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Association Between Postprandial Remnant-like Particle Triglyceride (RLP-TG) Levels and Carotid Intima-Media Thickness (IMT) in Japanese Patients with Type 2 Diabetes

Assessment by Meal Tolerance Tests (MTT)

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Our study evaluated the relationship between the pathologic changes associated with atherosclerosis, as primarily represented by postprandial remnant-like lipoproteins and carotid intima-media thickness (IMT), in type 2 diabetic patients. Meal tolerance tests (MTT) were performed in 68 patients with type 2 diabetes. The subjects were divided by pre-meal and 2-h postprandial triglyceride (TG) levels into the normotriglyceridemia (NTG) group; the postprandial hypertriglyceridemia (PHTG) group; and the fasting hypertriglyceridemia (FHTG) group. HOMA-R values were significantly higher in the FHTG group than in the NTG group, with the plasma pre-heparin LPL mass and serum adiponectin levels in the FHTG and PHTG groups significantly lower than in the NTG group. One- and two hour postprandial RLP-TG levels were significantly higher in the PHTG group than in the NTG group, while there was no significant difference in postprandial glucose levels between the two groups. The IMT values were significantly higher in both the FHTG and PHTG groups than in the NTG group. Logistics regression analysis of the 1- and 2-h RLP-TG values using IMT as an induced variable showed the odds ratio for high IMT values to be 5.17 ($p < 0.05$) for the 1-h RLP-TG values and 3.01 ($p = 0.105$) for the 2-h RLP-TG values. Our study results suggest that delayed TG metabolism leading to the retention of remnants in type 2 diabetic patients appears to be closely associated with atherosclerosis, and that postprandial hyperlipidemia is an independent risk factor for the early onset of atherosclerosis.

Key Words: Carotid intima-media thickness; remnant; postprandial hypertriglyceridemia; adiponectin; high-sensitive CRP.

Introduction

Patients with type 2 diabetes have latent postprandial hyperlipidemia despite the apparent absence of hyperlipidemia under fasting conditions. This postprandial elevation of triglyceride (TG) levels is assumed to trigger vascular endothelial disorders *(1)* by way of oxidative stress, while at the same time inducing the retention of intermediate density lipoproteins (IDL) *(2)* and remnant fractions *(3)*, the development of small-dense LDL *(4)*, and, ultimately, the sole reduction of HDL, leading to further progression of atherosclerosis. Of these risk factors, remnants—intermediate metabolites produced as a result of the hydrolysis of chylomicrons and VLDL by lipoprotein lipase—are reported to be a useful indicator of postprandial hyperlipidemia *(5)* and insulin resistance *(6,7)*, as well as a risk factor for cardiovascular disease *(8,9)*. Our study thus investigated the relationship between the pathology of hyperlipidemia, as reflected in the status of postprandial remnants and carotid intima-media thickness (IMT) in type 2 diabetic patients, using meal tolerance tests (MTT).

Results

Relationship Between Postprandial TG Levels and Carotid IMT

Postprandial TG levels in all subjects undergoing meal tolerance tests (MTT) peaked 2 h after meals. In addition, 42 subjects whose fasting TG levels were below 1.68 mmol/L were classified into four groups according to their 2-h postprandial TG levels—those with TG < 1.68; those with 1.68 ≤ TG < 2.24; those with 2.24 ≤ TG < 2.80; and those with TG ≥ 2.80—to compare IMT between these groups. As a result, IMT thickening was observed with increasing

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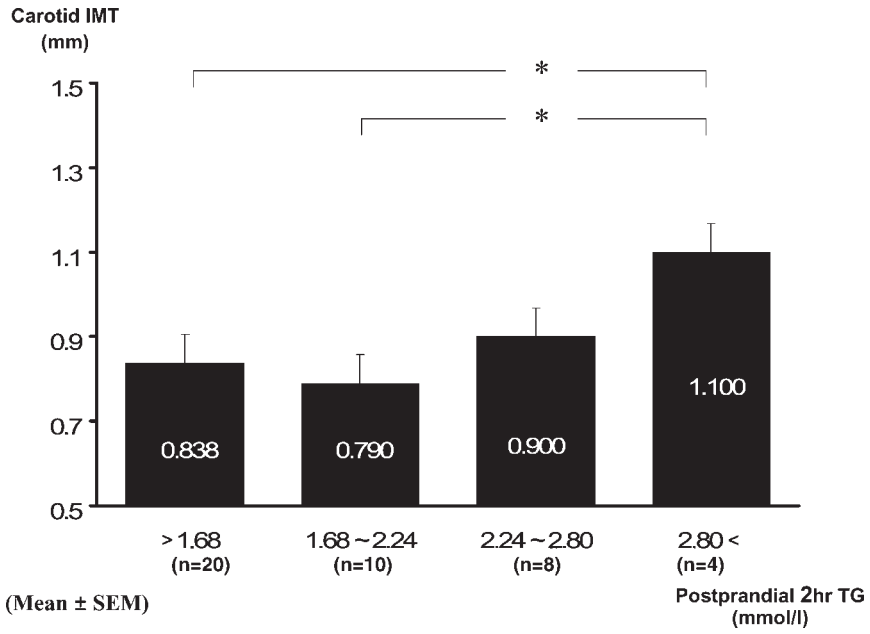

Fig. 1. Association between postprandial TG levels and carotid IMT in patients with a fasting TG level of < 1.68 mmol/L. *$p < 0.05$.

