# Association of angiopoietin-like protein 3 with hepatic triglyceride lipase and lipoprotein lipase activities in human plasma

# Association of angiopoietin-like protein3 (ANGPTL3) with HTGL and LPL activities in human plasma

Katsuyuki Nakajima,1,3,4,5　Takamitsu Nakano,1 Yoshiharu Tokita ,1　Takeaki Nagamine,1　Shigeyuki Imamura, 2　Junji Kobayashi, 3　Hiroshi Mabuchi,3　Masumi Ai ,4　Ernst Schaefer, 4.

1. School of Health Sciences, Faculty of Medicine, Gunma University, Maebashi, Gunma, Japan

2. Diagnostics Research & Development Department,  Diagnostic Division, Asahi Kasei Pharma Corporation, Izunokuni City, shizuoka,  Japan

3. Department of Lipidology and Division of Cardiology, Kanazawa University Graduate School of Medical Science, Kanazawa City, Ishikawa, Japan;

4. Lipid Metabolism Laboratory, Jean Mayer United States Department of Agriculture, Human Nutrition Research Center on Aging at Tufts University and Tufts University School of Medicine, Boston, MA, USA

5. Department of Molecular Biosciences., University of California Davis, Davis, CA, USA

Running title: ANGPTL3 and LPL, HTGL activity.

Keywords: Angiopoietin-like protein 3 (ANGPTL3); Lipoprotein lipase (LPL); Hepatic triglyceride lipase (HTGL); Remnant lipoprotein (RLP)-Cholesterol &TG, Small dense LDL-Cholesterol (sdLDL-C).

Correspondence to Dr. Katsuyuki Nakajima, Ph.D

E-mail address: nakajimak05@ybb.ne.jp

Mail address: School of Health Sciences, Faculty of Medicine, Gunma University,

3-39-15 Showa-machi, Maebashi, Gunma 371-8514, Japan

## Abstract

Background: The relationship between plasma angiopoietin-like protein 3 (ANGPTL3), and lipoprotein lipase (LPL) activity and hepatic triglyceride lipase (HTGL) activity has not been investigated in the metabolism of remnant lipoproteins and HDL.

Methods: Angiopoietin-like protein-3 (ANGPTL3), LPL activity, HTGL activity, remnant lipoproteins (RLP-C & RLP-TG), small dense LDL-Cholesterol (sd LDL-C) were measured in 20 overweight and obese subjects in the fasting and postprandial state.

Results: Plasma TG, RLP-C, RLP-TG and sd LDL-C were inversely correlated with LPL activity both in the fasting and postprandial state, but not correlated with HTGL activity and ANGPTL3. However, plasma HDL-C was positively correlated with LPL activity both in the fasting and postprandial state, while inversely correlated with HTGL activity. ANGPTL3 was inversely correlated with HTGL activity both in the fasting and postprandial state, but not correlated with LPL activity.

.Conclusion: Plasma remnant lipoproteins and sdLDL-C were inversely correlated with LPL activity but not correlated with HTGL activity and ANGPTL3, indicating that LPL, but not HTGL, plays a major role in the metabolism of remnant lipoproteins including small dense LDL. However, HDL-C was inversely correlated with HTGL activity and positively correlated with ANGPTL3, indicating that HTGL plays a major role in HDL

metabolism  but not remnant lipoprotein metabolism. These findings suggest that ANGPTL3 is strongly associated with the inhibition of HTGL activity and regulates HDL metabolism, but not associated with the inhibition of LPL activity for the metabolism of remnant lipoproteins in human plasma..

## 1 . Introduction

The regulation of TG-rich lipoprotein metabolism by angiopietin-like protein 3 (ANGPTL3) was first reported by Koishi et al (1) in KK obese mouse which is known to have abnormally high levels of plasma insulin (hyperinsulinemia), glucose (hyperglycemia) and lipids (hyperlipidemia). They observed abnormally low plasma lipid level in one strain (KK/San) which carried the defected hypolipidemia (hypl) locus in the middle of chromosome 4 encoded as ANGPTL3 gene. Over expression of ANGPTL3 or intravenous injection of the purified protein in KK/San mice elicited an increase in circulating plasma lipid levels, especially TG. These data suggested that ANGPTL3 regulates TG-rich lipoprotein metabolism in mice through inhibiting LPL and HTGL activities (2, 3). Therefore, we first paid attention to ANGPTL3 as an inhibitor of LPL activity associated with remnant lipoprotein metabolism in humans. However, Shimamura et al (4 ) and we (5) recently found that plasma ANGPTL3 levels were not correlated with TG levels in human plasma unlike in mice and was shown to be more strongly associated with HDL metabolism. Furthermore, we found that ANGPTL3 was more frequently associated with increased HDL-C rather than CETP deficient cases in plasma of Japanese hyperalphalipoproteinemic subjects, but not associated with TG levels (5).

