Novel insights into blood markers and cardiovascular disease: Results of the Netherlands Epidemiology of Obesity study

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CHAPTER 8

ASSOCIATION OF FASTING TRIGLYCERIDE CONCENTRATION AND POSTPRANDIAL TRIGLYCERIDE RESPONSE WITH THE CAROTID INTIMA MEDIA THICKNESS IN THE MIDDLE AGED: THE NEO STUDY

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Abstract

Background

People are in a postprandial state for the majority of the day, postprandial triglyceride response may be more important in the etiology of atherosclerosis than fasting triglycerides.

Objective

To investigate the associations of fasting triglyceride concentration (TGc) and postprandial TG response after a meal challenge with subclinical atherosclerosis, measured by intima media thickness (IMT) in a middle aged population.

Methods

5,574 participants (57% women) with a mean (SD) age of 56 (6) years were included in this cross-sectional analysis of baseline measurements of the Netherlands Epidemiology of Obesity study. Serum TGc was measured fasting, and 30 and 150 minutes after a liquid mixed meal and the incremental area under the curve (TGiAUC) was calculated. With linear regression analyses, we calculated the differences in IMT with 95% confidence intervals (CI), adjusted for confounding factors, and additionally for TGc or TGiAUC.

Results

Per standard deviation (SD) of TGc(0.82 mmol/L), IMT was 8.5 μm(2.1, 14.9) greater after adjustment for TGiAUC and confounding factors. Per SD of TGiAUC(24.0 mmol/L*min), the difference in IMT was -1.7 μm(-8.5, 5.0) after adjustment for fasting TG and confounding factors.

Conclusion

The association between TG response after a mixed meal and IMT disappeared after adjusting for TGc. The association between fasting TG concentration and IMT persisted after adjustment for postprandial TG response. These findings imply that it is not useful to perform a meal challenge in cardiovascular risk stratification. Our results support use of fasting TGc instead of postprandial TG responses for cardiovascular risk stratification in clinical practice.

Introduction

Atherosclerosis is a vessel disease that predisposes for the development of chronic or acute coronary heart disease and stroke [234]. Because clinically relevant atherosclerosis is present in almost half and coronary artery calcifications in one fifth of the middle-aged population, many individuals are at risk for the development of cardiovascular disease (CVD), the leading cause of death worldwide [1, 235, 236].

The progression of atherosclerosis is influenced by multiple cardiovascular risk factors, such as smoking, diet, body composition and genetic predisposition [234]. High fasting and non-fasting plasma TG concentrations (TGc) are well-known additional risk factors for cardiovascular disease [52]. The recently updated consensus statement by the European Atherosclerosis Society indicated that measuring TGc in a fasting state does not improve CV risk prediction compared to measurement of non-fasting TGc [194]. This implies that for clinical prediction of CVD, measuring fasting and non-fasting TGc may be exchangeable. However, the mechanisms that link fasting TGc with CVD may be different from the mechanism relating non-fasting TGc and CVD.

Since Zilversmit first proposed in 1979 that atherogenesis may be a predominantly postprandial process [53], studies have been undertaken to elucidate the underlying mechanisms. It has been hypothesized that the relation between postprandial hypertriglyceridemia and atherosclerosis depends on the size and TG content of lipid-transporting lipoprotein particles [211, 237]. Another mechanism between the TG response to a meal and atherosclerosis is the accumulation of lipoprotein remnant particles, which are responsible for TG transport [238]. The concentration of lipoprotein remnant particles is associated with postprandial hyperlipidemia [219] and strongly associated with coronary heart disease [220, 239]. Atherogenic effects of postprandial hypertriglyceridemia may be even more prominent when the vascular endothelium is exposed to oxidative stress as a result of smoking [240] or hyperglycemia [241]. Therefore smokers and (pre)diabetics may be more susceptible to the development of atherosclerosis as a result of hypertriglyceridemia.

To quantify the effects of TG metabolism on (subclinical) CVD in epidemiological studies, fasting TGc are often used as exposure measurement. However, people in the Western world are in a postprandial state for the largest part of the day [53], which may lead to prolonged high plasma TGc. We hypothesized that postprandial hypertriglyceridemia is associated with a larger carotid IMT as a measure of subclinical atherosclerosis and that this association might be stronger in strata of risk factors that are associated with deterioration of the vascular en-

dothelium. Therefore, the aim of the present study was to examine the associations of fasting TGc and postprandial TG response after a liquid mixed meal challenge with subclinical atherosclerosis in the total population and in subgroups based on sex, smoking status and fasting glucose.

