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RESEARCH ARTICLE

Effects of nateglinide and glibenclamide on postprandial lipid and glucose metabolism in type 2 diabetes

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Abstract

Background Postprandial hyperlipemia and small, dense LDL particles are features of dyslipidemia in type 2 diabetes. The purpose of this study was (1) to determine whether the oral insulinotropic drugs, nateglinide and glibenclamide, can overcome the defect of insulin action to suppress the hepatic VLDL release after a meal and decrease the postprandial lipemia and (2) to evaluate the acute effect of postprandial hypertriglyceridemia on LDL particle size in subjects with type 2 diabetes.

Methods Forty-three subjects with type 2 diabetes and mean baseline HbA_{1c} 7.6% (95% CI 7.3 to 7.9) were treated with nateglinide 120 mg three times daily or glibenclamide 5 mg once or twice daily for 12 weeks in a double-blind randomised trial. Insulin, glucose, and lipoprotein responses to a mixed fat-rich meal were determined for 8 h postprandially at baseline and at 12 weeks on-trial.

Results Nateglinide and glibenclamide significantly augmented the maximal response in serum insulin at 60 min postprandially compared with the response without the drug [additional increase 25.0 mU/l (95% CI 11.2–38.8) $p = 0.001$ and 12.5 mU/l (95% CI 4.6–20.3) $p = 0.003$, respectively] and reduced hyperglycemia. Neither drug affected fasting or postprandial lipid or lipoprotein levels. LDL size did not significantly change in the 8-h postprandial period.

Conclusions Although nateglinide and glibenclamide increase postprandial insulin secretion and attenuate hyperglycemia, they do not alleviate postprandial lipemia in subjects with type 2 diabetes and good glycemic control. Although small LDL particle size is associated with chronic hypertriglyceridemia, LDL size does not change during acute postprandial hypertriglyceridemia. Copyright © 2002 John Wiley & Sons, Ltd.

Keywords hypertriglyceridemia; small dense LDL; nateglinide; glyburide; apolipoprotein B

Introduction

Subjects with diabetes have an increased risk of cardiovascular disease compared to a non-diabetic population [1]. Conventional risk factors, such as elevated serum total and low density lipoprotein (LDL) cholesterol, low high density lipoprotein (HDL) cholesterol, and hypertension, explain only part of

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this excess risk [1]. Core elements of dyslipidemia in type 2 diabetes are mild to moderate hypertriglyceridemia, low HDL cholesterol, and preponderance of small, dense LDL particles. All these features are believed to increase the risk of cardiovascular complications [2,3]. In the postprandial period, diabetic subjects have exaggerated hyperglycemia and hypertriglyceridemia. The postprandial lipemia consists of triglyceride-rich lipoproteins (TRL) including chylomicrons and their remnants derived from intestine and hepatic released very low-density lipoprotein (VLDL) particles. Chylomicrons and VLDL particles compete for the same catabolic pathways that include lipolysis by lipoprotein lipase (LPL) and hepatic lipase (HL) and direct uptake of TRL by the liver. Consequently, conditions in which VLDL production is high favour the accumulation of postprandial TRL. Acute hyperinsulinemia suppresses the production of large triglyceride-rich VLDL1 particles (S_f 60–400) in the liver in healthy men, but this downregulation of VLDL1 production by insulin is defective in subjects with type 2 diabetes [4]. Accordingly, the inappropriate release of large VLDL1 particles in the postprandial phase is likely to be a contributing factor to hypertriglyceridemia.

Growing evidence suggests that TRL and their remnants are atherogenic, possibly owing to their direct effects on the vascular wall or owing to the secondary effects of TRL on HDL and LDL particles [5–7]. A number of cross-sectional and prospective studies have associated small, dense LDL particles with cardiovascular disease [3,8,9]. However, because of the close correlations between small, dense LDL, hypertriglyceridemia, and low HDL cholesterol [10], it is difficult to dissect out whether small LDL particles are an independent risk factor or are merely a marker of other factors with causal relationship to atherosclerosis.

