high reproducibility (Table 4) [20,21]. Likewise, total AUCs for total TGs from the full-length and abbreviated tests had high ICC values with the exception of the value for lean subjects from the full-length test. The ICCs for the incremental AUCs for total TGs were lower than those for the total AUC. The 4-hour incremental total TG AUCs had better reproducibility than the full-length test results.

Table 4
Intraclass correlation coefficients for the lipemic response to a high-fat meal conducted on 4 different days

	Lean	Obese	All subjects
Total TGs			
Fasting	0.89 (0.66, 0.99)	0.88 (0.60, 0.99)	0.90 (0.76, 0.97)
Total AUC	0.66 (0.25, 0.95)	0.89 (0.61, 0.99)	0.82 (0.60, 0.95)
Incremental AUC	0.32 (-0.06, 0.86)	0.38 (-0.06, 0.92)	0.36 (0.05, 0.74)
4-h total AUC	0.80 (0.46, 0.97)	0.93 (0.73, 1.0)	0.91 (0.78, 0.98)
4-h incremental AUC	0.52 (0.10, 0.92)	0.83 (0.46, 0.99)	0.71 (0.42, 0.91)
TRL TGs			
Fasting	0.82 (0.50, 0.98)	0.69 (0.23, 0.97)	0.70 (0.42, 0.91)
Total AUC	0.60 (0.17, 0.94)	0.49 (0.02, 0.94)	0.54 (0.22, 0.84)
Incremental AUC	0.42 (0.01, 0.89)	0.63 (-0.22, 0.81)	0.28 (-0.02, 0.62)
4-h total AUC	0.76 (0.40, 0.97)	0.59 (0.11, 0.96)	0.65 (0.34, 0.89)
4-h incremental AUC	0.60 (0.17, 0.94)	0.34 (-0.08, 0.92)	0.50 (0.17, 0.82)

Data are ICCs, with 95% confidence interval in parentheses. The ICCs with confidence intervals that do not include zero are significantly different from zero. Higher ICC values reflect greater reproducibility; outcomes with values of at least 0.75 were considered highly reproducible [20,21].

Table 5
Regression equations for predicting TG responses from 8-hour PPLTs from 4-hour tests and fasting TGs

Dependent variable	Independent variable	Intercept ± SE	Slope ± SE	R^2	P value for model
Total TGs					
8-h total AUC	4-h total AUC	−38.7 ± 87.9	2.1 ± 0.2	0.92	<.0001
8-h total AUC	Fasting TG	119 ± 239	687 ± 258	0.50	.03
8-h incremental AUC	4-h incremental AUC	−58.2 ± 57.3	2.4 ± 0.3	0.89	.0001
TRL TGs					
8-h total AUC	4-h total AUC	−74.5 ± 43.2	2.4 ± 0.2	0.96	<.0001
8-h total AUC	Fasting TRL TG	−84.6 ± 93.0	1180.9 ± 206.1	0.82	.0007
8-h incremental AUC	4-h incremental AUC	−48.8 ± 36.5	2.6 ± 0.31	0.91	<.0001

Analyses included data from each participant's first test only.

The reproducibility of fasting TRL TGs was modest, with the ICC values for obese subjects and all subjects combined not meeting the 0.75 cut point for high reproducibility [20,21]. Furthermore, the reproducibility for fasting TRL TGs was considerably lower than that for fasting total TGs. With 1 exception (4-hour total AUC), none of the ICCs for TRL-based postprandial lipemia measures met the 0.75 criterion for high reproducibility. In general, the TRL TG ICCs were greater for total AUCs as compared with incremental AUCs, indicating better reproducibility for total AUCs.

3.5. Validity of abbreviated 4-hour PPLTs

For total plasma TGs, the total and incremental AUCs from the 4-hour tests were highly predictive of the corresponding 8-hour test results, with coefficients of determination (R^2) of approximately 0.90 (Table 5). Likewise, the 4-hour results for TRL TG responses were highly predictive of the 8-hour TRL TG responses, with R^2 values of at least 91%. For comparison purposes, the ability of fasting TGs to predict TG AUCs was also assessed (Table 5). Although, fasting TG concentrations were significant predictors of 8-hour AUCs, the R^2 values were substantially lower than those for 4-hour AUCs.