Methods

Study design and population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based, prospective cohort study designed to investigate pathways that lead to obesity-related diseases. The NEO study started in 2008 and includes 6,671 individuals aged 45–65 years, with an oversampling of individuals with overweight or obesity. The study design and population are described in detail elsewhere [5].

Men and women living in the greater area of Leiden (in the West of the Netherlands) were invited through letters sent by general practitioners, by local advertisements and via registries of municipalities surrounding Leiden. They were invited to participate if they were aged between 45 and 65 years and had a self-reported BMI of 27 kg/m^2 or higher. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited to participate irrespective of their BMI, allowing for a reference distribution of BMI. Participants were invited for a baseline visit at the NEO study center of the Leiden University Medical Center (LUMC) after an overnight fast.

Prior to the study visit, participants completed a general questionnaire at home to report demographic, lifestyle and clinical information. The participants were asked to bring all medication they were using in the month preceding to the study visit. Research nurses recorded names and dosages of all medication. All participants underwent an extensive physical examination, including anthropometry, blood sampling and a meal challenge. In the present analysis, we excluded participants with a history of CVD (defined as angina pectoris, myocardial infarction, stroke, arrhythmias or congestive heart failure), missing values for fasting and postprandial TG and IMT. Because it is unknown how the TG response and atherosclerosis are influenced by glucose lowering medication, we additionally excluded participants that used this medication. Furthermore, participants that did not drink the meal challenge were excluded.

The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the design of the study and all participants gave their written informed consent.

Data collection

Fasting and postprandial triglycerides

After 5 minutes rest, fasting blood samples were collected from the antecubital vein. All participants consumed the same liquid mixed meal of 400 mL, that contained 600 kcal of which 16 percent (En%) was derived from protein, 50 En% from carbohydrates and 34 En% from fat. In total, the 400 mL meal contained 75.0 g of carbohydrates, 2.4 g of saturated, 13.7 g of monounsaturated and 6.8 g of polyunsaturated fat. Participants were instructed to ingest the mixed meal within 5 minutes. Blood was sampled 30 and 150 minutes after the meal ingestion. Triglyceride concentrations were determined using an enzymatic colorimetric assay using a TG GPO-PAP kit (11730711216, Roche) on on an automated analyser (Roche Modular P800, Roche Diagnostics, Almere, The Netherlands). Postprandial TG response was calculated as the TGiAUC using the trapezoidal method and was represented as mmol/L*min.

Postprandial triglyceride concentrations peak approximately 4 hours after the meal [242]. In the present study, the response was measured up until 150 minutes after the meal, a duration that may be too short to capture the complete triglyceride response. To validate whether 150 minutes is sufficient, we validated this measurement in a randomly selected subgroup of participants. Therefore, blood was sampled at 240 minutes after ingestion of the mixed meal (n=14) to investigate the agreement in TG response between 0-150 minutes and 0-240 minutes.

Plasma glucose and insulin concentrations, serum total cholesterol and high-density lipoprotein (HDL)-cholesterol were determined in the fasting blood samples at the central clinical chemistry laboratory of the LUMC using standard assays.

Impaired fasting glucose was defined as a fasting glucose concentration ≥6.1 mmol/L. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as the product of fasting glucose and insulin concentrations divided by 22.5 [129].

Carotid intima media thickness

The carotid IMT was measured in the far wall of the left common carotid artery (CCA) along a section with a length of 15 mm, located 10 mm proximal of the bifurcation with the participant in supine orientation. A 7.5-10 MHz linear array transducer in B-mode setting was used to visualize the distal CCA and the lu-

men-intima and intima-media limits were detected using an online wall-track system (Art.Lab version 2.1, Esaote, Maastricht, The Netherlands). IMT was measured in the transversal angle during six heartbeats.

