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Effects of Nateglinide on the Elevation of Postprandial Remnant-like Particle Triglyceride Levels in Japanese Patients with Type 2 Diabetes Assessment by Meal Tolerance Test

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To elucidate the role of early insulin response in postprandial hyperlipidemia, we examined triglyceride (TG) and remnant-like particle triglyceride (RLP-TG) levels, using a meal tolerance test (MTT) with or without the administration of nateglinide (NAT). The MTTs were performed 2 d apart in 36 drug-naive patients with type 2 diabetes who had been hospitalized for glycemic control while receiving diet therapy. Before the second MTT, patients were treated with 90 mg NAT. Treatment with NAT was associated with a significant increase in insulin levels in the treated patients 1 h after the test meal, compared to levels in non-treatment. NAT treatment was also associated with a significant decrease in the level of free fatty acids 1 and 2 h after the meal, and with a significant decrease in plasma glucose levels 1, 2, and 4 h after the meal, compared to those in non-treatment. During the first MTT with NAT non-treatment, 13 patients showed serum TG levels of 200 mg/dL or greater when measured 2 h after the meal. In these 13 patients, NAT administration produced a significant decrease in TG levels 1, 2, and 6 h after the meal, as well as a significant reduction in RLP-TG levels 1 and 2 h after the meal. NAT administration was also associated with significant reductions in area under the curve (ΔAUC) for TG and RLP-TG. These results suggest that, in a clinical setting, the early insulin response is closely associated with both postprandial glucose and postprandial lipid metabolism in Japanese patients with type 2 diabetes.

Key Words: Nateglinide; remnant-like particle (RLP) triglyceride; type 2 diabetes; postprandial hyperlipidemia; meal tolerance test.

Introduction

Recent epidemiologic evidence suggests that postprandial metabolic abnormalities, such as postprandial hyperglycemia and postprandial hyperlipidemia that characterize type 2 diabetes, have clearly been implicated as risk factors for progression of arteriosclerosis and development of cardiovascular events *(1–3)*. There are also indications that impaired vascular endothelial function is closely related to progression of arteriosclerosis from the very early stages *(4)*. Ceriello and his colleagues *(5)* have reported in their findings an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial function, suggesting oxidative stress as one of the common mediators of such effect.

Remnant-like particle (RLP) intermediate metabolites produced after hydrolysis of the intestinal chylomicron and hepatic very low density lipoprotein (VLDL) by lipoprotein lipase (LPL) present on the vascular endothelial surface may be useful as an indicator of postprandial hyperlipidemia *(6)* and insulin resistance *(7,8)*, as well as being a risk factor for cardiovascular disease *(9,10)*.

Presently, the remnant lipoprotein (CM or VLDL remnant-like particles) is recognized for its similarity to degenerated LDL in humans and laboratory animals, resulting in enhanced uptake by macrophages in the vascular endothelial surface, thereby causing foam cell formation and stimulation of arteriosclorotic lesion growth. The level of this lipoprotein is reported to increase in diabetic patients *(11)*. On the other hand, drugs that stimulate early insulin secretion are expected not merely to make improvements in controlling postprandial sugar level *(12)* but also to reduce postprandial hyperlipidemia *(13)* and are believed to have the potential of arresting arteriosclorosis progression *(14)*.

The present study was designed to investigate the role of early insulin secretion in postprandial triglyceride metabolism in patients with type 2 diabetes in a clinical setting, where meal tolerance testing (MTT) was performed to compare postprandial triglyceride (TG) and remnant-like particle (RLP)-TG levels with or without nateglinide administration.

