Nateglinide Suppresses Postprandial Hypertriglyceridemia in Zucker Fatty Rats and Goto–Kakizaki Rats: Comparison with Voglibose and Glibenclamide

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Postprandial hypertriglyceridemia, as well as postprandial hyperglycemia, are important factors contributing to the development of cardiovascular disease in patients with type 2 diabetes. Nateglinide is a recently approved antidiabetic that suppresses postprandial hyperglycemia by stimulating the early phase of insulin secretion. In the present study, we investigated the effects of nateglinide on postprandial hypertriglyceridemia in obese Zucker fatty (ZF) rats and non-obese diabetic Goto–Kakizaki (GK) rats. Administration of an oral fat load caused marked hypertriglyceridemia with a peak at 2 h in ZF and GK rats. Nateglinide (50 mg/kg) significantly suppressed the increase of plasma triglycerides after fat loading in both types of rat (*ΔAUC* [0—4 h]: 15±69 mg·h/dl for nateglinide *vs.* 838±100 mg·h/dl for vehicle in ZF rats; $p<0.01$, 81±22 mg·h/dl for nateglinide *vs.* 164±17 mg·h/dl for vehicle in GK rats; $p<0.01$). In contrast, other antidiabetic agents (voglibose and glibenclamide) did not show a significant effect on the increase of triglycerides after fat loading. The triglyceride components suppressed by nateglinide were mainly at the origin and in the pre β subfraction on agarose gel electrophoresis, suggesting that chylomicrons and very low density lipoproteins were decreased. Plasma insulin levels were significantly increased at 30 min in nateglinide-treated rats, but not in voglibose- or glibenclamide-treated rats. These results suggest that nateglinide not only suppresses postprandial hyperglycemia, but also suppresses postprandial hypertriglyceridemia, by promoting rapid and pulsatile insulin secretion in patients with type 2 diabetes.

Key words nateglinide; postprandial; triglyceride; insulin; Zucker fatty rat; Goto–Kakizaki rat

Postprandial hypertriglyceridemia is one of the characteristic pathophysiological abnormalities in type 2 diabetes.[1,2] Recent epidemiological studies have shown that postprandial triglyceride levels, as well as postprandial glucose levels, are an important contributing factor to development of atherosclerosis and cardiovascular disease.[3—5] Therefore, the drugs that suppress both postprandial hypertriglyceridemia and postprandial hyperglycemia may be beneficial for preventing the progression of diabetic macrovascular complications.

It is known that α-glucosidase inhibitors reduce postprandial glucose levels by delaying carbohydrate absorption. There should be little effect on lipid metabolism based on this mode of action, though some reports have indicated a slight suppression of postprandial triglyceride levels.[6,7] A recent clinical study showed that a long-acting sulfonylurea, glibenclamide could improve postprandial hypertriglyceridemia in patients with type 2 diabetes.[8] However, sulfonylureas have an inadequate effect on postprandial hyperglycemia. In addition, it has been pointed out that the optimum insulin secretagogue would be rapidly acting and has a short duration of effect, since the chronic hyperinsulinemia is undesirable.[9]

Nateglinide is a recently approved oral hypoglycemic agent of a new class, which stimulates the early phase of insulin secretion by pancreatic β-cells,[10,11] and consequently suppresses postprandial hyperglycemia in both animals and patients with type 2 diabetes.[12,13] In this study, we established postprandial hypertriglyceridemia models using two different diabetic animals, obese Zucker fatty (ZF) rats with insulin resistance and Goto–Kakizaki (GK) rats with impaired insulin secretion. Then we investigated the effects of nateglinide on plasma triglyceride levels after oral loading with fat emulsion, and compared the results with those obtained using an α-glucosidase inhibitor (voglibose) or glibenclamide.

MATERIALS AND METHODS

Animals Male ZF rats and Zucker lean (ZL) rats were purchased from Tokyo Experimental Animals (Tokyo, Japan). GK rats and normal Wistar rats were purchased from Charles River Japan (Yokohama, Japan). This study was reviewed and approved by the Animal Care and Use Committee of Ajinomoto Co., Inc.