Nateglinide is a D-phenylalanine derivative that acts by directly stimulating the release of insulin from pancreatic β-cells in a glucose-sensitive manner. Glibenclamide (glyburide) is a second-generation sulphonylurea. Both of these stimulate insulin secretion from pancreatic beta cells; however, the action of nateglinide is more rapid and shorter-lasting [11]. In human studies it has been shown that nateglinide restores early insulin secretion [12] and decreases postprandial hyperglycemia [13]. We hypothesised that the increased insulin secretion by nateglinide may suppress the release of hepatic VLDL1 particles in the postprandial phase and consequently reduce postprandial hyperlipemia. The objectives of the study were (1) to investigate whether an increased and timely portal concentration of insulin by nateglinide can overcome the defect of insulin action to suppress the VLDL1 release after a meal and decrease the postprandial lipemia and (2) to evaluate the acute effect of postprandial hyperlipemia on LDL particle size in subjects with type 2 diabetes mellitus.

Subjects and methods

Subjects and study protocol

We studied 48 men and women with type 2 diabetes in a single-centre, double-blind, randomised, active-controlled, parallel group study. All subjects were Caucasian. Inclusion criteria were age 18 to 75 years inclusive, body mass index (BMI) $\leq$33 kg/m^2, HbA_{1c} 6.5 to 10%, fasting serum glucose $<$15 mmol/l, total cholesterol $\leq$ 6.5 mmol/l, total triglycerides $\leq$ 4.5 mmol/l, and apolipoprotein E (apoE) 3/3 or 3/4 phenotype. Subjects with hepatic, renal, or thyroid disease were excluded. Other exclusion criteria were smoking, use of nicotine therapy, lipid lowering medication, insulin, and hormone replacement therapy. Seventeen patients were treated with diet alone, 20 with sulphonylurea, and 11 with metformin. The study protocol was approved by the Ethical committee of the Helsinki University Hospital, and all patients gave their informed consent.

Previous antidiabetic medication was withdrawn for at least six weeks in the washout phase before randomisation. At the randomisation visit (baseline, week 0), subjects had the first fat tolerance test (see details below). Study medication was started on the following morning. In the glibenclamide group (randomised $n = 24$), subjects received glibenclamide 5 mg at breakfast for the first 4 weeks. If fasting serum glucose at week 4 was $\geq$7.0 mmol/l, the glibenclamide dose was increased to 5 mg at breakfast and at dinner. They also received placebo tablets mimicking nateglinide for the whole 12-week study. In the nateglinide group (randomised $n = 24$), subjects received nateglinide 120 mg tablets before breakfast, lunch, and dinner and placebo capsules mimicking glibenclamide for the whole 12-week study. Postheparin lipolytic enzyme activities were measured from blood samples obtained at separate visits approximately seven days before the fat tolerance tests. From the nateglinide group, one subject discontinued the study owing to unsatisfactory therapeutic effect. From the glibenclamide group, one subject discontinued the study owing to unsatisfactory therapeutic effect, two subjects owing to repeated hypoglycemia, and one subject owing to convulsions that were possibly related to hypoglycemia. Only the subjects with both baseline and week 12 data available ($n = 23$ in the nateglinide group, $n =$ 20 in the glibenclamide group) are included in the analyses.

Oral fat tolerance test

The test meal consisted of bread, butter, cheese, sliced sausage, egg, paprika, soured whole milk, orange juice, and coffee. It contained 63 g fat (P/S ratio 0.08), 490 mg cholesterol, 25 g carbohydrate, and 35 g protein, and it was consumed within 10 min. After the test meal the

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participants were allowed to drink only water until the last blood samples were collected. Fat tolerance tests were performed at randomisation (baseline) and at 12-weeks on-trial (final visit). At baseline, subjects did not take any medication in the morning, and at the final visit, they took the randomised study drug 5 min before the test meal. Alcohol intake and vigorous exercise were not allowed during the previous 72 h. The test started at 0800 h after an overnight 12-h fast. Blood samples were drawn from a catheter placed in an antecubital vein at 0 h, 30 min, 1 h, 3 h, 4 h, 6 h, and 8 h [at 30 min and 1 h, only samples for glucose, insulin, and free fatty acids (FFA) were collected]. Plasma was separated within 20 min by low-speed centrifugation. The samples were stored at – 80 °C unless they were analysed immediately.