Population characteristics and other variables

Ethnicity was self-identified in the questionnaire and was regrouped into white (reference) and other. Highest completed level of education was reported in ten categories according to the Dutch education system and regrouped in two categories: low education (no education, primary education or lower vocational education) and high education (other). Participants reported the frequency, type, and duration of their usual physical activity in the past 4 weeks on the Short Questionnaire to Assess Health-enhancing physical activity, a method previously validated in the Dutch population [243, 244]. We calculated the energy expended during physical activity in leisure time in hours per week of metabolic equivalents. Participants reported their history of CVD, defined as myocardial infarction, angina, congestive heart failure, stroke or peripheral vascular disease. Smoking status was self-reported and grouped in three categories: never smoker, former smoker and current smoker. Quantification of long-term tobacco use was expressed in pack-years of smoking, which was defined as the product of the number of (20 cigarette)-packs per day and the number of years the person smoked. Habitual alcohol intake in grams per day was assessed using a semi-quantitative food frequency questionnaire (FFQ), which has been validated in the Dutch population [245], and calculated from the 2011 version of the Dutch food composition table [246].

Height was measured without shoes using a calibrated, vertically fixed tape measure. Body weight and percent body fat were measured by the Tanita bio impedance balance (TBF-310, Tanita International Division, UK) without shoes and one kilogram was subtracted to correct for the weight of clothing. Body mass index (BMI) was calculated by dividing body mass in kilograms by body height in meters squared. Waist circumference was measured in the middle of the distance between the crista iliaca and the lowest rib using a flexible steel tape measure. Blood pressure was measured seated on the right arm with a validated automatic oscillometric device (OMRON, Model M10-IT, Omron Health Care Inc, IL, USA). Three measurements with 5 min rest in between measurements were performed and the mean systolic and diastolic blood pressure were calculated

Statistical analysis

Baseline characteristics were presented as means with standard deviation, medians with 25th and 75th percentiles or as percentages stratified for fasting TGc. The cut-off value for increased fasting TGc was defined as 1.7 mmol/L [194]. In the NEO study, individuals with a BMI of 27 kg/m^2 or higher were oversampled. To correctly represent baseline associations in the general population, adjustments for the oversampling of individuals with a BMI ≥27 kg/m^2 were made. This was done by inverse probability weighting of all participants towards the BMI distribution of participants from the Leiderdorp municipality [60], whose BMI distribution was similar to the BMI distribution of the general Dutch population in the age range of 45–65 years [16].

We calculated Pearson's correlation coefficients between fasting TGc and TGc at 150 minutes and fasting TGc and TGiAUC. Fasting TGc and the TGiAUC were standardized to a mean of zero and a standard deviation of 1.We performed weighted linear regression analyses to examine the associations between fasting TGc and the TGiAUC as the exposure, with the IMT as the outcome variable and calculated regression coefficients with 95 % confidence intervals (CI). The regression coefficients were expressed as the difference in IMT in micrometers (µm) per standard deviation higher fasting TG or TGiAUC. Crude analyses were first adjusted for age and sex, then we added either the fasting TGc or TGiAUC response, and finally we adjusted all models for the potential confounding factors total body fat, physical activity, smoking status, pack years of smoking, alcohol consumption, use of alpha and beta blockers, use of lipid lowering drugs (fibrates, niacin or statins), fasting LDL-cholesterol and HOMA1-IR.

To investigate whether the associations were different between men and women, smokers and non-smokers and persons with and without hyperglycemia, we tested for interaction by including a product interaction term (TGiAUC*stratification factor) in the regression model. Subsequently, we performed all analyses stratified by sex, fasting glucose concentrations and smoking status.

We furthermore repeated all analyses including the participants with a history of CVD.

Results

Baseline characteristics

In total, 6,671 participants were included in the NEO study. After consecutive exclusion of participants with a history of cardiovascular disease (n=527), missing data on fasting (n=42), 30 minutes (n=90) and 150 minutes (n=72), missing IMT measurement (n=98), meal protocol violations (n=4) or use of glucose lowering medication (n=264), 5,574 participants were included in the analyses.