Drugs Nateglinide ((−)-*N*-(*trans*-4-isopropylcyclohexanecarbonyl)-D-phenylalanine; 50 mg/kg), voglibose (0.2 mg/kg), and glibenclamide (1 mg/kg) were suspended in 0.5% methylcellulose and administered to rats *via* a stomach tube in volume of 10 ml/kg. These doses of the drugs showed a similar suppressive effect on the peak blood glucose levels after oral sucrose or glucose loading of fasted normal rats in our previous study.[12] Control rats were treated with 0.5% methylcellulose alone (the vehicle).

Experimental Design Male Zucker rats and GK rats were used at the age of 12—15 and 32—34 weeks, respectively. After being fasting for 17 h, rats were orally given a fat emulsion (Intralipos, Welfide Co., Osaka, Japan) at a dose of 10 ml (containing 2 g soybean oil)/kg. Drugs were administered orally just before the fat load. In one experiment, rats received drug treatment alone without fat loading. Approximately 200 μl of blood was taken from the tail vein at 0, 30, 60, 120, 180, 240, and 300 min after fat loading, and plasma was separated for biochemical analysis.

Biochemical Analysis Blood glucose levels were deter-

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Table 1. Laboratory Data in Zucker Fatty Rats and GK Rats at the Beginning of the Experiment

Rat	Body weight (g)	Fasting plasma triglyceride (mg/dl)	Fasting blood glucose (mg/dl)	Fasting plasma insulin (ng/ml)
Zucker fatty rats (n=44)	499.6±7.3**	357.0±19.4**	136.8±2.7**	14.8±1.6**
Zucker lean rats (n=9)	342.7±2.9	55.8±1.4	98.6±2.4	1.0±0.1
GK rats (n=10)	412.9±10.8**	77.1±4.2	197.4±4.8**	1.1±0.1**
Wistar rats (n=5)	622.7±31.7	113.0±28.1	106.8±3.8	2.4±0.4

Fasting values were measured after 17 h fasting. Data are expressed as the mean±S.E. **p<0.01 *vs.* each normal rats.

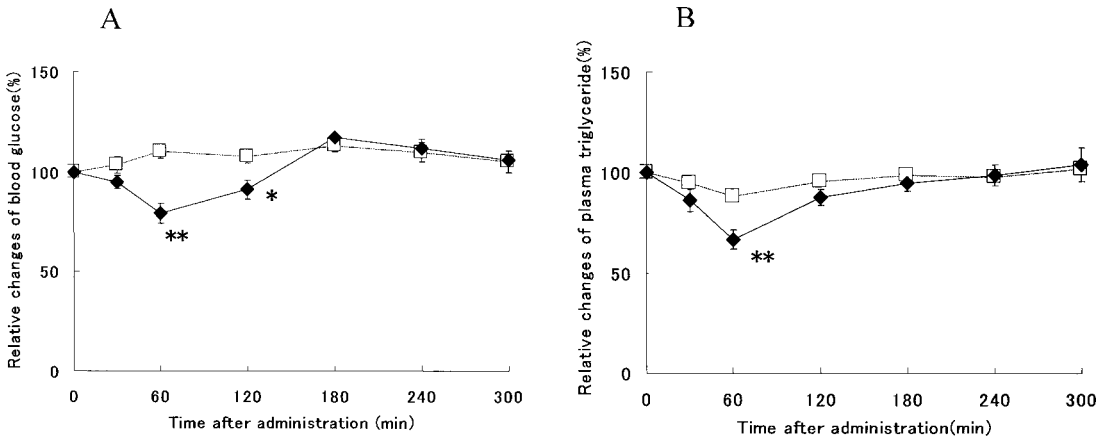

Fig. 1. Effect of Nateglinide on Blood Glucose (A) and Plasma Triglyceride (B) Levels in Fasted Zucker Fatty Rats

After 17 h of fasting, the vehicle (□, n=5) or nateglinide (◆, n=4) was administered to Zucker fatty rats by oral gavage. Blood samples were obtained from the tail vein at each specified time. All data are shown as percentages of the pretreatment value (mean±S.E.). *p<0.05; **p<0.01 *vs.* vehicle-treated Zucker fatty rats at the same time.

mined by the glucose oxidase method and plasma triglyceride levels were determined by the glycerol phosphodehydroxygenase method, both of which were performed using a Fuji Dri-Chem 5500 auto analyzer (Fuji Medical Systems, Tokyo, Japan). Plasma insulin levels were determined with a commercial enzyme immunoassay kit (Seikagaku Co., Tokyo, Japan) using rat insulin as the standard. The plasma triglyceride levels in lipoprotein fractions were determined by agarose gel electrophoresis, followed by triglyceride-specific staining using the Chol/Trig Combo system (Helena Laboratories, Saitama, Japan).