Biochemical analyses

Plasma lipoprotein fractions were isolated with density gradient ultracentrifugation as previously described [14]. Triglyceride and cholesterol concentrations were analysed in total plasma and in all lipoprotein fractions by automated enzymatic methods (Cobas Mira analyser, Hoffman-La Roche, Basel, Switzerland). Apolipoprotein B 48 (apoB 48) and apolipoprotein B 100 (apoB 100) were analysed in the plasma lipoprotein fractions separated by ultracentrifugation as previously described [14]. LDL peak particle diameter was measured from serum samples stored at – 80 °C using non-denaturing linear gradient gel electrophoresis as previously described [15]. All the samples of one individual were run in the same gel to eliminate the variation between gels. Serum glucose, insulin, FFA, and postheparin LPL and HL were analysed as previously described [16,17].

Statistical analyses

Data were analysed using SPSS 11 software (SPSS Inc., Chicago, Illinois). Between-group characteristics were compared by unpaired t-test. Within-group changes were compared by paired t-test. The effects of nateglinide and glibenclamide on postprandial insulin, glucose, FFA, and lipoprotein metabolism were analysed using repeated measures ANOVA [18]. The Greenhouse–Geisser adjustment was used when the sphericity assumptions were not fulfilled. Associations between LDL size and other variables were analysed using Pearson's correlation analysis. Variables with skewed distribution were log-transformed before the analyses, but the untransformed data are shown in text and tables for the ease of interpretation. Postprandial triglyceride, apoB 48, and apoB 100 responses over the 8-h period were calculated as areas under the curve (AUC) using the trapezoid rule [19]. Incremental areas under the curve (IAUC) were calculated by subtracting the fasting value from each postprandial value before area calculation. The p value <0.05 (two-tailed) was considered statistically significant. Data are shown as mean values with 95% confidence intervals (CI) in text and tables and mean ± standard error (SE) in figures.

Results

Subject characteristics and glycemic control

At baseline, subjects in nateglinide and glibenclamide groups had similar mean age, BMI, fasting serum glucose, HbA_{1c}, insulin, FFA, triglyceride, and cholesterol (Table 1). Postheparin LPL and HL activities were similar in both groups at baseline and on-trial (not shown). Nateglinide 120 mg before three meals daily decreased fasting serum glucose to 8.9 mmol/l (95% CI 8.1–9.7, $p = 0.005$), but it did not significantly change HbA_{1c} (7.4%, 95% CI 7.0–7.9). Glibenclamide 5 to 10 mg daily decreased fasting serum glucose to 7.5 mmol/l (95% CI 6.8–8.2, $p < 0.001$) and HbA_{1c} to 6.9% (95% CI 6.5–7.3, $p < 0.001$).

Changes in postprandial serum glucose, insulin, and FFA

Postprandial insulin, glucose, and FFA responses were similar in both groups at baseline (Figure 1). Postprandial insulin secretion was increased by both nateglinide and glibenclamide ($p < 0.001$, repeated measures ANOVA). The highest serum insulin levels were measured at 60 min postprandially. Both nateglinide and glibenclamide significantly augmented the maximal response in serum insulin compared with the response at baseline [25.0 mU/l (95% CI 11.2–38.8), $p = 0.001$ for nateglinide and 12.5 mU/l (95% CI 4.6–20.3), $p = 0.003$ for glibenclamide]. These differences were reflected in postprandial serum glucose concentrations. Accordingly, nateglinide decreased postprandial hyperglycemia especially during the first 3 h and glibenclamide at 3 h and onwards ($p < 0.001$ for both,

Table 1. Baseline characteristics in nateglinide and glibenclamide groups

	Nateglinide, $n = 23$	Glibenclamide, $n = 20$	p
Age, years	63 (59–66)	63 (58–67)	NS
BMI, kg/m^2	27.8 (26.4–29.3)	28.8 (27.2–30.4)	NS
Glucose, mmol/l	10.1 (9.0–11.3)	10.0 (8.8–11.2)	NS
HbA_{1c}, %	7.6 (7.2–8.0)	7.6 (7.2–8.1)	NS
Insulin, mU/l	10.7 (8.7–12.7)	10.3 (8.4–12.2)	NS
FFA, mmol/l	806 (713–899)	781 (657–905)	NS
TG, mmol/l	1.75 (1.56–1.94)	1.86 (1.53–2.19)	NS
TC, mmol/l	5.16 (4.86–5.46)	4.86 (4.59–5.12)	NS
LDL-C, mmol/l	2.85 (2.64–3.06)	2.58 (2.40–2.76)	NS
HDL-C, mmol/l	1.31 (1.19–1.44)	1.22 (1.09–1.04)	NS

Data are mean (95% CI). BMI, body mass index; FFA, free fatty acids; TG, total triglycerides; TC, total cholesterol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol.