Baseline characteristics of the NEO study population are presented in Table 1, stratified by fasting TGc. The mean (SD) age of the study population was 56 years, 57% were women and 16% were current smokers. The mean (SD) BMI was 26.1 (4.3) kg/m^2 and the mean IMT was 631 (143) µm, both were higher in the participants with increased concentrations of fasting TG. The population mean (SD) fasting TGc was 1.21 (0.82) mmol/L. The mean population TG response was 29.8 (24.0) mmol/L*min is represented in Figure 1. The correlation coefficient of fasting TGc with TGc at 150 minutes was 0.92 and with TGiAUC 0.36.

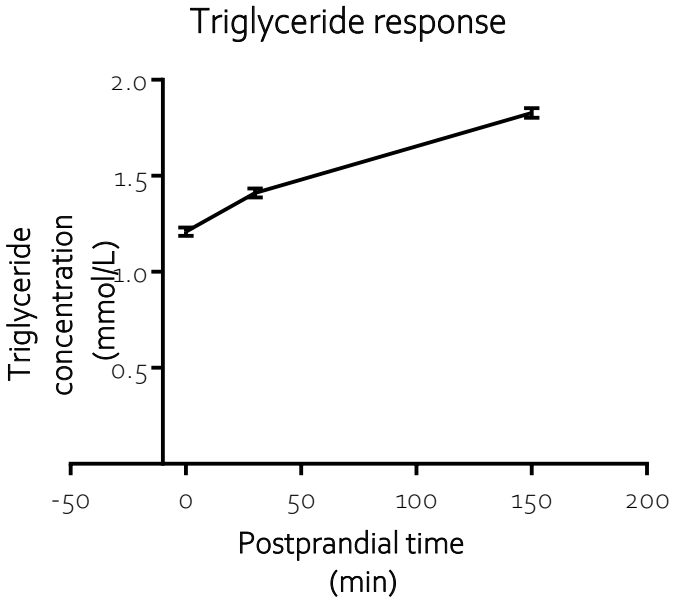


Figure 1 – Postprandial triglyceride response with 95% confidence intervals after a mixed meal in the participants of the Netherlands Epidemiology of Obesity study, men and women aged between 45 and 65 years who did not use glucose lowering therapy (n=5,574). Results are based on analyses weighted towards the BMI distribution of the general population.

In the validation population (n=14), the mean (SD) TGc at 150 minutes was 2.4 (1.1) mmol/L*min and at 240 minutes 2.3 (1.2) mmol/L*min. The area under the curve between 0 and 150 minutes postprandial was highly correlated with the area under the curve between 0 and 240 minutes; r^2=0.99 (Supplementary figure 1)

Table 1 – Baseline characteristics of the participants of the Netherlands Epidemiology of Obesity study, men and women aged between 45 and 65 years without medical history of cardiovascular disease of whom the TG response was assessed after a mixed meal challenge and who did not use glucose- and lipid lowering therapy (n=5,574).

	Fasting TG	
Characteristic	<1.7 mmol/L (83%)	≥1.7 mmol/L (17%)
Age (year)	55.5 (6.1)	55.5 (5.7)
Sex (% men)	39	61
Ethnicity (% whites)	95	95
BMI (kg/m^2)	25.6 (4.1)	28.5 (4.3)
Tobacco smoking (% never/former/current)	41 / 45 / 14	31 / 46 / 22
Pack years (packs*years)		
Current smokers	18 (10-29)	20 (12-32)
Former smokers	8 (3-17)	11 (5-23)
Physical activity (MET hours/week)	31 (17-51)	26 (12-45)
Education level (% high)[a]	48	41
Total cholesterol (mmol/L)	5.6 (1.0)	6.2 (1.2)
HDL cholesterol (mmol/L)	1.7 (0.4)	1.2 (0.3)
LDL cholesterol (mmol/L)	3.5 (0.9)	3.8 (1.1)
Fasting glucose (mmol/L)	5.3 (0.7)	5.7 (0.9)
Fasting glucose ≥6.1 mmol/L (%)	9	22
Fasting insulin (IU/L)	2.5 (2.0-5.6)	6.7 (3.3-11.7)
HOMA1-IR	1.6 (1.1-2.5)	2.9 (2.0-4.3)
Fasting TG (mmol/L)	0.94 (0.35)	2.53 (1.13)
TGiAUC (mmol/L*min)	25.6 (19.4)	50.3 (32.2)
IMT (µm)	625 (142)	663 (145)

BMI, body mass index; IMT, carotid Intima Media Thickness; HDL, high density lipoproteins; HOMA-IR, Homeostasis Model Assessment Insulin Resistance; TGiAUC, incremental area under the curve; LDL, low density lipoproteins; MET, metabolic equivalents of task; NEO, Netherlands Epidemiology of Obesity.