From these results, we investigated if ANGPTL3 really regulate TG-rich lipoprotein

metabolism through inhibiting LPL and HTGL activities in human plasma as in mice. LPL has been known to hydrolyze chylomicrons (CM) and very low density lipoproteins (VLDL) to form their remnants in human plasma (6,7), and HTGL has been known to hydrolyze β-VLDL or IDL to form small, dense LDL (sd LDL) (8). HTGL is also known to hydrolyze TG in LDL and HDL, especially to hydrolyze HDL2 to form HDL3 (9, 10). Therefore, we speculated that if ANGPTL3 regulate both LPL and HTGL activity to form remnant lipoproteins and sd LDL, the inverse correlations between ANGPTL3 concentration and LPL and HTGL activities could be found in human plasma.

Here, we have analyzed the concentrations of ANGPTL3, TG, remnant lipoproteins, sd LD-C and HDL-C in human plasma, together with LPL and HTGL activity recently developed by Imamura et al (11, 12). For the analysis of LPL and HTGL activities, we used post-heparin plasma in the fasting and postprandial state to measure the lipase activities associated with remnant lipoprotein metabolism. For small dense LDL analysis, we have used small dense LDL-cholesterol (sd LDL-C) assay which is a quantitative assay based on the density rather than particle size (13). The detection method of LPL activity, HTGL activity and sd LDL-C we used in this study are newly developed methods in Japan and different from the previous radioisotope methods reported on these interactions. The analysis was conducted in plasma of 20 overweight and obese volunteers who were generally healthy and susceptible to be postprandial remnant hyperlipoproteinemia, appropriate for the study of interactions among lipase activities and other parameters. Taken together, we have investigated the role of ANGPTL3 on LPL and HTGL activities in remnant lipoproteins, small dense LDL and HDL metabolism in humans and discussed the differences between the mouse studies reported previously (1-3 )..

## 2. Materials and methods

### 2.1 Study subjects

The study in healthy volunteers who were overweight and obese and were susceptible to be postprandial remnant hyperlipoproteinemia was performed in men (n=10) and post-menopausal women (n=10), age 40-71, BMI 25-35 at the University of California, Davis (Table 1). The UC Davis Institutional Review Board approved the experimental protocol, and subjects provided informed consent to participate in the study. All the volunteers were generally healthy and did not take any medications during this study. Bloods were provided to analyze for this study from the fructose and glucose comparison study reported by Stanhope et al (14).

Fasting blood samples were collected at 8:00 h in the morning and postprandial blood samples were collected at 20:00 h in the evening on the same day. During the day, the standardized foods were provided as breakfast, lunch and dinner to all the volunteers The energy content of the meals was based on each subject's energy requirement as determined by the Mifflin equation (15). Each volunteer participated in fasting and postprandial pre and post-heparin blood collections on two different days over a period of 12 weeks. Therefore, 20 volunteers under different conditions at each time were withdrawn two fasting bloods and two postprandial bloods for analysis on different days before and after fructose and glucose treatment. All the data were pooled for a total of 40 pairs of fasting and postprandial time points.

### 2.2. Measurements of lipids, lipoproteins, ANGPTL3 and lipase activities.

The plasma samples for the measurement of TC, TG, HDL-C, LDL-C, RLP-C, RLP-TG

and sd LDL-C were withdrawn immediately before heparin infusion and kept frozen at -80℃ until analysis. As LPL and HTGL activities were not detectable in pre-heparin plasma (12), all the lipase activities in this study were determined in post-heparin plasma. Post-heparin plasma in 15 min after intravenous injection of 50 units of heparin/kg body weight (BW) was withdrawn for the assay of LPL, HTGL activity and ANGPTL3. Plasma samples were kept frozen at -80℃ until analysis. TC and TG concentration were determined enzymatically. LDL-C and HDL-C concentration was measured using a homogenous method (Kyowa Medex, Tokyo). Glucose and insulin were determined by PolyChem (Polymedco, NY). RLP-C and RLP-TG were determined by immunoseparation method (JIMRO II, Otsuka, Tokyo) (16). Small dense LDL-C was determined by the method of Hirano et al (13 ). ANGPTL3 was measured by the ELISA method by Moon et al (5). LPL and HTGL activities were measured by the method of Imamura et al (12)

2.3 Statistical Analysis

Data were analyzed with Dr. SPSS II (SPSS, Chicago, IL).

Quantitative variables are reported as mean ± S.D values. The statistical significance of difference was determined by Mann-Whitney U-test. The correlation between variables is presented as Pearson's correlation coefficient (r-value). $P<0.05$ was statistically significant.

## 3. Results

3.1 Plasma lipids, lipoproteins, LPL, HTGL activity and ANGPTL3 analyses in the fasting and postprandial state.

The fasting and postprandial plasma levels of lipids and lipoproteins in 20 volunteers recruited in UC Davis, CA are shown in Table 2. Mean total cholesterol and LDL-C levels were within normal range and did not change between the fasting and postprandial state. Triglycerides, RLP-C, RLP-TG levels were significantly elevated and sd LDL-C and HDL-C significantly decreased in the postprandial state. LPL and HTGL activities did not change significantly. ANGPTL3 levels decreased significantly in the postprandial plasma compared with in the fasting plasma. Single regression analysis of these parameters in 20 volunteers was shown in Table 3. ANGPTL3 did not correlate significantly with TG and remnant lipoproteins in the fasting and postprandial state, while strongly and inversely correlated with HDL-C.