2-h postprandial TG levels and IMT values were significantly higher in those with TG ≥ 2.80 than those with TG < 1.68 ($p < 0.05$) and than those with 1.68 ≤ TG < 2.24 ($p < 0.05$) (Fig. 1). Furthermore, when the subjects were divided by 2-h postprandial TG levels above and below 1.68, 2.24, and 2.80, IMT values were 0.84 ± 0.04 and 0.89 ± 0.04 ($p = 0.40$), respectively, in those with a TG < 1.68 ($n = 20$) and in those with TG ≥ 1.68 ($n = 22$); 0.82 ± 0.03 and 0.97 ± 0.05 ($p < 0.05$), respectively, in those with TG < 2.24 ($n = 30$) and in those with TG ≥ 2.24 ($n = 12$); 0.84 ± 0.03 and 1.10 ± 0.12 ($p = 0.13$), respectively, in those with TG < 2.80 ($n = 38$) and in those with TG ≥ 2.80 ($n = 4$), suggesting that IMT values became significantly greater only when the subjects were divided by the 2-h postprandial TG threshold of 2.24. These results were used to classify the subjects into the following three groups based on their TG levels before meals and 2 h after meals: the normotriglyceridemia group (NTG Group; $TG_{0\ min} < 1.68$ mmol/L, $TG_{120\ min} < 2.24$ mmol/L), the postprandial hypertriglyceridemia group (PHTG Group; $TG_{0\ min} < 1.68$mmol/L, $TG_{120\ min} \geq 2.24$ mmol/L), and the fasting hypertriglyceridemia group (FHTG Group; $TG_{0\ min} \geq 1.68$ mmol/L, $TG_{120\ min} \geq 2.24$ mmol/L).

Clinical Profile of Each Group

Table 1 shows the clinical profile of each group. The proportion of men in each group rose from the NTG group to the PHTG group then to the FHTG Group. No significant differences were seen among these groups in terms of BMI or visceral fat area or in terms of systolic and diastolic pressure, HbAlc values or LDL cholesterol levels. Mean values of their blood pressure and LDL cholesterol showed that their blood pressure and LDL cholesterol levels were well controlled. These groups included patients who were on oral hypoglycemic drugs (α-glucosidase inhibitors and fast-acting insulin secretagogic agents); 37%, 23%, and 48% of the subjects in each group were given oral antihypertensive drugs, approx 70% of these being either antigotensin-receptor blockers (ARB) or angiotensin-converting enzyme (ACE) inhibitors; and 33%, 23%, and 32% of the subjects in each group were given oral lipid-lowering drugs, all of these being statins.

Laboratory Test Findings in Each Group

HOMA-R values were significantly higher in the FHTG group than in the NTG group, and also tended to be higher in the PHTG group although it was not significant. Likewise, pre-heparin LPL mass was significantly lower in both the FHTG and PHTG groups than in the NTG group (Table 1).

In MTT, the FHTG group showed significantly higher TG values than the NTG group at all measurement points, before meals, and 1, 2, 4, and 6 h after meals. On the other hand, the PHTG group showed significantly higher TG values than the NTG group, before meals, and 1, 2, and 4 h after meals (Fig. 2).

As with TG, the FHTG group showed significantly higher RLP-TG values at all measurement points, before meals, and 1, 2, 4, and 6 h after meals, while the PHTG group showed significantly higher RLP-TG values than the NTG group 1 and 2 h after meals (Fig. 2). The area under the TG curve (mmol·h/L) was 7.7 ± 0.3, 12.0 ± 1.0, and 16.7 ± 0.9, respectively, in the NTG, PHTG, and FHTG groups, while the area under the RLP-TG curve (mmol·h/L) was 2.0 ± 0.1, 3.8 ± 0.6, and 5.2 ± 0.5, respectively, in the NTG, PHTG,

Table 1
Patient Profile in Normotriglyceridemic (NTG),
Postprandial Hypertriglyceridemic (PHTG), and Fasting Hypertriglyceridemic (FHTG) Groups