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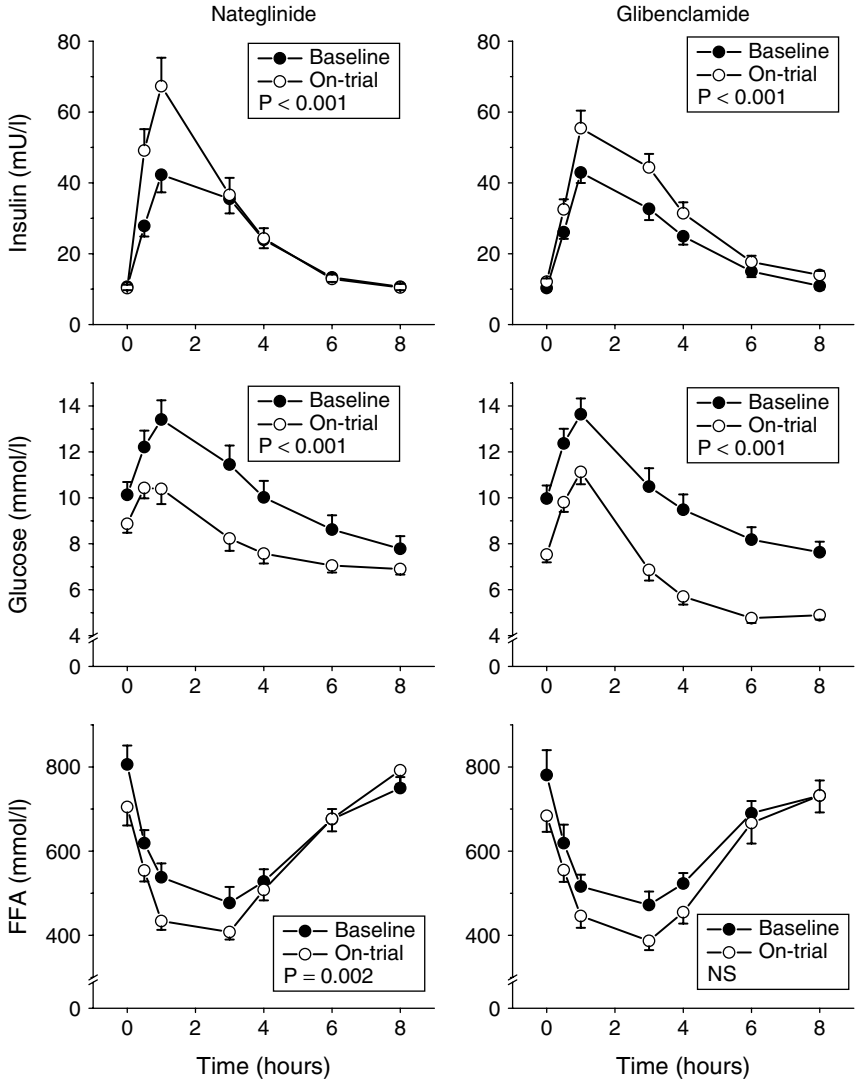

Figure 1. Baseline and on-trial postprandial insulin, glucose, and FFA concentrations in subjects treated with nateglinide (left column) and glibenclamide (right column)

Figure 1). Nateglinide significantly decreased the postprandial FFA values but the effect of glibenclamide did not reach statistical significance (Figure 1).

Changes in lipids and lipoproteins

Nateglinide and glibenclamide did not significantly change fasting lipid concentrations (not shown). Serum triglyceride, apoB 100, and apoB 48 responses in the VLDL1 (S_f 60–400) fraction to oral fat load at baseline and on-trial are shown in Figure 2. Treatment with nateglinide or glibenclamide did not affect postprandial plasma VLDL1 triglyceride, cholesterol, apoB 100, or apoB 48 concentrations. In addition, plasma chylomicron, VLDL2, IDL, LDL, and HDL responses were not affected by nateglinide or glibenclamide (not shown). IAUC for selected postprandial lipids and lipoproteins describing the total increase in plasma lipids during the 8-h postprandial period are given in Table 2. Neither nateglinide nor glibenclamide decreased the hyperlipemic response to a fat-rich mixed meal in these or other lipoproteins.

