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The correlation between TG vs remnant lipoproteins in the fasting and postprandial plasma of 23 volunteers

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ABSTRACT

Background: Two recent publications report that non-fasting triglycerides concentrations in plasma are more predictive of cardiovascular events than conventional measurements of fasting triglycerides. While these observations are consistent with the previous studies, direct correlations between remnant lipoprotein triglyceride (RLP-TG) and remnant lipoprotein cholesterol (RLP-C), which are also considered to be risk factors for cardiovascular disease, and fasting and postprandial TG have not been investigated.
Methods: On four different days, both fasting and postprandial blood samples were collected from twenty-three overweight to obese men and women at UC Davis and analyzed for plasma concentrations of TG, RLP-C and RLP-TG.
Results: Significantly higher correlations between plasma TG and RLPs were observed in the postprandial state (RLP-C $r^2=0.85$; RLP-TG $r^2=0.92$) than in the fasting state (RLP-C $r^2=0.61$; RLP-TG $r^2=0.73$). The differences in the correlations between the fasting and postprandial TG and RLPs were statistically significant ($p<0.001$). The increase of RLP-TG (postprandial RLP-TG minus fasting RLP-TG) consisted of approximately 80% of the total increase of TG (postprandial TG minus fasting TG).
Conclusion: Postprandial TG vs remnant lipoprotein concentrations were significantly more correlated when compared with fasting TG vs RLP concentrations. The increased TG in the postprandial state mainly consisted of TG in remnant lipoproteins. Therefore, the increased sensitivity of non-fasting TG in predicting the risk for cardiovascular events may be directly explained by the increase of remnant lipoproteins in the postprandial state.

1. Introduction

Nordestgaard et al. [1] and Bansal et al. [2] recently reported that triglycerides measured in non-fasting samples were more sensitive than more conventional measurements of fasting triglyceride concentrations in predicting the risk of cardiovascular events in the Copenhagen Heart Study and in the Women's Health Study as previously reported by Iso et al. [3] in Japanese population. Nordestgaard et al. [1] further reported that with increasing levels of non-fasting triglycerides there were increased levels of remnant lipoproteins, and that the association between non-fasting TG and risk of cardiovascular events may reflect the atherogenic effects of remnant lipoproteins. However, rather than directly measuring remnant lipoproteins (RLP), the investigators estimated RLP as "total cholesterol minus cholesterol in HDL and LDL". These results simply provided the plasma levels of either TG-rich lipoproteins or VLDL ($d<1.006$), but not measured remnant lipoproteins correctly. Furthermore, the Framingham Offspring Study previously reported by us [4] showed that TG was not a cardiovascular risk in the fasting, while RLP-C was an independent risk factor in the fasting state. Therefore it is interesting to know the relationship between the increased TG and remnant lipoproteins in the postprandial state for predicting the risk of cardiovascular events.

TG-rich lipoproteins contain apoB-48 carrying chylomicrons of intestinal origin and apoB-100 carrying VLDL of hepatic origin together with the remnants of both classes. Postprandial TG is known to increase highest in 3–6 h after food intake [5–8]. Therefore it is important to clarify the relationship between the increased TG and remnant lipoproteins measured directly in the fasting and postprandial states.

The relationship between one fifths of serum TG and VLDL-C ($d<1.006$) is known to be highly correlated, regardless of the fasting and postprandial states [9]. However, no reports have clarified the direct relationship between increased TG or VLDL-C and remnant lipoproteins in the postprandial states. Namely, what percentage of

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Table 1
Demographic data of the 23 volunteers (fasting state).

	Male ($n=12$)	Female ($n=11$)
Age	53±4	54±2
BMI	29±1	30±1
Waist circumference (cm)	101±2	92±6
Blood pressure (mm Hg)		
Systolic (mm Hg)	120±2	117±2
Diastolic (mm Hg)	76±1	76±2
T-Chol (mg/dl)	182±12	197±16
TG (mg/dl)	154±27	154±40
LDL-C (mg/dl)	117±9	126±16
HDL-C (mg/dl)	36±4	41±4
Glucose (mg/dl)	88±2	88±2
Insulin (μU/ml)	15±3	15±3

the increased TG or VLDL reflects the amount of remnant lipoproteins? Therefore, we measured plasma levels of TG and remnant lipoprotein in fasting and postprandial blood samples drawn from 23 volunteers who were overweight to obese and susceptible to be postprandial hyperlipidemia. To investigate the characteristics of increased TG in the postprandial state, measurement of RLP-TG was mainly focused in this study and discussed the component of TG correlated with the increased risk of cardiovascular disease in the postprandial state.