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Table 1
Changes in Postprandial Insulin, Plasma Glucose, and Free Fatty Acid Levels Following Single-Dose Nateglinide Administration

Meal tolerance test	Time (min)	Without nateglinide	With nateglinide
	0	8.3 ± 1.2	8.3 ± 1.3
	60	36.8 ± 5.8	47.6 ± 7.8*
Insulin (μU/mL)	120	31.7 ± 3.6	34.2 ± 5.3
	240	13.3 ± 1.9	13.89 ± 2.8
	360	8.1 ± 1.2	8.1 ± 1.4
	0	126.3 ± 4.0	125.8 ± 4.2
	60	190.8 ± 9.5	142.3 ± 10.2**
Glucose (mg/dL)	120	171.0 ± 9.6	129.5 ± 7.3**
	240	135.5 ± 7.0	115.9 ± 5.4**
	360	115.3 ± 4.8	112.8 ± 3.7
	0	0.45 ± 0.04	0.44 ± 0.02
	60	0.32 ± 0.03	0.25 ± 0.02**
FFA (mEq/L)	120	0.34 ± 0.03	0.29 ± 0.02*
	240	0.44 ± 0.03	0.49 ± 0.03
	360	0.58 ± 0.04	0.59 ± 0.03
ΔAUC for Insulin, 0–120 min (μU·h/mL)		40.2 ± 6.1	52.2 ± 8.3*
ΔAUC for Glucose, 0–360 min (mg·h/dL)		134.1 ± 23.4	25.9 ± 17.8**
ΔAUC for FFA, 0–120 min (mEq·h/L)		–0.18 ± 0.05	–0.26 ± 0.03
	0	120.5 ± 8.1	124.6 ± 7.3
	60	157.3 ± 9.4	155.5 ± 7.5
TG	120	194.6 ± 14.1	187.9 ± 11.5
	240	160.7 ± 18.3	170.4 ± 15.7
	360	119.3 ± 11.7	119.7 ± 9.9
	0	17.8 ± 1.9	19.1 ± 1.6
	60	43.3 ± 3.7	40.3 ± 2.8
RLP-TG	120	65.0 ± 6.9	60.4 ± 5.5
	240	46.0 ± 9.6	50.0 ± 7.9
	360	24.2 ± 4.0	25.0 ± 3.4

*$p < 0.05$.
**$p < 0.001$.

Results

Insulin levels were significantly higher 1 h after MTT in patients treated with nateglinide than those not treated with nateglinide ($p < 0.05$), while no significant difference was noted between treated and untreated patients (Table 1) 2 h after the meal. Nateglinide administration was also associated with significant reductions in free fatty acid levels 1 and 2 h after the meal ($p < 0.05$–$p < 0.001$), and with significant reductions in plasma glucose levels 1, 2, and 4 h after the meal compared to non-administration subjects ($p < 0.001$) (Table 1). MTT findings after nateglinide treatment showed a significant increase in ΔAUC for insulin levels ($p < 0.05$), and significant reductions in the ΔAUCs for plasma glucose ($p < 0.001$) and free fatty acids ($p < 0.05$) (Table 1). Nateglinide did not show a significant decrease in postprandial TG or RLP-TG in all subjects, including those who showed TG levels of 200 mg/dL or greater ($p > 0.05$). In 13 patients who showed serum TG levels of 200 mg/dL or greater as measured 2 h after the meal in the first MTT without NAT treatment, NAT produced a significant decrease in TG levels 1, 2, and 6 h after the meal, as well as a significant reduction in RLP-TG levels 1 and 2 h after the meal (Fig. 1). NAT treatment was also associated with significant reductions in ΔAUC for changes in TG and changes in RLP-TG.