Postprandial changes in LDL properties

LDL particle size did not change during the postprandial period at baseline (fasting diameter 25.5 nm, 8 h diameter 25.6 nm, $n = 43$). Both triglyceride and cholesterol concentrations in the LDL fraction increased in the late postprandial phase (6 to 8 h), as did the LDL triglyceride/LDL cholesterol ratio (Table 3). Similar changes in postprandial LDL properties were found when nateglinide and glibenclamide groups were analysed separately at week 12, that is, no significant drug effect was found (not shown).

Associations between LDL size and triglyceride-rich lipoproteins

Fasting triglyceride-rich lipid fractions were closely correlated with respective postprandial responses, as

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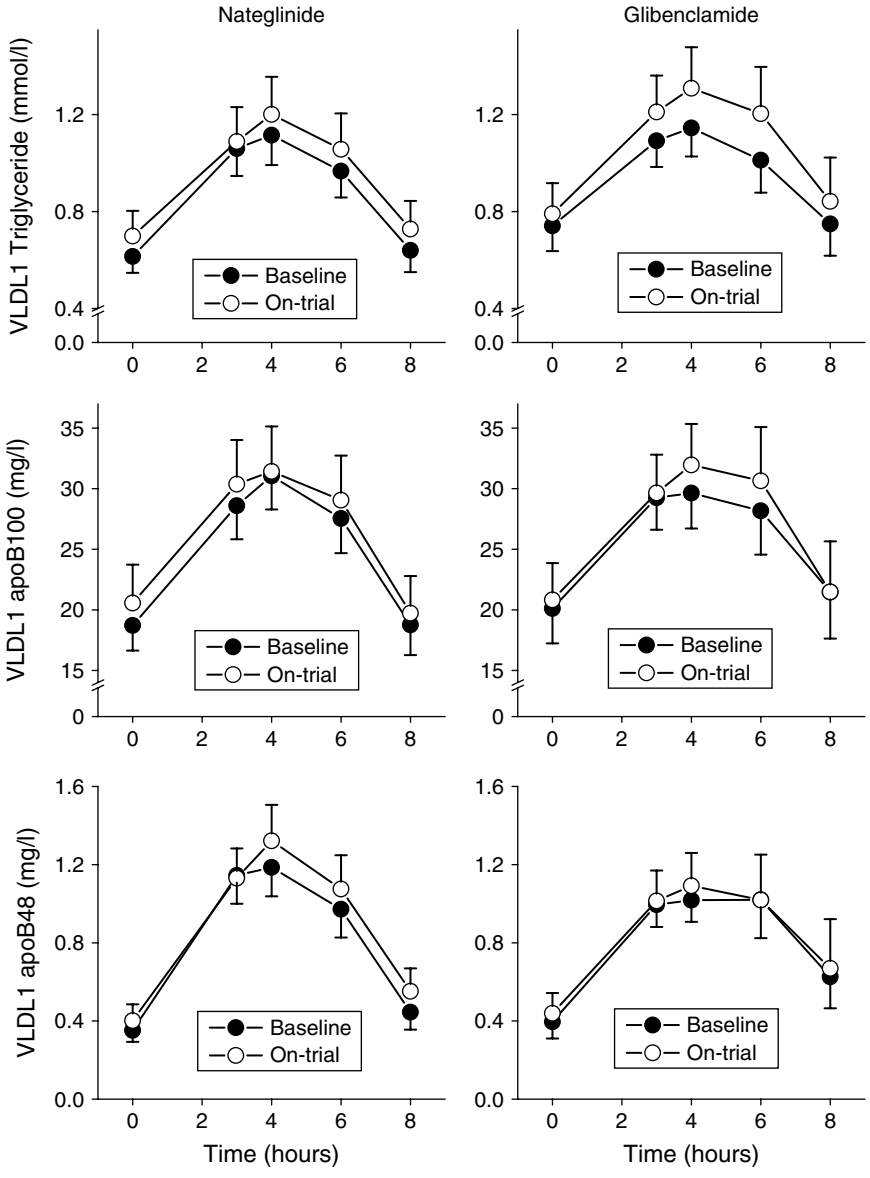

Figure 2. Baseline and on-trial postprandial VLDL1 (S_f 60–400) triglyceride, apoB 100, and apoB 48 concentrations in subjects treated with nateglinide (left column) and glibenclamide (right column). Nateglinide and glibenclamide did not affect postprandial lipoprotein responses