3.2. TG correlations between LPL activity, HTGL activity and ANGPTL3

Plasma TG concentration was inversely correlated with LPL activity (Figure 1A) both in the fasting ( $r=-0.63$, $P<0.001$) and postprandial ($r=-0.68$, $P<0.001$) state respectively, while no correlation was found with HTGL activity (Figure 1B) both in the fasting ($r=0.04$, NS) and postprandial ($r=0.18$, NS) state. No correlation was found with ANGPTL3 both in the fating and postprandial state (Figure 1C, Table 3).

3.2. RLP-C correlations between LPL activity, HTGL activity and ANGPTL3

RLP-C was inversely correlated with LPL activity (Figure 2A) both in the fasting ( $r=-0.42$, $P<0.001$) and postprandial ($r=-0.58$, $P<0.001$) state respectively, while no

correlation was found with HTGL activity both in the fasting and postprandial state (Figure 2B). No correlation was found with ANGPTL3 in the fasting and postprandial state (Table 3).

3.3. RLP-TG correlations between LPL activity, HTGL activity and ANGPTL3

RLP-TG was inversely correlated with LPL activity(Figure 2 C) both in the fasting ( r=-0.47, P<0.01) and postprandial (r=-0.68, P<0.001) state respectively, and positively correlated with HTGL activity in the postprandial state (r=0.33, p=0.03) (Figure 2 D) （Not correlated in the fasting state, but slightly correlated in the postprandial state）. The positive correlation between RLP-TG and HTGL activity means that HTGL does not hydrolyze RLP-TG. No correlation was found with ANGPTL3 in the fasting and postprandial state(Table 3).

3.4 Correlations of sd LDL-C between LPL activity, HTGL activity and ANGPTL3

Table 3 shows that sd LDL-C was inversely correlated with LPL activity, both in the fasting (r=-0.43, p<0.001) and postprandial (r=-0.35, P<0.05) state respectively, while no correlation was found with HTGL activity both in the fasting and postprandial state. No correlation was found with ANGPTL3 in the fasting and postprandial state. Furthermore, sd LDL-C with TG, RLP-C and RLP-TG were positively correlated both in the fasting (r=0.55 P<0.001; r=0.28, NS; r=0.38, P<0.01 ) and in the postprandial (r=0.38 P<0.01; r=0.33, P<0.05; r=0.27, P<0.05) state, respectively. Although sdLDL-C levels significantly decreased, and RLP-C and RLP-TG levels significantly increased in the postprandial state (Table 2), positive correlations were shown among them.

3.5. HDL-C correlations between LPL activity, HTGL activity and ANGPTL3

HDL-C was positively correlated with LPL activity in the fasting (0.38, $P<0.01$) and postprandial ($r=0.45$, $P<0.01$) (Figure 3A) state and inversely correlated with HTGL in the fasting ($r=-0.49$, $p<0.001$) and in the postprandial state ($r=-0.50$, $P<0.01$) (Figure 3 B). HDL-C was positively correlated with ANGPTL3 in the fasting ($r=0.36$, $p<0.05$) but not in the postprandial ($r=0.32$, NS) state (Figure 3C).

3.6. ANGPTL3 correlations between LPL activity and HTGL activity

As the plasma concentrations of ANGPTL3 were not affected by heparin infusion, ANGPTL3 was determined in post-heparin plasma together with LPL and HTGL activities. ANGPTL3 concentration was not correlated with LPL activity (Figure 4A) both in the fasting ($r=-0.21$, NS) and postprandial ($r=-0.20$, NS) state, however, inversely correlated with HTGL (Figure 4 B) both in the fasting ($r=-0.50$, $p<0.001$) and postprandial ($r=-0.31$, $p<0.05$). These results strongly suggest that ANGPTL3 is not an inhibitor for LPL activity in humans, but an inhibitor for HTGL activity in human plasma.

## 4. Discussion

Remnant lipoproteins have been recognized as the metabolized products of TG-rich lipoproteins by lipoprotein lipase and hepatic triglyceride lipase. ANGPTL3 was considered to be the inhibitor of both LPL and HTGL activity and to cause the delayed metabolism of TG-rich lipoproteins in animal studies (1-3). However, the interactive roles of ANGPTL3 on LPL and HTGL activity in humans have not been reported yet.

In this manuscript, we have shown the correlation between the lipase activities and its inhibitor ANGPTL3 in remnant lipoprotein and HDL metabolism in human plasma, using newly developed LPL, HTGL activity assay method (12). Especially, the previous investigators have all used radioisotope method for LPL and HTGL activity assay developed by Brunzell et al (17) in their studies, which are labor-intensive and known to typically have within and between run coefficients of variation of over 10 %. Our analyses of LPL and HTGL activity were carried with a high through-put activity assay performed on an automated analyzer, with coefficients of variation of 3% or less (11, 12). Therefore it was possible to measure a large number of plasma samples at one time to compare the correlations among these parameters.