	NTG	PHTG	FHTG
Number (female/male)	30 (16/14)	12 (4/8)	26 (11/15)
Age	58.5 ± 3.0	53.9 ± 4.7	65.3 ± 3.0
BMI	27.6 ± 0.9	27.0 ± 1.6	26.0 ± 0.9
Visceral fat area (cm^2)	136.5 ± 12.5	140.2 ± 16.6	121.7 ± 12.2
Subcuteneous fat area (cm^2)	197.7 ± 22.3	203.8 ± 41.1	144.6 ± 18.0
Systolic BP (mmHg)	128.7 ± 1.9	131.2 ± 3.0	128.4 ± 1.4
Diastolic BP (mmHg)	75.8 ± 1.6	76.1 ± 2.3	77.0 ± 1.5
HbA1c (%)	7.19 ± 0.29	7.44 ± 0.52	7.35 ± 0.3
HOMA-R	1.86 ± 0.22	2.24 ± 0.50	4.87 ± 0.63***
LPL (ng/mL)	50.7 ± 2.64	31.5 ± 4.63*	37.5 ± 2.43**
TC (mmol/L)	5.22 ± 0.20	5.57 ± 0.30	5.55 ± 0.18
TG (mmol/L)	1.01 ± 0.05	1.38 ± 0.08	2.21 ± 0.10***
HDL-C (mmol/L)	1.44 ± 0.07	1.29 ± 0.09	1.19 ± 0.06
LDL-C (mmol/L)	3.27 ± 0.20	3.64 ± 0.29	3.34 ± 0.18
hs CRP (ng/mL)	2088 ± 413	2396 ± 756	2306 ± 697
Adionectin (μg/mL)			
Male	6.23 ± 1.24	4.50 ± 0.31	5.54 ± 0.69
Female	10.8 ± 0.60	7.63 ± 0.54*	5.45 ± 0.88***
Smoking	5/30 (17%)	5/12 (42%)	3/26 (12%)
Duration of DM (year)	6.9 ± 0.9	5.2 ± 1.9	7.9 ± 1.6
Treatment of DM (diet/OHA)	20/10	10/2	19/7
Treatment of hypertention (medication)	11/30 (37%)	2/12 (17%)	13/26 (50%)
Treatment of hyperlipidemia (medication)	10/30 (33%)	3/12 (25%)	8/26 (31%)
IMT (mm)	0.822 ± 0.03	0.967 ± 0.05*	1.000 ± 0.04*

Mean ± SEM, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs NTG.

and FHTG groups, indicating that not only the FHTG group but also the PHTG group showed significantly higher RLP-TG values ($p < 0.001$, $p < 0.01$) than the NTG group. On the other hand, in MTT, the FHTG group showed significantly elevated plasma glucose levels than the NTG group before meals. A trend for elevated glucose levels was also observed 1, 2, 4, and 6 h after meals, although no significant differences were seen between the NTG group and the PHTG group at any measurement points (Fig. 2). The area under the plasma glucose curve (mmol·h/L) was 56.1 ± 3.0, 52.0 ± 2.3, and 68.3 ± 4.3 in the NTG, PHTG, and FHTG groups. While the FHTG Group showed a significantly higher value ($p < 0.05$) than the NTG group, no significant differences were seen between the PHTG and the NTG groups. While no significant intergroup differences were seen in high-sensitive CRP (hs-CRP), elevated hs-CRP levels were noted as the subjects progressed from NTG to PHTG, then to FHTG. Conversely, adiponectin levels were found to be significantly lower in both the FHTG and PHTG groups compared to the NTG group in the female patients (Table 1). The FHTG and PHTG group showed significantly higher carotid IMT values than the NTG group, while no significant differences were seen between the PHTG and FHTG groups (Table 1).

A logistic regression analysis of all test items (continuous variables) using IMT ≥ 1.1 mm as the target variable showed that there was a significant ($p < 0.05$) correlation between 1- and 2-h postprandial RLP-TG levels. Additionally, to determine the cut-off value for the 1- and 2-h postprandial RLP-TG levels, an ROC analysis of RLP-TG was conducted. As a result, the 1-h postprandial RLP-TG level of 50 mg/dL was associated with maximum sensitivity and specificity (sensitivity: 73%; specificity: 66%; concordance rate: 67.2%); and the 2-h postprandial RLP-TG level of 76 mg/dL was associated with maximum sensitivity and specificity (sensitivity: 64%; specificity: 64%; concordance rate: 63.8%). These values were used to perform a logistic regression analysis of the 1- and 2-h RLP-TG levels using IMT ≥ 1.1 as the target variable. This analysis showed the crude odds ratio for patients with high 1- and 2-h postprandial RLP-TG levels for IMT ≥ 1.1 and for those with low 1- and 2-h RLP-TG levels for IMT ≥ 1.1 was 5.167 ($p < 0.05$) and 3.088 ($p = 0.105$), respectively (Table 2). The odds ratios, when adjusted for age, were 8.575 ($p < 0.05$) and 2.570 ($p = 0.193$); when adjusted for sex, the odds ratios were 4.668 ($p < 0.05$) and 2.998 ($p = 0.123$); and when adjusted for age and sex, the odds ratios were 7.875 ($p < 0.05$) and 2.442 ($p = 0.250$) (Table 2).