Statistical Analysis Results were expressed as the mean±S.E. Statistical comparisons between two groups were done by the unpaired Student's *t*-test. Differences were defined as significant at $p<0.05$.

RESULTS

Laboratory Data Table 1 shows the laboratory data of the experimental animals. The mean body weight of ZF rats was significantly higher than that of ZL rats. Fasting plasma triglyceride and plasma insulin levels were obviously higher and fasting blood glucose was mild increased in ZF rats when compared with ZL rats. In contrast, the mean body weight and fasting plasma insulin levels of GK rat were significantly lower than those of normal Wistar rats. Fasting plasma triglycerides showed no significant difference between GK rats and Wistar rats, but fasting blood glucose was markedly higher when compared with the levels in Wistar rats. These data suggest that both ZF rats and GK rats were diabetic, but ZF rats were obese with insulin-resistance and GK rats were non-obese with impaired insulin secretion.

Effect of Nateglinide on Fasting Triglyceride Levels in Obese ZF Rats First, we examined the effect of nateglinide in fasted ZF rats. Nateglinide significantly reduced the blood glucose levels (by 24% with the nadir at 60 min after treatment) in a rapid-acting and short-duration manner, as reported previously (Fig. 1A). Nateglinide also significantly reduced high fasting plasma triglyceride levels by 33%, with the nadir at 60 min after treatment (Fig. 1B). Both effects were transient and these parameters returned to pretreatment levels within 120—180 min.

Effect of Nateglinide on Hypertriglyceridemia after Fat Loading in Obese ZF Rats Next, we examined the changes of plasma triglyceride levels after oral administration of a fat emulsion to ZF and ZL rats. There was a slight increase of the plasma triglyceride levels after oral fat loading in ZL rats (from 59.0±2.9 mg/dl at baseline to a peak of 92.7±7.4 mg/dl). On the other hand, the plasma triglyceride levels was markedly increased with a peak at 120 min in ZF rats and then returned to the fasting levels at 240 min (Fig. 2).

Nateglinide significantly suppressed the increase of plasma triglycerides in fat-loaded ZF rats at 120 min ($p<0.05$) and 180 min ($p<0.01$) (Fig. 3, top). We calculated the area under the curve from baseline (ΔAUC) of plasma triglyceride from 0 min to 240 min. ΔAUC of nateglinide-treated ZF rats was significantly lower when compared with vehicle-treated ZF rats (15±69 mg·h/dl for nateglinide *vs.* 838±100 mg·h/dl for vehicle; $p<0.01$). In contrast, the plasma triglyceride levels in voglibose- and glibenclamide-treated ZF rats showed no significant difference compared

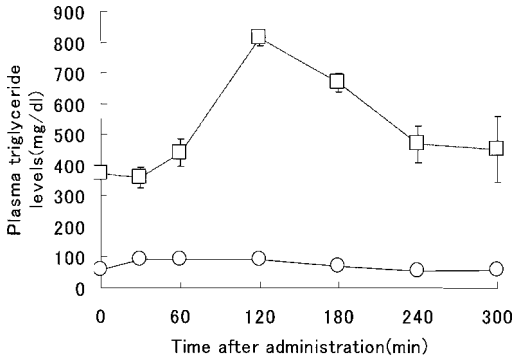

Fig. 2. Plasma Triglyceride Levels after Fat-Loading in Zucker Fatty Rats and Zucker Lean Rats

After 17 h of fasting, 2 g/kg of a fat emulsion was administered orally to Zucker fatty rats (□, $n=3$) or Zucker lean rats (○, $n=3$). Blood samples were obtained from the tail vein at each specified time. Data are expressed as the mean±S.E.