The study was conducted in the fasting and postprandial state in 20 healthy volunteers to investigate the relationship between lipase activities and ANGPTL3, mainly in remnant lipoprotein metabolism including small dense LDL.

ANGPTL3 was found as an inhibitor for both LPL and HTGL in mice (1-3). ANGPTL3-null mice revealed markedly low plasma lipid concentrations, especially plasma TG concentration with a normal, high-fat, or high calorie diet. Fujimoto et al (3) reported that lipoprotein lipase and hepatic lipase activities in the post-heparin plasma of ANGPTL3-null mice were 1.57 folds and 1.42 fold higher than those of wild-type mice,

respectively. When ANGPTL3 were over expressed or injected to mice, plasma TG and NEFA levels increased significantly within a few hours, indicating the inhibition of LPL and HTGL activity by ANGPTL3 (3). Therefore, ANGPTL3 has been recognized as an inhibitor for both LPL and HTGL in mice and associated with the metabolism of remnant lipoproteins. ANGPTL3 and TG concentrations in plasma were highly correlated in mice (1-3), however it was not observed in human plasma as reported by Shimamura et al (4) and by us (5).

In this study, we found that the plasma remnant lipoprotein and sd LDL-C concentrations were inversely correlated with LPL activities. Unexpectedly, HTGL activities were not correlated with remnant lipoproteins and sdLDL-C in human plasma, which may be different from the previous literatures (17-21). HTGL has been reported to have a role to hydrolyze small VLDL remnants or intermediate density lipoproteins (IDL) to form sd LDL (20). However, we could not find correlation between HTGL activity and sd LDL-C concentration as well as remnant lipoproteins. Previously, higher HTGL activity was reported to be correlated with higher small dense LDL concentration in plasma (18). Therefore, it has been considered acceptable that inhibition of HTGL activity decreases the formation of small dense LDL and reduces the risk of cardiovascular disease, The determination of small dense LDL in previous studies has been conducted by size or charge separation methods described as pattern A and B (22). However, we used a new detection method of small dense LDL in this study, which determines the cholesterol concentration quantitatively in small dense LDL fraction isolated by the density and particle size separation developed by Hirono et al (13). Using the quantitative sdLDL-C assay method, we could not find any correlation between HTGL activity and sd LDL-C concentration, although we found strong inverse

correlation between LPL activity and sd LDL-C in the same cases, similar with the correlation between LPL activity and RLPs. As it has been known that small dense LDL is positively correlated with remnant lipoproteins in plasma (23), these data stands on the same line that remnant lipoproteins are the precursor of sd LDL and metabolized in the same pathway by LPL,. However unexpectedly, it was not correlated with HTGL activity as reported previously (17-21).

From these data, HTGL seems not to play a significant role in the metabolic pathway of remnant lipoproteins unlike reported previously, but mainly plays a role in HDL metabolism in humans (18). Namely, ANGPTL3 showed a significant inverse correlation with HTGL activity as shown in Figure 4 B and positively correlated with HDL-C concentration (4, 5). Furthermore, Shimamura et al (4 ) and Jin et al (24 ) emphasized the role of ANGPTL3 on the inhibition of endothelial lipase (EL) in HDL metabolism. In this manuscript, we have first shown that ANGPTL3 is strongly associated with HTGL activity and HDL metabolism in human plasma. The inhibition of EL by ANGPTL3 in human plasma is still unknown because of the difficulty of measuring EL activity.

An interesting possibility of different role of ANGPTL3 on LPL activity in humans and mice is the cleavage of ANGPTL3 in plasma. The cleavage of ANGPTL3 by proprotein convertase is reported to be important for the activation of ANGPTL3 to inhibit LPL activity (24). Ono et al (25) reported that a majority of ANGPTL3 detected in mouse plasma was cleaved fragments of N-terminal ANGPTL3 (17-165), while only a small amount of N-terminal fragments with a majority of full length ANGPTL3 were detected in human plasma. They speculated that full length ANGPTL3 is not able to inhibit LPL in vivo unless it is cleaved to N-terminal fragment. Therefore, full length ANGPTL3

which present mainly in human plasma may not play a role for inhibiting LPL activity. We are now studying the role of full length and N-terminal fragment of ANGPTL3 on lipase activities in human plasma.

Probucol is known to decrease plasma HDL-C levels significantly. As a new mechanism of probucol on HDL-C reduction in addition to the previously known mechanisms (26), Miida et al have shown that probucol treatment significantly decreased serum ANGPTL3 concentration (27). Decreasing ANGPTL3 may enhance HTGL or endothelial lipase (EL) activity (4) and decreased HDL-C significantly. Therefore, probucol could increase HTGL activity through the decrease of ANGPTL3. Homma et al (28) reported that probucol markedly reduced plasma HDL2 levels. The reduction rates of TC, TG and apo A-I levels in HDL2 were 43.0%, 43.6% and 47.0%. These results suggested the increased HTGL activity by probucol treatment, possibly through the decrease of ANGPTL3. These findings also suggest that ANGPTL3 does not inhibit LPL activity in human plasma, however it inhibits HTGL or EL activity to increase HDL-C. As plasma ANGPTL3 levels reflect HTGL activity inversely and HDL-C positively, ANGPTL3 could be a therapeutic target for raising HDL-C levels like CETP. Because HTGL activity assay requires heparin infusion before blood withdrawal, ANGPTL3 may take place of HTGL activity assay as a therapeutic target, which is not required heparin-infusion for analysis.