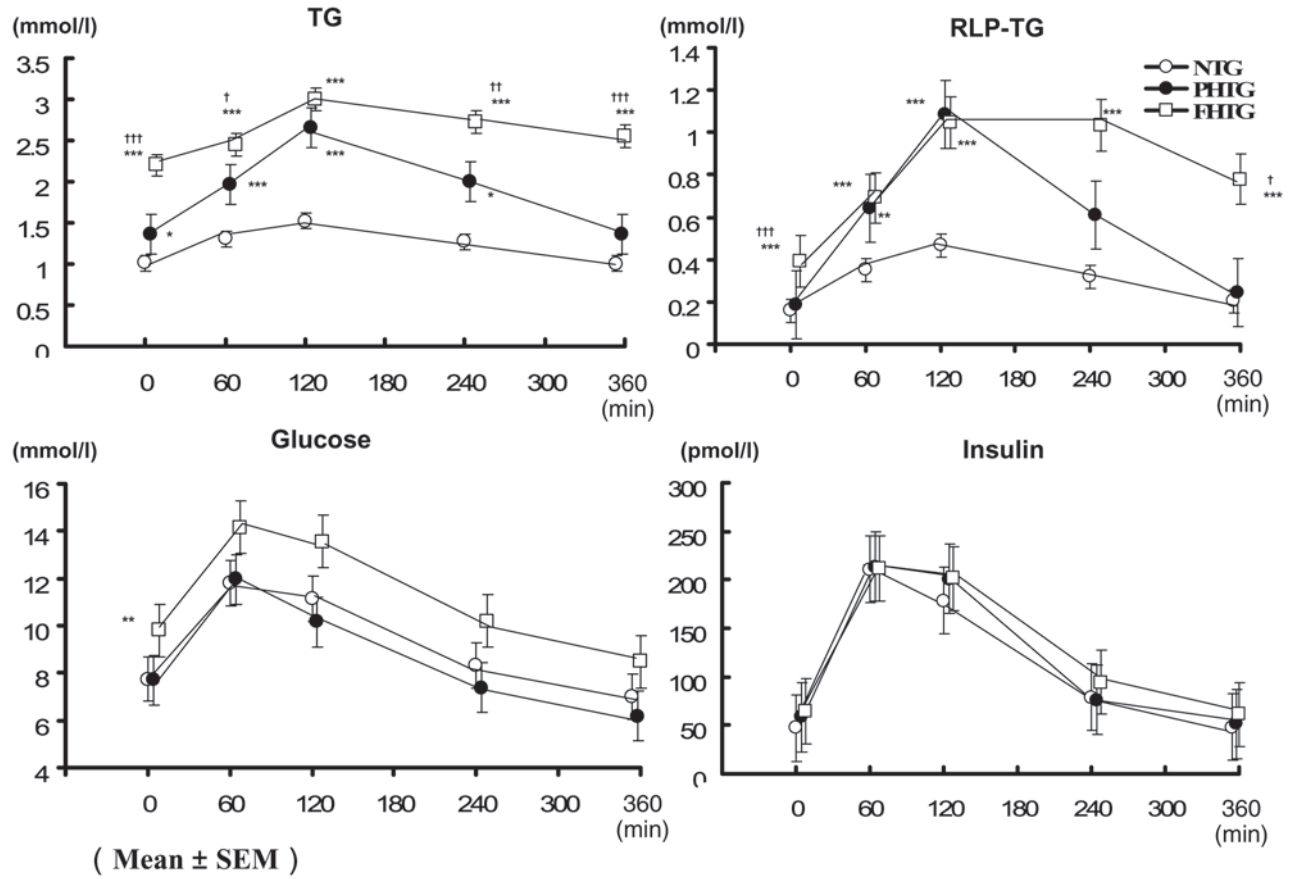

Fig. 2. Changes in postprandial TG and RLP-TG levels, and changes in postprandial plasma glucose levels and insulin values in the NTG, PHTG, and FHTG groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs NTG; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs PHTG (NTG, normotriglyceridemic; PHTG, postprandial hypertriglyceridemic; FHTG, fasting hypertriglyceridemic).