Results are based on analyses weighted towards the BMI distribution of the general population (n = 5,574). Data are shown as mean (SD), median (IQR) or weighted percentage.

a; Low eduation: none, primary school or lower vocational education as highest level of education.

Associations of fasting TG concentrations and carotid intima media thickness

The associations between fasting TGc and IMT (µm) are presented in Figure 2. Per SD of fasting TGc, the difference in IMT was 18.8 (95% confidence interval: 13.4-24.1) µm. After adjustment for age and sex this difference attenuated to 15.3 (10.1-20.5) µm and after additional adjustment for TGiAUC this coefficient was 14.4 (8.9-20.0) µm. After additional adjustment for all confounding factors the coefficient further attenuated to 8.5 (2.1-14.9) µm.

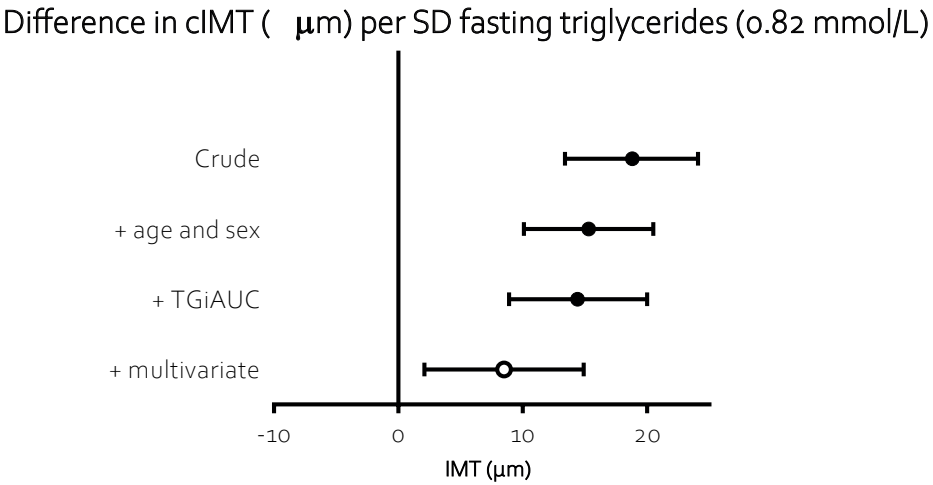


Figure 2 – Difference in carotid intima media thickness (µm) per standard deviation of fasting triglyceride concentrations (0.82 mmol/L) Multivariate: additionally adjusted for LDL-cholesterol, total body fat, alcohol consumption, use of alpha and beta blockers, use of lipid lowering drugs, physical activity, pack years of smoking, HOMA1-IR, TGiAUC. TGiAUC, incremental area under the curve of postprandial triglycerides; cIMT, carotid intima media thickness; LDL, Low density lipoprotein; HOMA-IR, Homeostastis assessment of insulin resistance. Results are based on analyses weighted towards the BMI distribution of the general population.

Associations between postprandial TG response and carotid intima media thickness

One SD of TGiAUC was associated with a 13.2 (7.0-19.4) µm difference in IMT. After adjustment for age and sex this difference attenuated to 7.2 (1.0-13.5) µm per SD. After additional adjustment for fasting TGc the coefficient attenuated to 2.7 (-3.6-9.0) and after full adjustment for confounding factors TGiAUC was not associated with IMT (-1.7, 95% CI: -8.5-5.0 µm per SD). (Figure 3).