2. Materials and methods

The current study was performed in 23 overweight to obese men ($n=12$) and postmenopausal women ($n=11$), age 40–71 y, with a BMI range of 25–35 (Table 1). The experimental protocol was approved by the UC Davis Institutional Review Board and the subjects provided informed consent to participate in the study. Fasting blood samples were collected at 9:00 h and postprandial blood samples were collected at 22:00 h on the same day. Between sampling, each subject consumed a standardized breakfast (9:00 h), lunch (13:00 h) and dinner (18:00 h) provided by the study staff containing 55% of energy as carbohydrate, 30% fat, and 15% protein. The energy content of the meals was based on each subject's energy requirement as determined by the Mifflin equation [10]. The subjects participated in 4 blood collection trials over a period of 12 weeks and the meals were identical at all four trials except that the carbohydrate portions of the meals varied with respect to the starch and sugar (fructose or glucose) content of the diets. While the type of carbohydrate consumed had significant effects on absolute triglycerides, RLP and other lipid/lipoprotein parameters, it did not affect the correlations or proportional changes reported in the present comparison. Therefore all data from 23 volunteers were pooled for a total of 92 pairs of fasting and postprandial time-points. Plasma samples were kept frozen at −80°C until analysis. TC and TG were measured enzymatically and LDL-C and HDL-C were measured by homogeneous methods (Daiichi Chemicals, Tokyo, Japan). Glucose and insulin were measured by PolyChem system (Polymedco, NY). RLP-C and RLP-TG were measured by the method of Nakajima et al. [11].

2.1. Statistics

Data were analyzed with Stat View software (Ver. 5.0; SAS Institute Inc, Cary, NC).The statistical significance of differences was determined by Mann–Whitney *U*-test. The correlation between variables is presented as Pearson's correlation coefficient (*r*-value). A $p<0.05$ was statistically significant.

3. Results

Table 2 shows that postprandial TG and remnant lipoprotein levels were significantly increased in 23 volunteers compared with those in the fasting state. Each volunteer plotted 4 times in all figures under different conditions. Significantly higher correlations between TG and

Table 2
Fasting and postprandial plasma lipids and lipoproteins in 23 volunteers.

	Fasting (F)	Postprandial (P)	*p* value(F vs P)
Cholesterol (mg/dl)	180±14	179±20	NS
Triglycerides (mg/dl)	155±76	232±122	$p<0.0001$
HDL-C (mg/dl)	38±9	36±9	$p<0.0001$
RLP-C (mg/dl)	9±7	13±9	$p<0.000l$
RLP-TG (mg/dl)	32±27	96±71	$p<0.0001$

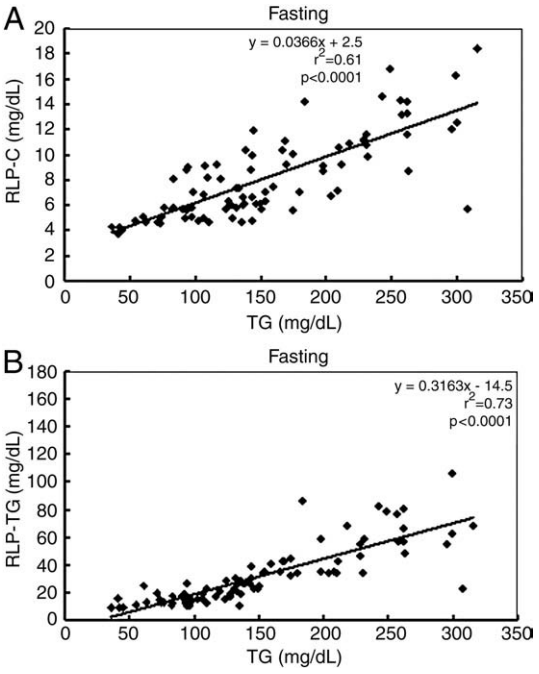

Fig. 1. Correlations between fasting TG and remnant lipoproteins (A: RLP-C $r^2=0.61$ and B: RLP-TG $r^2=0.73$, respectively). The correlation between fasting TG and VLDL-C is consistently shown to be approximately $r^2=0.90$ [8], however the correlation between fasting TG and RLP-C was shown to be correlated significantly less.