Table 2
Odds Ratio for Patients with High 1- and 2-h Postprandial RLP-TG Levels for IMT ≥ 1.1 and for Those with Low 1-and 2-h RLP-TG Levels for IMT ≥ 1.1

	Odds ratio	*p* value	CI
1-h postprandial RLP-TG			
Crude	5.167	0.0272	1.203–22.198
Adjusted for age	8.575	0.0124	1.592–44.171
Adjusted for sex	4.668	0.0445	1.038–20.983
Adjusted for age and sex	7.875	0.0224	1.339–46.323
2-h postprandial RLP-TG			
Crude	3.088	0.1054	0.789–12.049
Adjusted for age	2.570	0.1928	0.621–10.634
Adjusted for sex	2.998	0.1225	0.744–12.080
Adjusted for age and sex	2.442	0.2498	0.534–11.168

Discussion

In this study, HOMA-R values were shown to be significantly higher in the FHTG group than in the NTG group, while pre-heparin LPL mass and adiponectin values were shown to be low in both the FHTG and PHTG groups. They also showed a significant increase in postprandial RLP-TG levels, as well as in IMT. Logistic regression analysis further revealed that postprandial RLP-TG levels were a significant risk factor for increased carotid IMT. The RLP measured in this study represent lipoproteins that exist in unbound fractions in human serum (RLP fractions) in immunoaffinity mixed gels containing human anti-Apo A-1 and human Apo B-100 monoclonal antibodies, developed to measure the amount of remnant lipoproteins (remnants) in the blood *(10)*. Remnants are defined as intermediate metabolites of TG-rich lipoproteins, such as VLDL and chylomicrons. Under normal conditions, these remnants are rapidly metabolized, and their plasma concentrations remain low. However, there are a variety of pathologies for which elevated remnant levels have been reported, and some reports indicate that RLP can be a useful indicator of postprandial hyperlipidemia *(5)* and insulin resistance *(6,7)*, which are important risk factors for cardiovascular disease.

Lipoprotein lipase (LPL), an enzyme produced by adipose and muscle cells, is present on the surface of vascular endothelial cells, and responsible for the intravascular hydrolysis of triglycerides, where an impaired LPL function is known to result in the onset of hypertriglyceridemia. In past clinical evaluations, LPL production used to be measured and assessed as part of the so-called postheparin plasma lipolytic activity, where LPL is known to be released

from the vascular surface following intravenous heparin administration. In recent years, however, more sensitive LPL quantitation methods have been developed, and helped further clarify that LPL is present, although in a relatively inactive form, prior to intravenous heparin administration *(11)*. Although how preheparin LPL becomes metabolized or in what form it exists remains to be clarified, pre-heparin LPL mass is currently assumed to reflect indirectly the ability of an individual to produce LPL. Additionally, preheparin LPL mass was shown to be lower in remnant-positive cases *(12)*. Krapp et al. *(13)* reported that LPL is a remnant ligand for the VLDL receptor, and enhances the uptake of remnants into cells. Therefore, in the low pre-heparin LPL mass group, remnants may have tended to remain in the blood stream, resulting in the presence of remnant or midband lipoproteins.

Published studies show that postprandial TG is a significant risk factor for increased IMT, suggesting an association between postprandial hyperlipidemia and arteriosclerosis *(14–19)*. We believe our study results provide further support for the current view that, in patients with type 2 diabetes, retention of postprandial remnants caused by delayed triglyceride metabolism in the presence of insulin resistance is closely associated with atherosclerosis.

Currently, despite the pressing need for meal (fat) loading tests as a diagnostic tool for postprandial hyperlipidemia, the details of an appropriate meal load, including its nutritional composition, quality, and amount, as well as the method required for its evaluation, remain to be established. The meal (fat) loads reported in the literature *(20–24)* vary greatly in composition, each being a different mixture of four to nine different dietary ingredients, where the total nutritional contents of a test meal is in the 890–1400 kcal energy range, with lipid-energy ratios of 50–85% and cholesterol accounting for 85–900 mg. These test meals are similarly rich in energy, with a correspondingly high lipid-energy ratio, and are therefore considered inappropriate as a test meal for Japanese subjects. As our study was performed on an outpatient basis, the study employed a commercially available dietary supplement (Caloriemate®, Otsuka, Tokushima, Japan), as it was easy to take, did not offer excessive feeding, and was associated with fewer adverse effects, while at the same time allowing a certain level of fat loading with a lipid-energy ratio of 49%.