As for LPL inhibitors associated with remnant lipoprotein metabolism, ANGPTL4 reported by Kersten et al (29) or recently discovered glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (30) may inhibit LPL activity directly in human plasma and increase the plasma concentration of remnant lipoproteins, independent from ANGPTL3.

In conclusion, ANGPTL3 was not associated with LPL activity and remnant lipoproteins in human plasma unlike in mice, however ANGPTL3 was strongly associated with HTGL activity. Therefore, ANGPTL3 may be strongly associated with HDL metabolism by inhibiting HTGL activity, but not associated with remnant lipoprotein metabolism in humans.

.Acknowledgement

Authors greatly thank Dr. John Brunzell, University of Washington, Seattle for his valuable discussions and comments and Dr. Peter Havel and Dr. Kimber Stanhope, University California Davis, for providing the clinical samples used for this study (NIH grant: HL-075675).

## 5 References

1. Koishi R, Ando Y, Ono M, Shimamura M,, Yasumo H, Fujiwara T, Horikoshi,H, Furukawa H.Angptl3 regulates lipid metabolism in mice. Nat Genet.2002; 30(2):151-7.

2. Shimizugawa T, Ono M, Shimamura M, Yoshida K, Ando Y, Koishi R, Ueda K, Inada T, Minekawa H, Kodama T, Furukawa H. ANGPTL3 decreases very low density lipoprotein triglyceride clearance by inhibition of lipoprotein lipase. J Biol Chem. 2002; 277: 33742-8.

3. Fujimoto K, Koishi R, Shimizugawa T and Ando Y. Angptl3-null mice show low plasma lipid concentrations by enhanced lipoprotein lipase activity. Exp. Anim. 2006; 55: 27-34.

4. Shimamura M, Matsuda M, Yasumo H, Okazaki M, Fujimoto K, Kono K, Shimizukawa T, Ando Y, Koishi R, Kohama T, Sakai N, Kotani K, Ishida T, hirata k, Yamahsita S, Furukawa H.. Angiopoietin-like protein3 regulates plasma HDL cholesterol through suppression of endothelial lipase. Arterioscler Thromb Vasc Biol. 2007; 27:366-72.1.

5. Moon HD, Nakajima K, Kamiyama K, Takanashi K, Sakurabayashi I,Nagamine T. Higher frequency of abnormal serum angiopoietin-like protein 3 than abnormal cholesteryl ester transfer protein in Japanese hyperalphalipoproteinemics. Clin Chim Acta. 2008; 398: 93-104.

6. Havel RJ. Remnant lipoproteins as therapeutic targets. Review Curr Opin Lipidol. 2000; 11: 615-20.

7. Kobayashi J, Nakajima K, Nohara A, Kawashiri M, Yagi K, Inazu A, Koizumi J, Yamagishi M, Mabuchi M. The relationship of serum lipoprotein lipase mass with fasting serum apolipoprotein B-48 and remnant-like particle triglycerides in type 2 diabetic patients. Horm Metab Res. 2007; 39: 612-6.

8. Dzien A, Breier C, Lisch HJ, Braunsteiner H. Stimulating effect of intermediate-density lipoproteins (IDL) from hyperlipemic plasma on hepatic lipase. Klin Wochenschr. 1986; 64: 530-3.

9. Applebaum-Bowden D, Haffner SM, Hazzard WR The dyslipoproteinemia of anabolic steroid therapy: increase in hepatic triglyceride lipase precedes the decrease in high density lipoprotein2 cholesterol. Metabolism. 1987 ;36(10):949-52

10 Kobayashi J, Kusunoki M, Murase Y, Kawashiri M, Higashi kata T, Miwa K, Katsuda S, Takata M, Asano A, Nohara A, Inazu A, Mabuchi. Relationship of

lipoprotein lipase and hepatic triacylglycerol lipase activity to serum adiponectin levels in Japanese hyperlipidemic men. Horm Metab Res. 2005 ;37:505-9.

11. Imamura S, Kobayashi J, Sakasegawa S, Nohara A, Nakajima K, Kawashiri M, Inazu A, Mabuchi M.. A novel method for measuring human hepatic lipase activity in postheparin plasma. J Lipd Res 2007; 48: 453-7.

12. Imamura S, Kobayashi J, Nakajima K, Sakasegawa S, Nohara A, Noguchi T, Kawashiri M, Inazu a, Deeb SS, Mabuchi M, Brunzell JD. A novel method for measuring human lipoprotein lipase and hepatic lipase activities in postheparin plasma. J Lipid Res. 2008 ; 49:1431-7.

13. Hirano T, Ito Y, Saegusa H, Yoshino G.. A novel and simple method for quantification of small, dense LDL. J Lipid Res. 2003;44:2193-201.

14. Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, Hatcher B, Cox CL, Dyachenko A, Zhang W, McGahan JP, Seibert A, Krauss RM, Chiu S, Schaefer EJ, Ai M, Otokozawa S, Nakajima K, Nakano T, Beysen C, Hellerstein MK, Berglund L, Havel PJ., Effects of consuming fructose- or glucose-sweetened beverages for 10 weeks on lipids, insulin sensitivity and adiposity. J Clin Invest 2009 ;119:1322-34

15. Mifflin, M.D., St Jeor, S.T., Hill, L.A., Scott, B.J., Daugherty, S.A., and Koh, Y.O. A new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr 1990; 51:241-47.

16. Nakajima K, Saito T, Tamura A, Suzuki M, Nakano T, Adachi M, Tanaka A, Tada N, Nakamura H, Campos E, Havel RJ. 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

17. Babirak, S. P., P. H. Iverius, W. Y. Fujimoto, and J. D. Brunzell.. Detection and characterization of the heterozygote state for lipoprotein lipase deficiency. Arteriosclerosis. 1989; 9: 326–334..

18. Zambon A, Deeb SS, Pauletto P, Crepaldi G, Brunzell JD. Hepatic lipase: a marker for cardiovascular disease risk and response to therapy. Curr Opin Lipidol. 2003; 14: 179-89.

19. Deeb SS, Zambon A, Carr MC, Ayyobi AF, Brunzell JD. Hepatic lipase and dyslipidemia: interactions among genetic variants, obesity, gender, and diet. J. Lipid Research 2003; 44:1279-86,

20. Oi K, Hirano T, Sakai S, Kawaguchi Y, Hosoya T. Role of hepatic lipase in intermediate-density lipoprotein and small, dense low-density lipoprotein formation in hemodialysis patients. Kidney Int Suppl. 1999;71: S227-8.

21. Murase T, Itakura H. Accumulation of intermediate density lipoprotein in plasma after intravenous administration of hepatic triglyceride lipase antibody in rats. Atherosclerosis. 1981;39: 293-300.

22. Austin, M. A., J. L. Breslow, G. H. Hennekens, J. E. Buring, W. C. Willett, and R. M. Krauss. Low-density lipoprotein subclass patterns and risk of myocardial infarction. *JAMA.* 1988; 260: 1917–1921.

23. Deighan CJ, Caslake MJ, McConnell M, Boulton-Jones JM, Packard CJ. The atherogenic lipoprotein phenotype: small dense LDL and lipoprotein remnants in nephrotic range proteinuria. Atherosclerosis. 2001; 157: 211-20.

24. Jin W, Wang X, Millar JS, Quertermous T, Rothblat GH, Glick JM, Rader DJ. Hepatic Proprotein convertases modulate HDL metabolism. Cell Metabolism, 2007; 6: 129-136

25. Ono M, Shimizugawa T, Shimamura M, Yoshida K, Noji-Sakikawa C, Ando Y, Koishi R, Furukawa H. Protein region important for regulation of lipid metabolism on

angiopoietin-like 3 (ANGPTL3). J. Biol. Chem. 2003; 278; 41804-41809

26. Yamashita S, Matsuzawa Y .Where are we with probucol: A new life for an old drug? Atherosclerosis. 2009; 207: 16-23.

27. Miida T, Seino U, Miyazaki O, Hanyu O, Hirayama S, Saito T, Ishikawa Y, Akamatsu S, Nakano T, Nakajima K, Okazaki M, Okada M. Probucol markedly reduces HDL phospholipids and elevated prebeta1-HDL without delayed conversion into alpha-migrating HDL: Putative role of angiopoietin-like protein 3 in probucol-induced HDL remodeling. Atherosclerosis. 2008; 200: 329-35.

28.Homma Y, Moriguchi EH, Sakane H, Ozawa H, Nakamura H, Goto Y. Effects of probucol on plasma lipoprotein subfractions and activities of lipoprotein lipase and hepatic triglyceride lipase. Atherosclerosis. 1991;88:175-81

29. Kersten S, Mandard S, Tan NS, Escher P, Metzger D, Chambon P, Gonzalez FJ, Desvergne B, Wahli W. Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene.
J Biol Chem. 2000; 275: 284-90

30. Beigneux AP, Davies BS, Gin P, Weinstein MM, Farber E, Qiao X, Peale F, Bunting S, Walzem RL, Wong JS, Blaner WS, Ding ZM, Melford K, Wongsiriroj N, Shu X, de Sauvage F, Ryan RO, Fong LG, Bensadoun A, Young SG. Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 plays a critical role in the lipolytic processing of chylomicrons. Cell Metab. 2007; 5:279-91.

## Figure legends

Figure 1. TG correlations between LPL activity, HTGL activity and ANGPTL3 in 20 subjects.

Plasma TG was inversely correlated with LPL activity (A). No correlation was found between TG and HTGL activity (B). No correlation was found between TG and ANGPTL3 (C). Eighty plots reflect the total of 4 plots in each volunteer in the fasting (n=40) (open circle) and postprandial (n=40) (closed circle) state, before and after glucose and fructose treatment study during 12 weeks.