RLPs (Fig. 2) were observed in the postprandial state (RLP-C $r^2=0.85$; RLP-TG $r^2=0.92$), than in the fasting state (RLP-C $r^2=0.61$; RLP-TG $r^2=0.73$) (Fig. 1). Similar less correlations between TG and RLP-C in the fasting ($r=0.79$) were previously reported in an analysis of samples from Framingham Offspring Study [4]. The differences in the correlations between the fasting and postprandial TG vs RLPs were

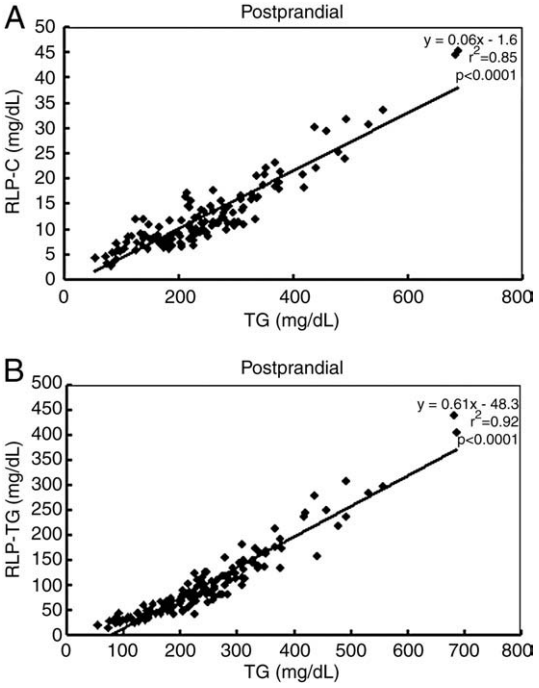

Fig. 2. Correlations between postprandial TG and remnant lipoproteins (A: RLP-C $r^2=0.85$ and B: RLP-TG $r^2=0.92$, respectively). The correlation between postprandial TG and VLDL-C is consistently shown to be approximately $r^2=0.90$ [8]. Therefore the correlation between postprandial TG and RLPs was shown to be highly correlated as the correlation between VLDL-C and RLP-C. The correlations between postprandial TG and remnant lipoproteins were significantly higher than those in the fasting (Fig. 1 vs 2) ($p<0.0001$).

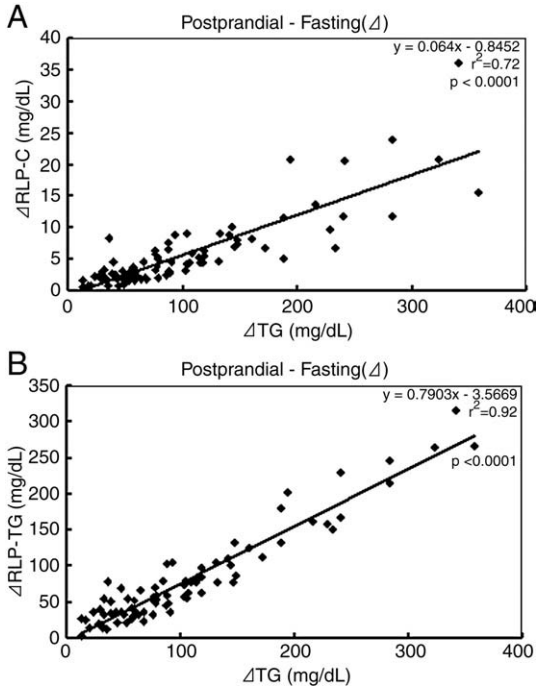

Fig. 3. Correlation between delta TG (total postprandial minus fasting TG) and delta RLP (total postprandial minus fasting remnant lipoproteins) (A: RLP-C, $r^2 = 0.72$, B: RLP-TG, $r^2 = 0.92$ respectively). Delta RLP-TG occupied approximately 80% of delta total TG. Significantly high correlation was shown between delta TG and delta RLP-TG, but shown lesser correlation between delta TG and delta RLP-C.

significantly different ($p<0.0001$). The increase of RLP-TG (postprandial minus fasting RLP-TG) contributed to approximately 80% of the increase of total TG (total postprandial minus fasting TG) and the correlation between these increases ($r^2 = 0.92$; $p<0.0001$; Fig. 3) was the same as the correlation between postprandial TG and RLP-TG (Fig. 2). Increases of RLP-C (postprandial minus fasting RLP-C) were less well correlated with the increase of TG (postprandial minus fasting TG) ($r^2 = 0.72$; $p<0.0001$) (Fig. 3), compared with RLP-TG.

4. Discussions

The plasma TG level is known to reflect VLDL ($d<1.006$) level strongly, regardless of the fasting and postprandial states [9]. The correlation between total TG and VLDL-C is known to be above $r = 0.95$ as VLDL measurement always moves parallel with TG. Furthermore, one fifth of total TG level is often used clinically as VLDL-C level. However, VLDL-C has not been recognized as an established CHD risk as RLP-C [12,13] because of the inconsistent results in many clinical studies. The clinical significance of plasma TG and VLDL-C has often shown very similar inconsistency as a risk for CHD, especially in the fasting state.