In our study, postprandial TG levels were shown to peak in all subjects 2 h after meals. Therefore, we classified 42 subjects whose fasting TG levels were below 1.68 mmol/L according to their 2-h postprandial TG levels into four groups, i.e., those with TG < 1.68; those with 1.68 ≤ TG < 2.24; those with 2.24 ≤ TG < 2.80; and those with TG ≥ 2.80, to compare IMT between these groups. This comparison revealed a tendency for markedly increased IMT, noted in those with a TG level of 2.24 mmol/L or greater. Furthermore, IMT values became significantly different only when divided by the 2-h postprandial TG threshold level of 2.24. Thus, in this study, the 2-h postprandial TG level of 2.24 mmol/L was tentatively used as an indicator for postprandial hyperlipidemia, with the value of 2.24 mmol/L or greater defined as representing postprandial hyperlipidemia. Our study results revealed that postprandial hyperlipidemia was associated with increased IMT, in agreement with the report of Teno et al. *(17)* who studied the relationship between postprandial TG levels and IMT using 2-h postprandial TG levels as an indicator of postprandial hyperlipidemia in type 2 diabetic patients.

In our study, significantly lower adiponectin values were noted only in female subjects in the PHTG and FHTG groups compared to the NTG group. As generally recognized, adiponectin values vary between men and women, thus this may account for our study findings that lower adiponectin values were noted in males compared to females in the NTG group and that there were no significant differences between males in the NTG, PHTG, and FHTG groups in our study.

There were several reports concerning the relationship between hypertriglyceridemia and blood TNF-α levels or TNF-α production *(25,26)*. Therefore, a possibility exists that the increase in VLDL in the PHTG and FHTG group may have led to an acceleration of TNF-α production, and increased the ability of TNF-α to suppress the adiponectin production, thus decreasing the level of adiponectin. On the other hand, experiments in cultured adipocytes have shown that elevated free fatty acid levels lead to an increase in NADPH oxidase, followed by an increase in reactive oxygen species in the presence of decreased antioxidant enzyme activity, where oxidative stress induces an abnormal production of adipocytokines, such as adiponectin, in local adipose tissues *(27)*. While FFA levels in MTT were not investigated in our study, changes in postprandial FFA levels in the PHTG and FHTG groups may have influenced adiponectin production in adipose tissues through oxidative stress.

Again, adiponectin is known to inhibit the expression of the adhesion molecules VCAM-1, ICAM-1, E-selectin, thereby blocking the attachment of monocytes to vascular endothelial cells *(28)*, while at the same time suppressing the expression of the scavenger receptor SRA-1 and keeping macrophages from becoming foamy *(29)*. Additionally, adiponection has been reported to have the ability to inhibit the proliferation and migration of vascular endothelial cells that result from the production of several growth factors and their action *(30)*. Thus, adiponectin is considered a molecule that plays a key role in inhibiting all pathological processes leading to the development of atherosclerosis, suggesting that the presence of hypoadiponectinemia also affected IMT values in both the FHTG and PHTG groups in our study.

Epidemiological insights have been accumulated in recent years into the association between IGT and cardiovascular disease *(31,32)* or IMT *(33)*, as well as the association between postprandial hyperglycemia and cardiovascular disease *(34)*. Logistic regression analysis using IMT ≥ 1.1 mm

as the target variable showed that 1- and 2-h postprandial RLP-TG levels were significantly correlated, while postprandial plasma glucose levels, LDL cholesterol levels, and blood pressure showed no significant correlation. This was thought to be due to the fact that this study included patients who were on oral hypoglycemic agents, statins, and antihypertensive drugs, their mean plasma glucose, LDL cholesterol, and blood pressure levels indicated that their conditions had been well controlled, resulting in no significant correlation seen between these factors and IMT. It was not possible to draw any conclusions regarding this from our study alone, or to compare among the various risk factors examined (postprandial hyperlipidemia, postprandial hyperglycemia, high LDL cholesterolemia, and hypertension) for their potential contribution to increased IMT.

Our study suggests that the delayed metabolism of TG in the presence of insulin resistance in type 2 diabetic patients, which leads to remnants being retained postprandially, is closely associated with carotid IMT.

Patients and Methods

Subjects

Meal tolerance tests (MTT) *(35)* were performed in a total of 68 type 2 diabetic patients who were being treated at our hospital on an outpatient basis. Patients receiving insulin therapy, oral insulin-sensitizing agents, or fibrates, and those with a history of ischemic heart disease and/or stroke were excluded from the study. The study was conducted with the approval of the ethics committee of the National Hospital Organization, Utsunomiya National Hospital, and all subjects gave their informed consent to the protocol.

Protocol

Blood samples were drawn from the subjects early in the morning under fasting conditions, before meals, and 1, 2, 4, and 6 h after meals to measure plasma glucose, insulin, triglyceride, and remnant-like particle (RLP) TG levels, and meal tolerance tests were performed in these subjects. The nutritional composition of the test meal *(35)* was 400 kcal of energy, 8.2 g of protein, 22.2 g of fat, and 41.7 g of carbohydrate, while the fatty acid composition was 39.8% oleic acids, 15.1% palmitic acids, 6.5% stearic acids, and 11.9% linoleic acids. Furthermore, pre-meal blood samples were used to measure the baseline pre-heparin LPL mass, high-sensitive CRP (hs-CRP), and adiponectin levels.