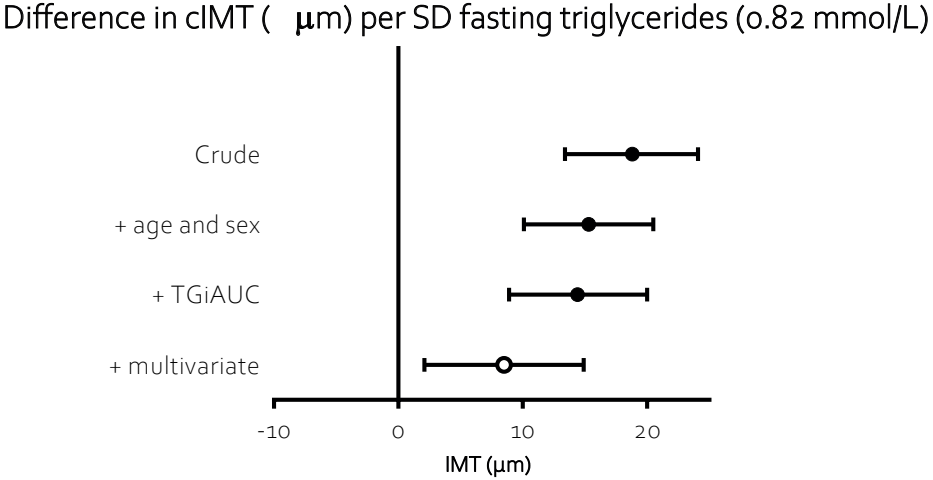


*Figure 3 – Difference in carotid intima media thickness (μm) per standard deviation of incremental area under the curve of triglyceride response (24.0mmol/L*min) Multivariate: additionally adjusted for LDL-cholesterol, total body fat, alcohol consumption, use of alpha and beta blockers, use of lipid lowering drugs, physical activity, pack years of smoking, HOMA1-IR, TGiAUC. TGiAUC, incremental area under the curve of postprandial triglycerides; cIMT, carotid intima media thickness; LDL, Low density lipoprotein; HOMA-IR, Homeostastis assessment of insulin resistance. Results are based on linear regression analyses weighted towards the BMI distribution of the general population.*

Associations between postprandial TG response and carotid intima media thickness in high-risk subgroups

Stratified results are presented in Table 2.

In men, the crude difference of 5.6 (-4.4-15.5) μm IMT per SD TGiAUC attenuated to 4.5 (-5.2-14.1) μm after adjustment for age and fasting TG and to -0.3 (-10.3-9.6) μm after additional adjustment for confounding factors. In women, the crude difference of 12.3 (4.5-20.1) μm IMT per SD TGiAUC disappeared after adjustment for age and fasting TG (0.7; -7.8-9.2 μm) and additional adjustment for confounding factors (-1.3; -10.1-7.5 μm). There was no significant interaction between TG response and sex (p= 0.23).

Crude effects in persons with normal and impaired fasting glucose concentrations of 12.0 (5.0-19.0) and 8.1 (-4.2-20.5) μm per SD attenuated to 6.0 (-1.1-13.1) and 8.7 (-3.1-20.6) μm per SD after adjustment for age and sex. After adjustment for fasting TGc, these coefficients changed to 1.3 (-5.8-8.5) μm per SD in persons with normal fasting glucose and 7.5 (-4.5-19.4) μm per SD in persons with impaired fasting glucose concentrations. After additional adjustment for confounding factors the differences were -2.3 (-10.0-5.3) μm per SD for persons

with normal and 3.2 (-8.9-15.3) µm per SD for persons with impaired fasting glucose concentrations. The interaction between TG response and glycemia was not significant (p=0.35).

The crude differences per SD of TGiAUC in never smokers (15.8; 6.0-25.5 µm), former (8.3; -0.1-16.7 µm) and current smokers (14.8; -1.8-31.3 µm) attenuated to 3.5 (-7.1, 14.1) µm (never), -1.7 (-10.1, 6.7) µm (former) and 8.5 (-6.6, 23.6) µm (current) after adjustment for age, sex and fasting TG. Additional adjustment for confounding factors resulted in differences of 0.8 (-10.0-11.6) µm (never), -6.6 (-15.5-2.2) µm (former) and 7.7 (-8.5-23.9) µm IMT (current) per SD of TGiAUC. The interaction between the postprandial TG response and smoking status was not significant (p=0.41 for never vs former smoking and p=0.70 for never vs current smoking).

When we included participants with prior or prevalent cardiovascular disease in the analyses, the results were similar (data not shown).