The increase of postprandial TG has been long known as the increase of remnant lipoproteins in plasma [14]. However, the precise comparative studies between increased TG or VLDL and remnant lipoproteins in the fasting and postprandial states were not reported yet. Therefore, the direct correlations between increased TG or VLDL-C and remnant lipoproteins, a part of VLDL which may increase or decrease separately from total VLDL, were studied in this manuscript.

Postprandial TG and remnant lipoprotein concentrations were shown to be significantly more correlated when compared with fasting TG vs RLP concentrations. These results indicated that the amount and ratio of remnant lipoproteins in the postprandial VLDL increased significantly compared with those in the fasting VLDL. Therefore, the correlation between RLP and TG in the fasting vs postprandial state was significantly different. In particular, the increase of postprandial RLP-TG concentrations compared with fasting RLP-TG concentrations contributed to approximately 80% of the increase of postprandial total TG concentrations compared with total fasting TG concentrations. Marcoux et al. [6], Ooi et al. [7] and Nakajima et al. [15] previously reported the similar results in small number of Caucasian and Japanese healthy volunteers, in whom approximately 60–80% of RLP-TG in total TG increased in 3–6 h after fat loading. These results have provided the direct proof that most of the increased TG in the postprandial state was consisted of remnant lipoproteins. The other 20–30% of increased TG may consist of increased HDL-TG and LDL-TG in the postprandial state [16].

The postprandial increase of TG consists of increased levels of TG-rich lipoproteins (TRL or VLDL; $d<1.006$) containing apoB-48 and apoB-100 carrying particles. The increase in the number of RLP apoB-100 particles (VLDL remnants) is actually far greater than that of apoB-48 containing lipoproteins (chylomicron remnants) in the postprandial state [17,18]. Of note, the accumulation of large TRL apoB-100 particles seems to be a particular characteristic for hypertriglyceridemic patients with coronary heart disease (CHD) compared with healthy hypertriglyceridemic subjects, suggesting a link between accumulation of large VLDL and development of atherosclerosis [19,20].

The Framingham Offspring Study and other studies have reported the differences in clinical significance between TG and remnant lipoproteins in the fasting state [4,20–22]. Namely, fasting remnant lipoproteins are shown to be significantly more predictive of cardiovascular events than fasting TG concentrations. These studies suggest that when samples are collected in the fasting state, measurement of remnant lipoprotein levels, especially RLP-C, may also be necessary in addition to TG. These results revealed the same conclusion with the recent JAMA studies that triglycerides measured in fasting samples were less sensitive than the measurements of non-fasting triglyceride concentrations in predicting the risk of cardiovascular events. Given the high correlations between postprandial RLP and postprandial total TG we have demonstrated (Fig. 2), measurement of postprandial TG could obviate the need for measuring remnant lipoproteins.

However, in the Honolulu Heart Study [23] it was reported that RLP levels did not provide additional information about CHD incidence over and above total triglyceride levels. Therefore, this study did not support the need for testing of remnants if measures of fasting triglycerides are available. This study reported that, unlike the studies above [4,20–22], the fasting TG in Honolulu Heart Study group was shown to be an independent predictor of CHD incidence as well as RLP-C. Therefore, Imke et al. [23] mentioned that RLP-C and TG showed the same clinical significance in the fasting state and not necessary to measure the same parameter duplicate. However, this discussion was not based on the fact that RLP occupies proatherogenic fraction of TG-rich lipoproteins [13] and varies differently in lipid disorders, which is the same relationship between TC and LDL-C. Although this study provided the criticism for the measurement of remnant lipoproteins, the meanings of fasting TG should be discussed more together with the recent results of prospective studies reported previously [1,2].

In summary, these results support the conclusion that non-fasting TG levels more strongly and directly reflect remnant lipoprotein concentrations than fasting TG levels [1]. The correlations between postprandial TG and remnant lipoprotein levels were significantly more robust when compared with fasting TG vs remnant lipoprotein levels. The greater predictive values of non-fasting TG concentrations for cardiovascular events have been proved directly by the increased levels of remnant lipoproteins in the postprandial state. Therefore, non-fasting TG measurements performed in 3–6 h after food intake could replace direct measurement of the remnant lipoproteins for the assessment of cardiovascular disease risk. However, the establishment of new cut-off value of non-fasting TG (above 150 mg/dl) may be necessary to use as a marker of CHD risk.