Plasma glucose levels were determined by the glucose dehydrogenase methods, and hemoglobin A1c (HbA1c) was measured by cation exchange high-performance liquid chromatography. The estimate of insulin resistance was based on a homeostasis model assessment (HOMA-R) as described by Matthews et al. *(36)*. Serum lipids (triglycerides, total cholesterol, HDL cholesterol) were measured enzymatically using enzyme reagents (L-Type TG H, Wako Pure Chemicals, Osaka, Japan; Tokyo, L-Type CHO H, Wako Pure Chemicals, Osaka, Japan; Cholestest N HDL, Daiichi Pure Chemicals, Tokyo, Japan). An estimate of the LDL cholesterol concentration was then made from these three measurements using the Friedewald formula when TG levels were below 4.5 mmol/L. Serum RLP were measured by using an immunoabsorption kit (RLP-cholesterol, JIMRO II, Japan Immunoresearch Laboratories, Takasaki, Japan) *(10)*. Pre-heparin LPL mass was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using specific monoclonal antibody against bovine milk LPL, as described by Kobayashi et al. *(37)*. For the assay, a kit from Daiichi Pure Chemicals (Tokyo, Japan) was used. Insulin and adiponectin levels were determined using commercial enzyme immunoassay kits (LS Eiken Insulin Kit, Eiken Chemical, Tokyo, Japan and adiponectin ELISA kit, Otsuka, Tokushima, Japan). High-sensitive C-reactive protein (hs-CRP) was measured by using a latex nephelometry assay (N High Sensitivity CRP, Dade Behring, Marburg GmbH, Marburg, Germany).

Abdominal computed tomography (CT; Hitachi model, CTW550, Hitachi Medical Co., Tokyo Japan) scans at the umbilical level were also performed on all patients at the same time. Abdominal visceral fat area and subcutaneous fat area were measured, as described elsewhere *(38)*.

Measurement of Carotid IMT

Ultrasonography of the carotid arteries was performed with an echotomography system (SSA-370; Toshiba, Tokyo, Japan) and an electrical linear transducer (mid-frequency of 8.0 MHz) as reported previously *(39,40)*. The intima-media thickness (IMT) at the carotid bulb, at the common carotid artery 10 mm proximal to the bulb, and the internal carotid artery 10 mm distal to the carotid bulb were measured from the leading edge of the first echogenic line to that of the second. The first line represented the lumen-intima interface, with the second representing the collagen-containing upper layer of the adventitia. Then, the mean maximum values were estimated. Obvious plaques were excluded from the measurement. The scanning time averaged 30 min, and all images were photographed.

Statistical Analysis

All data were represented as means ± SEM. An estimation of the reliability of intragroup differences was made using one-way analysis of covariance. Scheffe's method was used to adjust for multiple comparisons in the *post hoc* analysis. IMT values as classified by 2-h postprandial TG levels for patients with a fasting plasma TG level of < 1.68 mmol/L were compared and tested for significance using unpaired *t*-test. A p value < 0.05 was considered significant. Using IMT ≥ 1.1 as the target variable, a logistic regression analysis of all test items (continuous variables) was performed to examine these variables for significance. Then, an ROC analysis of 1- and 2-h postprandial RLP-TG values associated with IMT ≥ 1.1 was conducted, and the values at

which sensitivity and specificity were maximal were considered cut-off values. The cut-off values thus established were used to perform multiple logistic analysis, and the relative risk of postprandial RLP-TG levels for IMT ≥ 1.1 was evaluated in terms of odds ratios.