*Table 2 – Differences in IMT (µm) with 95% confidence intervals per standard deviation of postprandial TG response (24.0 mmol/L*min) in participants of the NEO study stratified by sex, glycaemia and smoking status.*

		IMT		
Postprandial TGiAUC (SD = 24.0 mmol/L*min)		Model	Difference in IMT (µm)	95% Confidence interval
Men	(43%)	Crude	5.6	-4.4, 15.5
		Adjusted for age and sex	7.9	-2.2, 17.9
		+fasting TG	4.5	-5.2, 14.1
		Multivariate adjusted	-0.3	-10.3, 9.6
Women	(57%)	Crude	12.3	4.5, 20.1
		Adjusted for age and sex	6.6	-0.9, 14.1
		+fasting TG	0.7	-7.8, 9.2
		Multivariate adjusted	-1.3	-10.1, 7.5
Fasting glucose <6.1 mmol/L	(89%)	Crude	12.0	5.0, 19.0
		Adjusted for age and sex	6.0	-1.1, 13.1
		+fasting TG	1.3	-5.8, 8.5
		Multivariate adjusted	-2.3	-10.0, 5.3
Fasting glucose ≥6.1 mmol/L	(11%)	Crude	8.1	-4.2, 20.5
		Adjusted for age and sex	8.7	-3.1, 20.6
		+fasting TG	7.5	-4.5, 19.4
		Multivariate adjusted	3.2	-8.9, 15.3
Never smoker	(39%)	Crude	15.8	6.0, 25.5
		Adjusted for age and sex	8.5	-1.1, 18.2
		+fasting TG	3.5	-7.1, 14.1
		Multivariate adjusted	0.8	-10.0, 11.6
Former smoker	(45%)	Crude	8.3	-0.1, 16.7
		Adjusted for age and sex	2.6	-5.4, 10.5
		+fasting TG	-1.7	-10.1, 6.7
		Multivariate adjusted	-6.6	-15.5, 2.2
Current smoker	(16%)	Crude	14.8	-1.8, 31.3
		Adjusted for age and sex	10.9	-5.9, 27.8
		+fasting TG	8.5	-6.6, 23.6
		Multivariate adjusted	7.7	-8.5, 23.9

IMT, carotid Intima Media Thickness; TGiAUC, incremental area under the curve;
Multivariate: additionally adjusted for LDL-cholesterol, total body fat, alcohol consumption, use of alpha and beta blockers, use of lipid lowering drugs, physical activity, pack years of smoking, HOMA1-IR and fasting TGc in additional models
Results are based on analysis weighted towards the BMI distribution of the general population.

Discussion

In this population-based study of men and women without prevalent CVD we observed a clear association between fasting TGc and IMT, which persisted after adjustment for postprandial TG response over 150 minutes. The association between the TG response and IMT however disappeared after adjusting for fasting TGc. These findings imply that in general it is not useful to perform a meal challenge in order to estimate a person's risk of atherosclerosis and thereby add to the ongoing debate on the importance of fasting TGc measurements in cardiovascular risk. Although our results have no implications for random non-fasting TGc, the association between fasting TGc and IMT persisted beyond postprandial TG response.

Previous studies showed that postprandial TG response is a risk factor for cardiovascular disease and atherosclerosis in certain subgroups of the general population [247-251]. It has been shown that smoking and diabetes aggravate the effect of other cardiovascular risk factors [251, 252]. Also, the mechanisms behind the additional risk in the presence of the atherogenic risk factors smoking and diabetes has been studied in smaller experimental studies [219, 253]. Because the vascular endothelium is exposed to elevated TGc in the postprandial range for a large part of the day, we hypothesized that the postprandial TG response would be stronger associated with (subclinical) atherosclerosis than fasting TGc. However, our findings suggest that fasting TGc are responsible for the observed crude association between postprandial TG response and IMT. However, the suggestion of a remaining association between TG response and IMT in smokers and (pre)diabetics after adjustment for fasting TGc may indicate that these conditions increase the susceptibility of the endothelial wall to either postprandial TG response or higher concentrations of remnant particles.

Besides the importance of TG response in certain subgroups prone to atherosclerosis, our findings indicate an important role of fasting TGc in the etiology of atherosclerosis beyond TG response. These results are in line with clinical practice, but are not in complete agreement with the recent consensus statement [194], because the association between fasting TGc and IMT persists after adjustment for TG response.