Discussion

The present study indicates that, in the patients with type 2 diabetes showing postprandial TG elevation, the fast-acting insulin secretagogue nateglinide acts not only to reduce postprandial hyperglycemia but also to reduce postprandial elevation of TG and RLP-TG. In other words, early insulin secretion not only affects postprandial glucose elevation, but is also closely linked to a variety of other pathologies seen

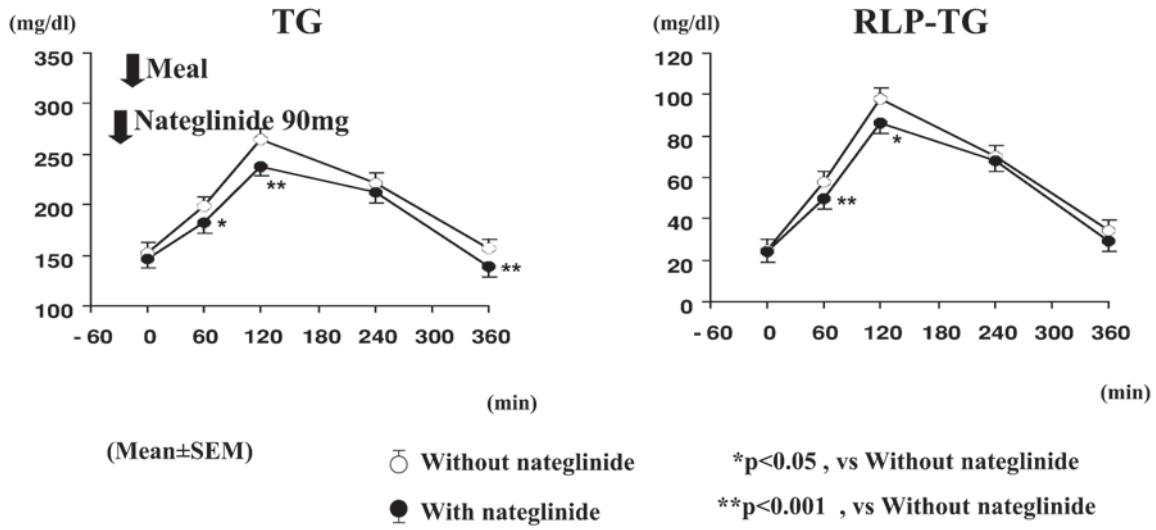

Fig. 1. Changes in postprandial TG and RLP-TG levels following single-dose nateglinide administration. Analysis performed for data from 13 patients who showed postprandial TG of 200 mg/dL or greater 2 h after MTT without nateglinide administration.

in type 2 diabetes, such as postprandial TG elevation. Clearly, improving the early insulin response is likely to be an important therapeutic measure against postprandial hyperglycemia and postprandial hyperlipidemia. On the other hand, Vakkilainen et al. reported that nateglinide had no significant effect on postprandial hyperlipidemia or LDL size in type 2 diabetes patients *(15)*. It is not clear why their findings are in sharp contrast to our results reported here. Notwithstanding, the difference could possibly be due to difference in the amount of fat loaded, difference in responsiveness of insulin secretion to nateglinide, or the fact that we analyzed only those cases in which postprandial triglycerides were elevated beyond a certain degree during a food loading test.

When we investigated the effects of single doses of nateglinide and glibenclamide on TG levels following fat loading, in an OLETF rat model with spontaneous type 2 diabetes, our results showed that only the nateglinide-induced rapid spike of insulin secretion led to the increased expression of LPL mRNA in adipose tissues, and to the decreased TG elevation after fat loading *(16)*. Analysis of the lipoprotein fraction showed reduced levels of both endogenous TG-rich lipoprotein VLDL and exogenous TG-rich lipoprotein chylomicron. Furthermore, a fat loading study in Zucker fatty rats was consistent with our findings that TG levels after fat loading were reduced by nateglinide-induced rapid insulin secretion, accompanied by reductions in VLDL and chylomicron levels *(13)*.