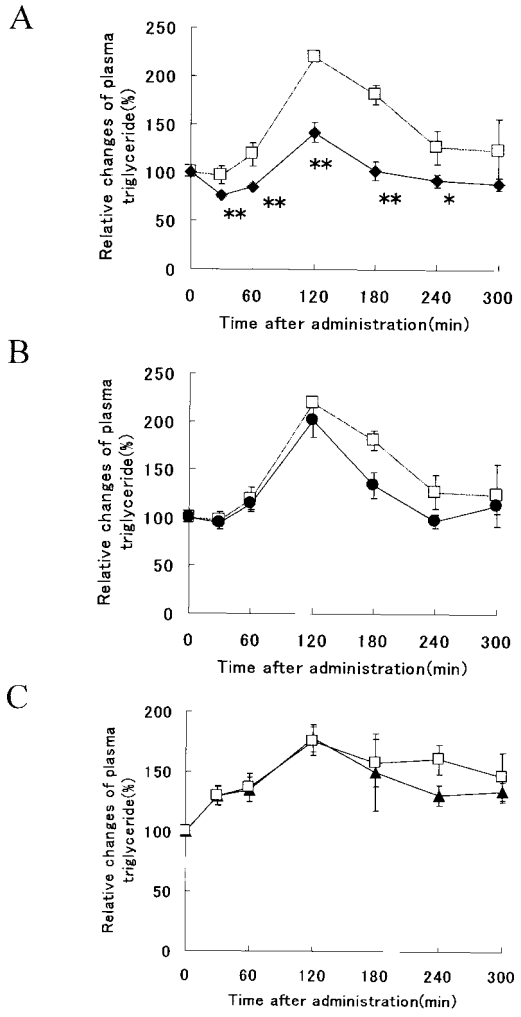

Fig. 3. Effect of Nateglinide (A), Voglibose (B), and Glibenclamide (C) on Hypertriglyceridemia in Fat-Loaded Zucker Fatty Rats

After 17 h of fasting, vehicle (panel A—C, □, $n=3$—6), nateglinide (panel A, ◆, $n=10$), voglibose (panel B, ●, $n=10$), or glibenclamide (panel C, ▲, $n=6$) were administered to Zucker fatty rats by oral gavage just before loading with 2 g/kg of a fat emulsion. Blood samples were obtained from the tail vein at each specified time. All data are shown as percentages of the pretreatment value (mean±S.E.). $*p<0.05$; $**p<0.01$ *vs.* vehicle-treated Zucker fatty rats at the same time.

with those in vehicle-treated ZF rats at any time (Fig. 3, middle and lower panels). In voglibose- and glibenclamide-treated rats, *ΔAUC* was also not significantly different from

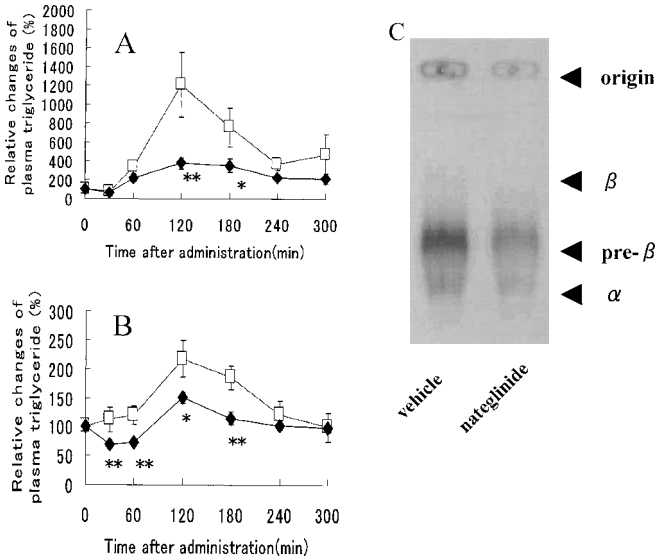

Fig. 4. Electrophoresis Analysis of Plasma Lipoproteins in Nateglinide-Treated Zucker Fatty Rats

After 17 h of fasting, the vehicle (□, $n=3$) or nateglinide (◆, $n=10$) was administered to Zucker fatty rats by oral gavage just before loading with 2 g/kg of a fat emulsion. Blood samples were obtained from the tail vein at each specified time. The plasma triglyceride levels in each lipoprotein subfraction was determined by agarose gel electrophoresis, followed by triglyceride-specific staining using the Chol/Trig Combo analysis system (Helena Laboratories, Saitama, Japan). The positions of the origin (A), *β*, pre-*β* (B), and *α* are shown, which include chylomicrons, LDL, VLDL, and HDL, respectively. All data are shown as percentages of the pretreatment value (mean±S.E.). Pretreatment value of origin was 17.4±3.3 mg/dl and that of pre *β* was 226.4±14.6 mg/dl. $*p<0.05$; $**p<0.01$ *vs.* vehicle-treated Zucker fatty rats at the same time. Electrophoresis analysis of plasma lipoproteins at 120 min after administration in vehicle-treated and nateglinide-treated Zucker fatty rats is shown in panel C.

that in the vehicle-treated group.