Figure 2. RLP-C and RLP-TG correlations between LPL and HTGL activity in 20 subjects

Plasma RLP-C was inversely correlated with LPL activity (A). No correlation was found between RLP-C and HTGL activity (B). Plasma RLP-TG was inversely correlated with LPL activity (C) and positively correlated with ANGPTL3 in the postprandial state(D). Eighty plots reflect the total of 4 plots in each volunteer in the fasting (n=40) (closed circle) and postprandial (n=40) (open circle) states, before and after glucose and fructose treatment study during 12 weeks.

Figure 3 HDL-C correlations between LPL activity, HTGL activity and ANGPTL3 in 20 subjects.

HDL-C was positively correlated with LPL activity (A) and inversely correlated with HTGL (B). HDL-C was positively correlated with ANGPTL3 in the fasting state (C). Eighty plots reflect the total of 4 plots in each volunteer in the fasting (n=40) (open

circle) and postprandial (n=40) (closed circle) states, before and after glucose and fructose treatment study during 12 weeks.

Figure 4. ANGPTL3 correlations between LPL and HTGL activity in 20 subjects. ANGPTL3 concentration was not correlated with LPL activity (A) both in the fasting and postprandial state, however inversely correlated with HTGL (B) both in the fasting ($r=-0.50$, $p<0.001$) and postprandial ($r=-0.31$, $p<0.05$). Eighty plots reflect the total of 4 plots in each volunteer in the fasting (n=40) (open circle) and postprandial (n=40) (closed circle) states, before and after glucose and fructose treatment study during 12 weeks.

Table 1. Demographic data of the 23 volunteers

| | Male (n=12) | Female (n=11) |
|---|---|---|
| Age | 53±4 | 53.6±2 |
| BMI | 29±1 | 29.6±1 |
| aist Circumference(cr | 101±2 | 91.5±6 |
| Body Fat (%) | 29±1 | 41±2 |
| olic Blood Pressure(mr | 120±2 | 117±2 |
| colic Blood Pressure(m | 77±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±3 |
| Insulin(μU/ml) | 15±2 | 15±3 |

The values presented are mean ±SD

Table 2. Plasma Lipid, lipoproteins, lipases and ANGPTL3 levels 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 < 0.0001 |
| RLP-TG(mg/dl) | 32±27 | 96±71 | P < 0.0001 |
| LPL(U/L) | 90±38 | 87±40 | NS |
| HTGL(U/L) | 296±117 | 318±127 | NS |
| ANGPTL3(ng/ml) | 199±62 | 151±56 | P < 0.0001 |

Table 3. Single Regression Analysis of the parameters in the fasting and postprandial state.

**Fasting**

| | r | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TG | | RLP-C | | RLP-TG | | sdLDL-C | | HDL-C | | LPL | | HTGL | | ANGPTL3 | |
| TG | – | | 0.718 | *** | 0.882 | *** | 0.564 | *** | −0.374 | ** | −0.429 | ** | −0.057 | | 0.153 | |
| RLP-C | 0.718 | *** | – | | 0.911 | *** | 0.279 | | −0.194 | | −0.274 | | −0.010 | | 0.198 | |
| RLP-TG | 0.882 | *** | 0.911 | *** | – | | 0.428 | ** | −0.229 | | −0.246 | | 0.018 | | 0.158 | |
| sdLDL-C | 0.564 | *** | 0.279 | | 0.428 | ** | – | | −0.355 | ** | −0.221 | | −0.101 | | −0.117 | |
| HDL-C | −0.374 | ** | −0.194 | | −0.229 | | −0.355 | ** | – | | 0.331 | * | −0.488 | *** | 0.361 | ** |
| LPL | −0.429 | ** | −0.274 | * | −0.246 | | −0.221 | | 0.331 | * | – | | 0.076 | | −0.129 | |
| HTGL | −0.057 | | −0.010 | | 0.018 | | −0.101 | | −0.488 | *** | 0.076 | | – | | −0.497 | *** |
| ANGPTL3 | 0.153 | | 0.198 | | 0.158 | | −0.117 | | 0.361 | ** | −0.129 | | −0.497 | *** | – | |

**Postprandial**

| | r | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TG | | RLP-C | | RLP-TG | | sdLDL-C | | HDL-C | | LPL | | HTGL | | ANGPTL3 | |
| TG | – | | 0.863 | *** | 0.954 | *** | 0.435 | ** | −0.419 | ** | −0.384 | ** | 0.094 | | 0.000 | |
| RLP-C | 0.863 | *** | – | | 0.860 | *** | 0.363 | ** | −0.319 | * | −0.400 | ** | −0.053 | | 0.218 | * |
| RLP-TG | 0.954 | *** | 0.860 | *** | – | | 0.330 | * | −0.422 | ** | −0.418 | ** | 0.238 | | −0.044 | |
| sdLDL-C | 0.435 | ** | 0.363 | ** | 0.330 | * | – | | −0.299 | | −0.209 | | −0.195 | | −0.116 | |
| HDL-C | −0.419 | ** | −0.319 | ** | −0.422 | ** | −0.299 | * | – | | 0.349 | * | −0.469 | *** | 0.243 | |
| LPL | −0.384 | ** | −0.400 | ** | −0.418 | ** | −0.209 | | 0.349 | * | – | | −0.193 | | −0.268 | * |
| HTGL | 0.094 | | −0.053 | | 0.238 | | −0.195 | | −0.469 | *** | −0.193 | | – | | −0.254 | |
| ANGPTL3 | 0.000 | | 0.218 | | −0.044 | | −0.116 | | 0.243 | | −0.268 | * | −0.254 | | – | |