Table 2. Postprandial responses of triglycerides and apolipoproteins*

	Group	Baseline	On-trial
Total TG, mmol/l.h	Nateglinide ($n = 23$)	4.7 (3.5–5.9)	4.7 (3.1–6.3)
	Glibenclamide ($n = 20$)	3.5 (2.5–4.5)	4.7 (3.2–6.2)
Chylomicron TG, mmol/l.h	Nateglinide ($n = 23$)	2.0 (1.5–2.4)	1.9 (1.3–2.6)
	Glibenclamide ($n = 20$)	1.5 (1.1–1.9)	1.9 (1.3–2.5)
VLDL1 TG, mmol/l.h	Nateglinide ($n = 23$)	2.4 (1.6–3.2)	2.3 (1.4–3.2)
	Glibenclamide ($n = 18$)	2.1 (1.4–2.7)	2.4 (1.6–3.2)
VLDL1 apoB 100, mg/dl.h	Nateglinide ($n = 23$)	56 (36–76)	52 (31–73)
	Glibenclamide ($n = 18$)	56 (34–77)	55 (36–73)
Chylomicron apoB 48, mg/dl.h	Nateglinide ($n = 23$)	0.7 (0.5–0.9)	0.7 (0.4–1.0)
	Glibenclamide ($n = 20$)	0.6 (0.4–0.8)	0.7 (0.3–1.0)
VLDL1 apoB 48, mg/dl.h	Nateglinide ($n = 23$)	4.2 (2.8–5.5)	4.3 (3.0–5.7)
	Glibenclamide ($n = 18$)	3.9 (2.2–5.5)	3.6 (1.9–5.2)

*Postprandial incremental AUC. Data are mean (95% CI). Nateglinide and glibenclamide did not have significant effect on postprandial lipoprotein responses.

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Table 3. Postprandial changes in LDL properties at baseline ($n = 43$, nateglinide and glibenclamide groups combined)

Time (h)	LDL size (nm)	LDL-C* (mmol/l)	LDL-TG* (mmol/l)	LDL-TG/LDL-C* (ratio)
0	25.47 ± 1.23	2.72 ± 0.46	0.162 ± 0.036	0.060 ± 0.014
4	25.49 ± 1.20	2.74 ± 0.48	0.164 ± 0.036	0.061 ± 0.015
6	25.52 ± 1.21	2.77 ± 0.48	0.169 ± 0.036**	0.062 ± 0.014**
8	25.55 ± 1.19	2.82 ± 0.49**	0.174 ± 0.038**	0.063 ± 0.015**

LDL-C, LDL cholesterol; LDL-TG, LDL triglycerides.
*$p < 0.001$, repeated measures ANOVA.
**$p < 0.05$ versus fasting value.

expected (not shown). At baseline, LDL size was strongly associated with fasting VLDL1 cholesterol ($r = -0.72$, $p < 0.001$, $n = 43$), VLDL1 triglyceride ($r = -0.67$, $p < 0.001, n = 43$), and HDL cholesterol ($r = 0.67, p < 0.001$, $n = 43$). Similar associations were detected between fasting LDL size and postprandial AUC for TRL and HDL cholesterol. Strongest associations were between fasting LDL size and VLDL1 cholesterol AUC ($r = -0.71$, $p < 0.001$, $n = 43$), VLDL1 triglyceride AUC ($r = -0.69$, $p < 0.001$, $n = 43$), with HDL cholesterol AUC ($r = 0.68$, $p < 0.001$, $n = 43$). Correlations between LDL size and other lipid or lipoprotein fractions were weaker, as were the correlations of LDL size and IAUC values (not shown). Treatment with nateglinide or glibenclamide did not significantly influence the aforementioned associations (not shown).

Discussion

The main purpose of this study was to find out whether treatment with oral hypoglycemic agents can decrease postprandial hyperlipemia in subjects with type 2 diabetes. Although nateglinide and glibenclamide increased postprandial insulin secretion and decreased postprandial glycemia, they did not attenuate postprandial lipemia in type 2 diabetic subjects with good glycemic control. Glibenclamide 5 to 10 mg daily decreased fasting serum glucose and HbA_{1c} more efficiently than nateglinide 120 mg before three meals daily, but nateglinide decreased the postprandial rise in serum glucose more than glibenclamide did. This is in accordance with a previous study [13].