We further analyzed the subfractions of triglycerides after fat loading. Analysis of lipoprotein subfractions by agarose gel electrophoresis showed that the incremental triglycerides at 120 min after fat loading was mainly at the origin and in the pre *β* subfractions (Fig. 4C), which includes chylomicrons and very low density lipoprotein (VLDL). Nateglinide significantly suppressed the increase both at the origin and in the pre-*β* subfractions (Figs. 4A, B). In contrast, the *α* and *β* subfractions were almost unchanged from baseline after fat loading and nateglinide did not affect these subfractions.

We measured plasma insulin levels at the same times. Oral fat administration did not change the plasma insulin levels from baseline. In nateglinide-treated rats, a significant, rapid and pulsatile increase of plasma insulin was observed at 30 min, with a return to basal levels at 60 min (Fig. 5). Plasma insulin levels at 30 min were not significantly different in voglibose- and glibenclamide-treated rats compared with vehicle-treated rats (data not shown).

Effect of Nateglinide on Hypertriglyceridemia after Fat Loading in Non-obese Diabetic GK Rats Finally, we examined the effect of nateglinide on postprandial lipid metabolism in non-obese GK rats. Oral administration of a fat emulsion induced slight, but significant, hypertriglyceridemia with a peak at 120 min. Nateglinide significantly suppressed the increase of plasma triglyceride levels after fat loading in GK rats (Fig. 6). The *ΔAUC* of plasma triglycerides from 0 to 240 min was also significantly reduced in nateglinide-treated GK rats compared with vehicle-treated GK rats (81±22 mg·h/dl for nateglinide *vs.* 164±17 mg·h/dl for vehicle; $p<0.01$).

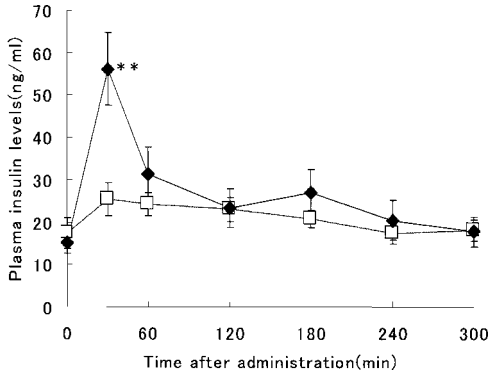

Fig. 5. Plasma Insulin Levels after Fat Loading and Nateglinide Administration in Zucker Fatty Rats

After 17 h of fasting, the vehicle (□, $n=3$) or nateglinide (◆, $n=10$) was administered to Zucker fatty rats by oral gavage just before loading with 2 g/kg of a fat emulsion. Blood samples were obtained from the tail vein at each specified time. All data are shown as percentages of the pretreatment value (mean±S.E.). $^{**}p<0.01$ *vs.* vehicle-treated Zucker Fatty rats at the same time.

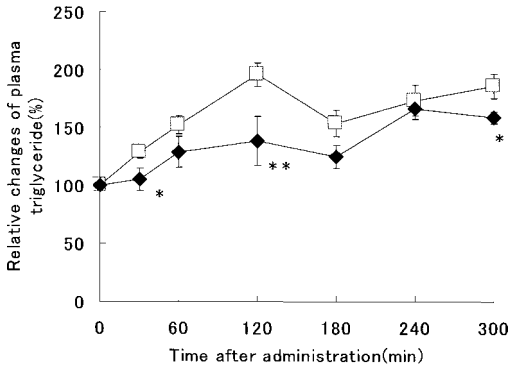

Fig. 6. Effect of Nateglinide on Hypertriglyceridemia after Oral Fat Loading in Non-obese GK Rats

After 17 h of fasting, the vehicle (□, $n=10$) or nateglinide (◆, $n=10$) was administered to GK rats by oral gavage just before loading with 2 g/kg of a fat emulsion. Blood samples were obtained from the tail vein at each specified time. All data are shown as percentages of the pretreatment value (mean±S.E.). $^{*}p<0.05$; $^{**}p<0.01$ *vs.* vehicle-treated GK rats at the same time.