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References

[1] Nordestrgaad BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. JAMA 2007;298:299–308.
[2] Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting tryglycerides and risk of cardiovascular events in women. JAMA 2007;298:309–16.
[3] Iso H, Naito Y, Sato S, et al. Serum triglycerides and risk of coronary heart disease among Japanese men and women. Am J Epidemiol 2001;153:490–9.
[4] McNamara JR, Shah PK, Nakajima K, et al. Remnant-like particle (RLP) cholesterol is an independent cardiovascular disease risk factor in women: results from the Framingham Heart Study. Atherosclerosis 2001;154:229–36.
[5] Tanaka A, Tomie N, Nakano T, et al. Measurement of postprandial remnant-like particles following a fat-loading test. Clin Chim Acta 1998;275:43–52.
[6] Marcoux C, Hopkins PN, Wang T, et al. Remnant-like particle cholesterol and triglyceride levels of hypertriglyceridemic patients in the fed and fasted state. J Lipid Res 2000;41:1428–36.
[7] Ooi TC, Cousins M, Ooi DS, et al. Postprandial remnant- like lipoproteins in hypertriglyceridemia. J Clin Endocrinol Metab 2001;86:3134–42.
[8] Schaefer EJ, McNamara JR, Tayler T, et al. Effects of Atorvastatin on fasting and postprandial lipoprotein subclasses in coronary heart disease patients versus control subjects. Am J Cardiol 2002;90:689–96.
[9] Rifai N, Merrill JR, Holly RG. Postprandial effect of a high fat meal on plasma lipid, lipoprotein cholesterol and apolipoprotein measurements. Ann Clin Biochem 1990;27:489–93.
[10] Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr 1990;51:241–7.
[11] Nakajima K, Saito T, Tamura A, et al. Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apo B-100 and anti apo A-I immunoaffinity mixed gel. Clin Chim Acta 1993;223:53–71.
[12] Twickler TB, Dallinga-Thie GM, Cohn JS, Chapman MJ. Elevated remnant-like particle cholesterol concentration: a characteristic feature of the atherogenic lipoprotein phenotype. Circulation 2004;109:1918–25.
[13] Nakajima K, Nakano T, Tanaka A. Article title: the oxidative modification hypothesis of atherosclerosis: the comparison of atherogenic effects on oxidized LDL and remnant lipoproteins in plasma. Clin Chim Acta 2006;367:36–47.
[14] Zilversmit DB. Atherogenic nature of triglycerides, postprandial lipidemia, and triglyceride-rich remnant lipoproteins. Clin Chem 1995;41:153–8.
[15] Nakajima K, Yamashita S, kanatani K, et al. Characterization of remnant-like particles (abstract in Japanese). J Atheroscler Thromb 2002;34(suppl):192.
[16] Franceschini G, Moreno Y, Apebe P, et al. Alterations in high-density lipoprotein subfractions during postprandial lipidaemia induced by fat with and without ethanol. Clin Sci (Lond) 1988;75:13–9.
[17] Schneeman BO, Kotite L, Todd KM, Havel RJ. Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoprotein B-48 and B-100 to a fat-containing meal in normolipidemic humans. Proc Natl Acad Sci USA 1993;90:2069–73.
[18] Nakajima K, Nakajima Y, Takeichi S, Fujita MQ. ApoB-100 carrying lipoprotein, but not apoB-48, is the major subset of proatherogenic remnant-like lipoprotein particles detected in plasma of sudden cardiac death cases. Atherosclerosis 2007;194:473–82.
[19] Karpe F. Postprandial lipoprotein metabolism and atherosclerosis. J Intern Med 1999;246:341–55.
[20] Nakajima K, Nakajima Y, Takeichi S, Fujita MQ. Plasma remnant-like lipoprotein particles or LDL-C as major pathologic factors in sudden cardiac death cases. Atherosclerosis 2008;198:237–46.
[21] Lamon-Fava S, Herrington DM, Reboussin DM, et al. Plasma levels of HDL subpopulations and remnant lipoproteins predict the extent of angiographically-defined coronary artery disease in postmenopausal women. Arterioscler Thromb Vasc Biol 2008;28:575–9.
[22] Arai H, Yamamoto A, Matsuzawa Y, et al. Polymorphisms of apolipoprotein and methylenetetrahydrofolate reductase in the Japanese population. J Atheroscler Thromb 2007;14:167–71.
[23] Imke C, Rodriguez BL, Grove JS, et al. Are remnant-like particles independent predictors of coronary heart disease incidence? The Honolulu heart study. Arterioscler Thromb Vasc Biol 2005;25:1718–22.