4. Discussion

4.1. Reproducibility of total TG responses

Results from the present study demonstrate that the total TG responses during PPLTs are highly reproducible in lean and obese subjects when calculated as the total area under the response curve and may therefore be of clinical utility for assessing CVD risk. These findings support those of Brown et al [22] who reported that postprandial total TG concentrations measured 3.5 and 9 hours after a high-fat meal were highly reproducible (ICCs = 0.76 and 0.85, respectively). Gill et al also found that the total TG responses to a high-fat meal were highly reproducible in men (ICC = 0.93), but found more modest reproducibility in young women (ICC = 0.65) if one test was performed during the follicular phase of the menstrual cycle and the second test on the same subjects was performed during the luteal phase [23]. In light of this finding in women, it is somewhat surprising that reproducibility was high in our study, despite the fact the most of our subjects (78%) were young women and menstrual cycle phase was not measured or controlled for. Furthermore, based on an analysis on women only, total AUC for total TGs was highly reproducible (WCV = 7.6%, ICC = 0.85) in our study.

4.2. Reproducibility of incremental and TRL TG responses

It is often argued that incremental AUC should be studied instead of total AUC because fasting TGs and total AUCs for TGs are correlated [24,25]. Furthermore, for a variety of reasons, it may be desirable or necessary to measure TRL TG responses during PPLTs. However, to our knowledge, the reproducibility of incremental TG responses and TRL TG responses has not been previously assessed. In contrast to our findings for total AUC for total TGs, we found that reproducibility is relatively low when lipemic responses are calculated as incremental areas above baseline or when TRL TG concentrations are measured instead of total TGs.

The reason for greater variability in incremental AUCs and TRL-based lipemic responses is not entirely clear. However, this may be partly attributable to more sources of measurement error. For the incremental AUCs, fasting TG concentrations are subtracted from postprandial concentrations; and as a consequence, this introduces measurement error from the fasting value into the postprandial values. For TRL TGs, the additional analytic steps may contribute to greater variability. In either case, it would be expected that, with greater analytic precision, the reproducibility of these outcomes would improve. However, until improvements occur, the low reproducibility of incremental and TRL-based measures of postprandial lipemia precludes their use as clinical measures. This does not imply that these measures should not be used for research purposes; however, it may be necessary to use more subjects or use more powerful experimental designs to overcome this variability and avoid type 2 statistical errors (ie, false negative results).

4.3. Validity of abbreviated 4-hour PPLTs

A secondary objective was to determine if an abbreviated 4-hour test could be used to validly predict results from a full-length 8-hour test. Indeed, the lipemic responses from the

4-hour test accounted for 89% to 96% of the variance in the 8-hour test results. It is important to recognize that this validity assessment does not include day-to-day variability in lipemic responses because the 4-hour test results were derived from the 8-hour test data. However, this approach is appropriate, given that our objective was to compare short and long tests independent of day-to-day variation in biological function. From a reproducibility perspective, findings from the present study suggest that postprandial lipemia from a 4-hour test is just as reproducible as that from an 8-hour test. Taken together, these findings indicate that an abbreviated 4-hour PPLT is a valid and reproducible alternative to full-length tests and may therefore be useful in clinical settings or when large study sample sizes preclude the use of longer tests. Certainly, the 4-hour test is still a considerable time burden for patients or study participants; and the evaluation of shorter protocols may be warranted in the future. However, from the perspective of medical expense and time burden on medical personnel, a 4-hour test is reasonable, as it only requires the insertion of an intravenous catheter, the administration of the test meal, and the acquisition of hourly blood samples.

4.4. Lipemic responses in lean vs obese individuals

It is somewhat surprising that the lipemic responses were not significantly greater in obese subjects than in lean subjects. However, to avoid the potentially confounding effect of disease treatments (eg, medications) on the postprandial lipemia reproducibility, we excluded individuals with chronic diseases and, as a consequence, may have had obese subjects who were unusually healthy from a metabolic perspective. However, a more likely explanation is that our study was not powered to detect differences between groups. In support of this, it is noteworthy that the mean lipemic responses were all approximately 60% higher in obese subjects than in lean subjects, with weak tendencies for significant differences (P = .08-.17).

5. Conclusion

Data from the present study show that PPLTs are highly reproducible when the total TG response is calculated as total AUC. Although there is still a need for meal standardization (ie, energy and nutrient content) and a need for standards to classify lipemic responses as normal or abnormal, the reproducibility of total TG responses is sufficient for use in a clinical setting. In contrast, when the incremental AUC is used, or when TRL TGs are used in lieu of total TGs, the reproducibility is substantially lower. Unless the variability in these methods for quantifying lipemic responses can be improved, for example, through refinement of analytic methods, the large variability in incremental AUCs and TRL TG responses precludes their use for clinical purposes. Finally, results of the present study show that an abbreviated 4-hour PPLT is a valid and reproducible surrogate for an 8-hour test.

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