Remnants are defined as intermediate metabolites of TG-rich lipoproteins, such as VLDL and chylomicron. 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 *(6)* and insulin resistance *(7,8)*, which are important risk factors for cardiovascular disease. The nateglinide-induced reduction in postprandial RLP-TG in this study indicates accelerated metabolism of TG-rich VLDL and chylomicron lipoproteins, and is in agreement with the findings in OLETF rats, where the use of nateglinide has been associated with increased expression of LPL mRNA in adipose tissue *(16)*. Also, in the present research, nateglinide administration produced a transient decrease in peripheral free fatty acids, consistent with the study in OLETF rats that showed that nateglinide-induced rapid insulin secretion brought a transient decrease in portal free fatty acids *(17)* and with another clinical study on the food-loading test conducted on type 2 diabetes patients, which showed decrease in free fatty acid level as a result of improved early insulin secretion reducing postprandial blood sugar level and arresting endogenous lipolysis *(18)*.

Alternatively, as mechanisms by which nateglinide inhibits postprandial TG elevation, nateglinide-induced rapid secretion of insulin into the portal blood causes reduction in portal blood free fatty acids, which in turn affects the process of TG synthesis in the liver, while at the same time nateglinide affects the metabolic process of TG-rich lipoproteins through elevation of lipoprotein lipase.

These observations suggest that Nateglinide should be regarded not only as an agent for improvement of postprandial hyperglycemia but also as a drug that has the potential to arrest progression of arteriosclerosis in type 2 diabetes. A large-scale clinical trial, the Nateglinide and Valsartan in Impaired Glucose Tolerance Outcomes Research (NAVIGATOR) study *(19)*, is currently underway to investigate

the effectiveness of nateglinide, vis-à-vis the angiotensin II receptor antagonist valsartan, in preventing the development of type 2 diabetes or cardiovascular disease in IGT patients either 50 yr of age or older with cardiovascular disease or 55 yr of age or older with more than one risk factor for cardiovascular disease. This study may provide further insight into the role of nateglinide in preventing type 2 diabetes and cardiovascular disease.

Patients and Methods

Thirty-six patients with newly diagnosed type 2 diabetes were enrolled in this study. The patients were hospitalized for glycemic control, and were being treated with diet therapy alone (age, 54.0 ± 2.5 yr; BMI, 31.7 ± 1.3%; HbA1c, 7.8 ± 0.4%). MTT (energy 500 kcal, protein 10.9 g, fat 28 g, sugars 51.3 g) *(20)* was performed twice with an interval of 2 d between tests. Patients who had previously received pharmacotherapy for diabetes and those with fasting plasma glucose levels of 160 mg/dL or greater were excluded from the study. At the second MTT, patients were orally administered 90 mg of nateglinide immediately before test meals. For both tests, blood samples were drawn before, 1, 2, 4, and 6 h after meals, for assay of plasma glucose, insulin, triglycerides, free fatty acids, and RLP-TG levels.

Plasma glucose levels were determined using a glucose dehydrogenase method as described preciously *(21)*, and serum lipids (triglycerides and free fatty acids) were measured using enzyme reagents (L-Type TG H, Wako Pure Chemicals, Osaka; NEFA-SS, Eiken Chemical, Tokyo) as the standard technique. Serum RLP was measured by an immunoabsorption kit (RLP-cholesterol, JIMRO II, Japan Immunoresearch Laboratories, Takasaki) according to the manufacturer's instructions. Briefly, RLP-TG measurement was made by a specific solid-phase double-monoclonal antibody immunoseparation assay in RLP samples separated from other lipoproteins (HDL, LDL, nascent VLDL, etc.). In principle, serum samples were applied to either anti apo A-I, or apo B-100 antibodies coupled column, which can absorb normal lipoproteins containing apo A-I, or most lipoproteins containing apo B-100. Unbound lipoproteins enriched for RLP were used for measurement of TG *(22)*. Serum insulin levels were determined by using a enzyme immunoassay kit (LS Eiken Insulin Kit, Eiken Chemical, Tokyo).

For statistical analysis, all numerical values were presented as mean ± SEM. The paired *t*-test was used to test for significance ($p < 0.05$). The study was conducted with the approval of the Ethics Committee of the National Hospital Organization, Utsunomiya National Hospital. Informed consent was obtained from all subjects who participated in the study.

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