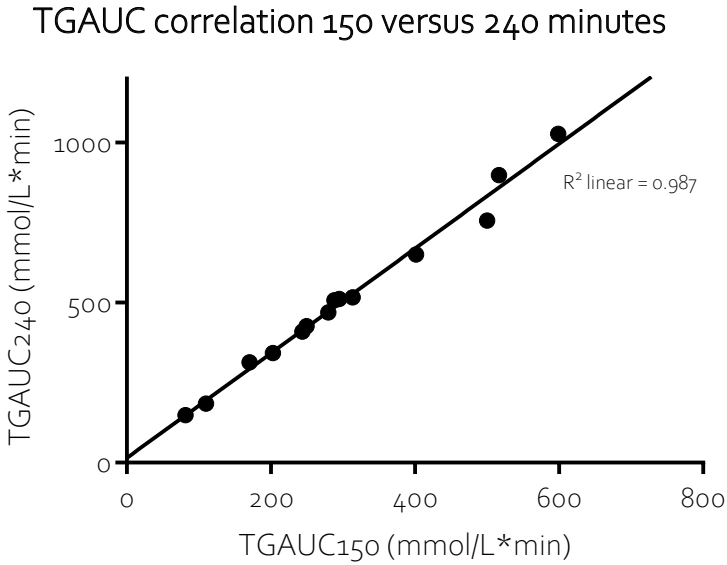
Not all modulators of fasting TGc are completely known. Excess of TG and cholesterol in the diet most likely play a role, as well as a limited hydrolysing capacity of lipoprotein lipase (LPL) due to low systemic LPL expression [222] or other lipoproteins competing for hydrolysis by LPL [52, 254, 255]. The present study, together with other studies indicate that TG response [241] and its effects on IMT [252] are different between persons with and without hyperglycemia.

Strengths of this study are that all participants were challenged to a mixed meal to represent daily dietary challenges instead of a meal challenge with isolated carbohydrates or lipids. Other strengths of this study are the availability of extensive phenotypic data regarding atherosclerosis and confounding variables in a large study population.

There are also some limitations that need to be considered. First, the TG response was assessed during a 150 minute period, while the peak of TGc after a meal may occur even 4 hours after a meal. Therefore a residual association may exist between a longer TG response and subclinical atherosclerosis. However, in a subgroup of participants we showed that the TG response over 240 minutes strongly correlated with the TG response over 150 minutes. Second, we assessed the TG response to one single meal with 34 energy percent from fat, which was not individualized with regard to body surface area, which may not fully represent daily exposure to postprandial TG or provoke a substantial triglyceride response. Elevated TGc due to multiple meals during the day may be more important in vascular wall damage than the TG response to one meal. The results did not change when additionally adjusting for body surface area (data not shown). Future studies are needed to investigate the relation between daily TGc and atherosclerosis and cardiovascular disease. Third, the confidence intervals of the observed associations in the subgroups were large and include null indicating large variation or insufficient power in these specific subpopulations. However, the calculated association between TG response and IMT in smokers and persons with increased fasting glucose concentrations did not reach zero after adjustment for other confounding factors, implicating a loss of power in the subgroup analyses rather than chance findings. Fourth, the cross-sectional design of this study precludes causal inference. As a result of the observational design, residual confounding may be present due to remaining unknown, unmeasured or inaccurately measured confounding factors, such as the use of dietary supplements.

In conclusion, this study shows that in the general population, the association between TG response after a mixed meal and subclinical atherosclerosis disappeared after adjusting for fasting TGc. Our findings suggest that non-fasting and fasting TGc may not be exchangeable but that a higher fasting TGc is related to a larger IMT beyond TG response. These results confirm the clinical practice of using fasting TGc for cardiovascular risk stratification in the general population. More research is needed to specifically study the effect of the postprandial TG response during the day, in particular in susceptible individuals as smokers and (pre)diabetics.

Supplementary tables and figures



Supplementary figure 1 – Correlation plot of the area under the blood concentration curve of triglycerides measured over 150 minutes (TGAUC150) versus the area under the blood concentration curve of triglycerides measured over 240 minutes of time (TGAUC240) after a mixed meal challenge in 14 participants of the Netherlands Epidemiology of Obesity study.