DISCUSSION

In this study, we demonstrated that nateglinide suppressed the increase of plasma triglycerides after fat loading in rats with impaired insulin secretion and/or insulin resistance.

Postprandial hypertriglyceridemia is an inherent feature of diabetic dyslipidemia and is common in diabetic patients even when the fasting triglyceride concentration is normal, being characterized by delayed removal of chylomicrons and VLDL remnants from the circulation, together with enhanced synthesis of VLDL in the liver.[1—4)] Nateglinide slightly reduced the plasma triglyceride levels at 60 min after administration to fasted ZF rats (Fig. 1). Though chylomicron triglyceride levels were negligible in fasted ZF rats (data not shown), this transient reduction of plasma triglyceride levels might be mainly due to the modification of endogenous VLDL. In contrast, nateglinide suppressed the increase of postprandial triglyceride levels that showed a peak at 120 min after fat loading (Fig. 3). Our fat-loaded rats showed an increase at the origin and in the pre β subfractions, including chylomicrons and VLDL (Fig. 4). In addition, endogenous lipogenesis was minimized or negligible after overnight fasting and loading with carbohydrate-free fat, in contrast to previous postprandial hyperlipididemia studies using rats.[14,15)] The selective reduction of the abnormal fractions (Fig. 4) suggests that nateglinide accelerated the removal of exogenous chylomicrons and VLDL remnants from plasma. It is noteworthy that neither α-glucosidase inhibitor, voglibose nor glibenclamide had a significant effect in our model.

It is generally accepted that the exaggerated postprandial response is at least partly caused by relatively inadequate early phase of insulin secretion.[1—4)] Recent studies have revealed the mechanisms of glucose-induced biphasic insulin secretion and the rational for the unique *in vivo* and *ex vivo* insulinotropic activity profile of nateglinide, especially for the rapid insulin release.[16,17)] Nateglinide ameliorated postprandial hypertriglyceridemia, which suggests that an early phase of insulin secretion could overcome insulin resistance in ZF rats (Figs. 3, 5). We have previously reported that nateglinide induced early phase of insulin secretion and suppressed postprandial hyperglycemia in GK rats with defective glucose-induced insulin secretion by pancreatic β-cell.[18)] Nateglinide also reduced postprandial triglyceride levels in GK rats (Fig. 6).

The mechanisms by which nateglinide regulates abnormal postprandial metabolism in these rats are still unclear, but our results suggested the importance of early pulsatile insulin secretion, which is stimulated by nateglinide. The role of early phase insulin has not been clarified since insulin has pleiotropic effects (both directly and indirectly) on exogenous lipoprotein metabolism and endogenous lipoprotein synthesis.[19,20)] One of the well-defined molecules that influence the levels of triglycerides is lipoprotein lipase (LPL), which hydrolyzes the triglyceride component of circulating lipoprotein, and decreases the blood triglyceride levels. Insulin is a major stimulator of the postprandial increase of LPL activity, but other reports have suggested that decreased LPL gene expression is mediated by the increase of insulin in rats with postprandial hyperlipidemia.[21,22)] In addition, some recent transgenic or knockout mice studies have suggested a role of apolipoproteins, receptors for lipoproteins and other enzymes in the mechanisms of lipid abnormalities. Investigation of enzyme activities and/or the expression of these molecules in nateglinide-treated animals is planned to address these issues.

Some recent epidemiological studies have indicated that impaired glucose tolerance is one of the risk factors for cardiovascular disease.[23)] Tominaga suggested that macrovascular diseases, unlike microvascular complications, might not be solely due to hyperglycemia.[24)] In other words, factors other than hyperglycemia also seem to be related to the development of macrovascular disease. Although further studies will be needed to elucidate the effect of long-term control of postprandial lipid metabolism, our results suggested that restoring early phase of insulin secretion by administration of nateglinide might be a beneficial therapeutic approach for reducing of the risk of macrovascular complications in patients with type 2 diabetes.

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