References

1. Ceriello, A., Taboga, C., Tonutti, L., et al. (2002). *Circulation* **106,** 1211–1218.
2. Kasama, T., Yoshino, G., Iwatani, I., et al. (1987). *Atherosclerosis* **63,** 263–266.
3. Watanabe, N., Taniguchi, T., Taketoh, H., et al. (1999). *Diabetes Care* **22,** 152–156.
4. Iwai, M., Yoshino, G., Matsushita, M., et al. (1990). *Diabetes Care* **13,** 792–796.
5. Tanaka, A., Tomie, N., Nakano, T., et al. (1998). *Clin. Chim. Acta* **275,** 43–52.
6. Ai, M., Tanaka, A., Ogita, K., et al. (2000). *J. Clin. Endocrinol. Metab.* **85,** 3557–3560.
7. Ai, M., Tanaka, A., Ogita, K., et al. (2001). *J. Am. Coll. Cardiol.* **38,** 1628–1632.
8. McNamara, J. R., Shah, P. K., Nakajima, K., et al. (2001). *Atherosclerosis* **154,** 229–236.
9. Kugiyama, K., Doi, H., Takazoe, K., et al. (1999). *Circulation* **99,** 2858–2860.
10. Nakajima, K., Saito, T., Tamura, A., et al. (1993). *Clin. Chim. Acta* **223,** 53–71.
11. Olivecrona, G., Hultin, M., Savonen, R., et al. (1995). In: *Atherosclerosis X.* Woodford, F. P., Davignon, J., and Sniderman, A. (eds.). Elsevier, New York, pp. 250–253.
12. Watanabe, H., Miyashita, Y., Murano, T., Hiroh, Y., Itoh, Y., and Shirai, K. (1999). *Atherosclerosis* **145,** 45–50.
13. Krapp, A., Zhang, H., Ginzinger, D., et al. (1995). *J. Lipid Res.* **35,** 2363–2374.
14. Jacqueline, E. R., Howard, G., Craven, T. E., Bond, M. G., Hagaman, A. P., and Crouse, J. R. III. (1992). *Stroke* **23,** 823–828.
15. Karpe, F., de Faire, U., Mercuri, M., Bond, M. G., Hellénius, M.-L., and Hamsten, A. (1998). *Atherosclerosis* **141,** 307–314.
16. Boquist, S., Ruotolo, G., Tang, R., et al. (1999). *Circulation* **100,** 723–728.
17. Teno, S., Uto, Y., Nagashima, H., et al. (2000). *Diabetes Care* **23,** 1401–1406.
18. Chen, X., Tian, H., and Liu, R. (2003). *Chin. Med. J.* **116,** 1933–1935.
19. Iso, H., Naito, Y., Sato, S., et al. (2001). *Am. J. Epidemiol.* **153,** 490–499.
20. Karpe, F., Hellenius, M., and Hamsten, A. (1999). *Metabolism* **48,** 301–307.
21. Guerci, B., Verges, B., Durlach, V., Hadjadj, S., Drouin, P., and Paul, J. (2000). *Int. J. Obesity* **24,** 468–478.
22. Schrezenmeir, J., Keppler, I., Fenselau, S., et al. (1993). *Ann. NY Acad. Sci.* **683,** 302–314.
23. Uiterwaal, C. S. P. M., Grobbee, D. E., Witteman, J. C. M., et al. (1994). *Ann. Intern. Med.* **121,** 576–583.
24. Reznik, Y., Pousse, P., Herrou, M., et al. (1996). *Metabolism* **45,** 63–71.
25. Jovinge, S., Hamsten, A., Tornvall, P., et al. (1998). *Metabolism* **47,** 113–118.
26. Mohrschladt, M. F., Weverling-Rijnsburger, A. W., de Man F. H, et al. (2000). *Atherosclerosis* **148,** 413–419.
27. Furukawa, S., Fujita, T., Shimabukuro, M., et al. (2004). *J. Clin. Invest.* **114,** 1752–1761.
28. Ouchi, N., Kihara, S., Arita, Y., et al. (1999). *Circulation* **100,** 2473–2476.
29. Ouchi, N., Kihara, S., Arita, Y., et al. (2001). *Circulation* **103,** 1057–1063.
30. Arita, Y., Kihara, S., Ouchi, N., et al. (2002). *Circulation* **105,** 2893–2898.
31. DECODE Study Group. (1999). *Lancet* **354,** 617.
32. Tominaga, M., Eguchi, H., Manaka, H., Igarashi, K., Kato, T., and Sekikawa, A. (1999). *Diabetes Care* **22,** 920–924.
33. Hanefeld, M., Koehler, C., Henkel, E., Fuecker, K., Schaper, F., and Temelkova-Kurktschiev, T. (2000). *Diabet. Med.* **17,** 835–840.
34. Hanefeld, M., Fischer, S., Julius, U., et al. (1996). *Diabetologia* **39,** 1577–1583.
35. Ogawa, S., Takeuchi, K., Sugimura, K., et al. (2000). *Metabolism* **49,** 331–334.
36. Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., and Turner, R. C. (1985). *Diabetologia* **28,** 412–419.
37. Kobayashi, J., Hashimoto, H., Fukamachi, I., et al. (1993). *Clin. Chim. Acta* **16,** 113–123.
38. Yoshimizu, T., Nakamura, T., Yamane, M., et al. (1999). *Radiology* **211,** 283–286.
39. Pionoli, P., Tremoli, E., Poli, A., Oreste, P., and Paoletti, R. (1986). *Circulation* **74,** 1399–1406.
40. Mercuri, M., Tang, R., Phillips, R. M., and Bond, M. G. (1996). *Blood Press* **5(Suppl. 4),** 20–23.