*; $p < 0.05$, **; $p < 0.01$, ***; $p < 0.001$

Fig. 1

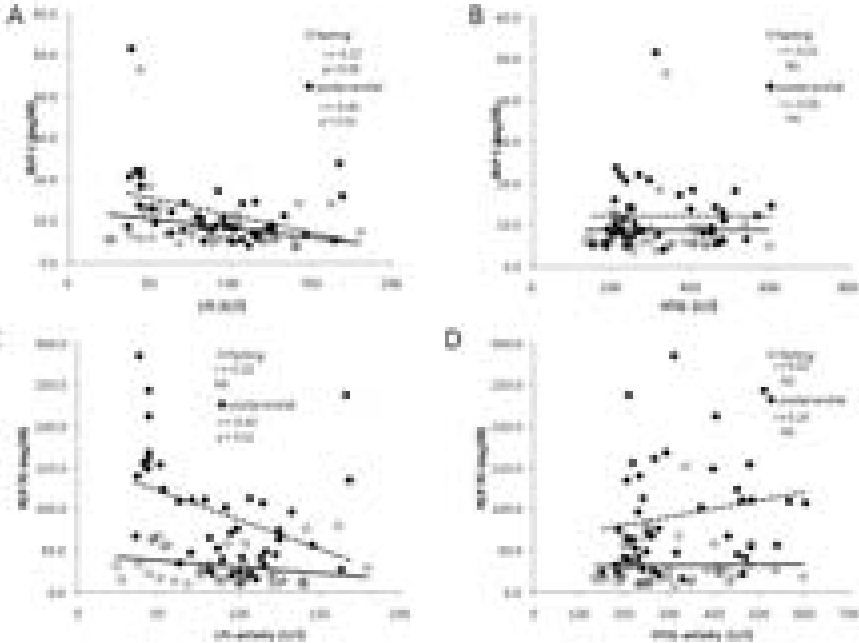
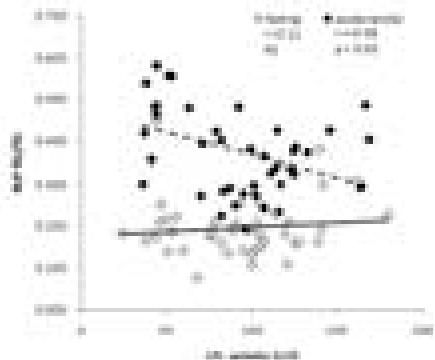


Fig.2



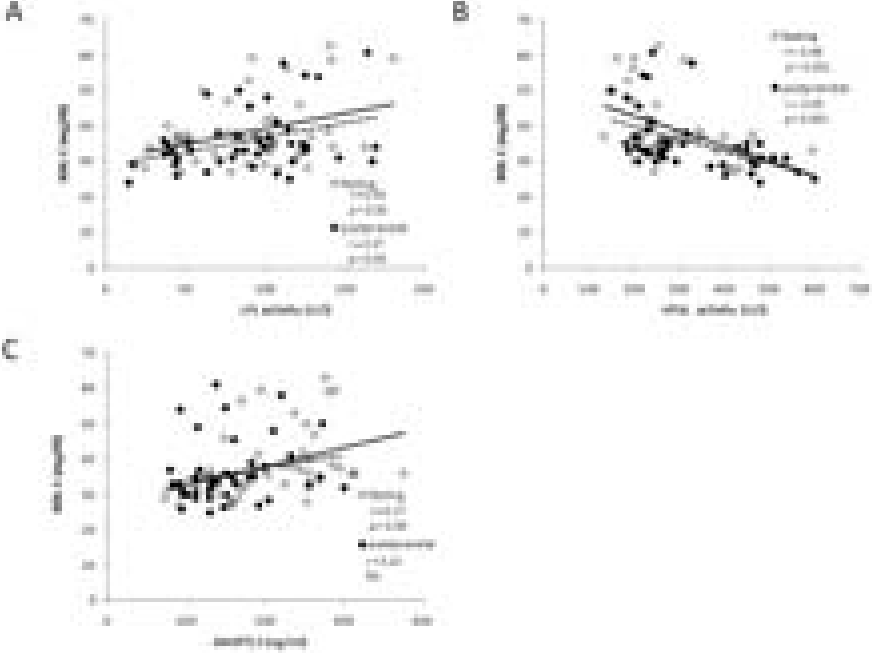

A
B
C

Fig. 4