Nateglinide stimulates insulin secretion rapidly for a short period of time [11] and restores at least part of the early insulin secretion in men with diabetes [12]. Although nateglinide was effective in preventing postprandial hyperglycemia, it did not decrease postprandial hypertriglyceridemia as we expected. The majority of plasma triglycerides are carried in VLDL1 particles secreted by the liver. Previously, Malmström *et al*. have reported that while hepatic VLDL1 secretion decreases during a hyperinsulinemic euglycemic clamp in healthy men, this action of insulin is defective in men with type 2 diabetes [4]. Although their study was performed in experimental settings and in the fasting state, the data are in accordance with the results of the current study.

Several factors influence apoB and triglyceride secretion from hepatocytes. Nascent apoB protein is rapidly degraded, and therefore lipidation of apoB protein is a critical regulator of apoB secretion from hepatocytes. In insulin-resistant conditions, lipolysis in adipose tissue is increased and FFA uptake and esterification is decreased leading to an increased FFA flux to the liver and the skeletal muscle. Part of the FFA taken up by hepatocytes is stored in the cytosolic triglyceride pool in which they provide a high amount of substrate for VLDL assembly and secretion. Microsomal triglyceride transfer protein (MTP) is essential in catalysing the lipidation of apoB in the endoplasmic reticulum. Importantly, the MTP gene has a negatively regulated insulin response element [20], and it can be suggested that in insulin-resistant conditions, MTP activity is chronically upregulated leading to overproduction of triglyceride-rich VLDL [21]. In the study by Malmström *et al*. lowering of FFA by acute hyperinsulinemia did not affect hepatic VLDL1 production in men with type 2 diabetes [4]. In the current study, on-trial FFA level was decreased by nateglinide but fasting or postprandial triglyceride or apoB 100 concentrations did not change. This is a clinically significant result, which suggests that the insulin response achieved by recommended doses of oral insulinotropic agents does not decrease plasma FFA levels sufficiently to attenuate VLDL production in the liver. It is also possible that defects in hepatic insulin signalling pathways may determine fasting and postprandial hyperlipidemia in type 2 diabetes and other insulin-resistant states.

The subjects in our study had relatively good metabolic control at baseline and they represent typical type 2 diabetic patients treated with monotherapy. Although especially nateglinide therapy caused a significant decrease in postprandial hyperglycemia, neither agent had significant effect on postprandial hyperlipidemia. It is possible that in subjects with type 2 diabetes, insulin resistance and other metabolic disturbances may be so advanced that the increase in endogenous insulin production cannot overcome the defects in lipoprotein metabolism. It is not known whether in subjects with impaired glucose tolerance a hypolipidemic effect of nateglinide or glibenclamide may be detected. However, the risk of hypoglycemic side effects is higher in these

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subjects. On the other hand, in diabetic patients with poor glycemic control a significant improvement in glucose control can attenuate both fasting and postprandial dyslipidemia [22].

The second aim of this study was to investigate the acute effect of hypertriglyceridemia on properties of LDL particles. Only few studies have assessed acute changes in postprandial LDL size in subjects with type 2 diabetes [23,24] and the data have been controversial, possibly because the number of subjects in these studies has been relatively small. We did not detect a significant change in LDL size. If anything, the size of the most prevalent LDL subfraction tended to increase slightly. Postprandially between 6 to 8 h, cholesterol and especially triglyceride concentrations increased in the LDL fraction. It must be noted, though, that since we did not measure LDL apoB concentrations, we cannot estimate the number of LDL particles. Consequently, it remains unknown whether the increase in plasma LDL cholesterol and LDL triglyceride concentrations merely reflect the change in LDL particle number. In all, the data suggest that LDL particles are only marginally affected by acute postprandial hypertriglyceridemia. Packard *et al*. have proposed that LDL particles need approximately three days exposure to hypertriglyceridemia until cholesterol ester/triglyceride exchange by cholesteryl ester transfer protein (CETP) is mediated in such amount that HL can hydrolyse triglycerides and phospholipids from LDL particles rendering them small and dense [7]. Our results show that 8-h acute hypertriglyceridemia does not significantly change LDL size in type 2 diabetic subjects and support the concept that chronic hypertriglyceridemia is a key determinant of LDL size.

In conclusion, nateglinide and glibenclamide increase postprandial insulin secretion. Although both drugs attenuate hyperglycemia, they do not change fasting or postprandial plasma lipid concentrations in type 2 diabetic patients with good glycemic control.

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