CLINICAL STUDY

Impact of metformin versus the prandial insulin secretagogue, repaglinide, on fasting and postprandial glucose and lipid responses in non-obese patients with type 2 diabetes

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Abstract

Objective: Non-obese patients with type 2 diabetes (T2DM) are characterized by predominant defective insulin secretion. However, in non-obese T2DM patients, metformin, targeting insulin resistance, is non-inferior to the prandial insulin secretagogue, repaglinide, controlling overall glycaemia (HbA_{1c}). Whether the same apply for postprandial glucose and lipid metabolism is unknown. Here, we compared the effect of metformin versus repaglinide on postprandial metabolism in non-obese T2DM patients.

Design: Single-centre, double-masked, double-dummy, crossover study during 2 × 4 months involving 96 non-obese (body mass index ≤ 27 kg/m²) insulin-naïve T2DM patients. At enrolment, patients stopped prior oral hypoglycaemic agents therapies and after a 1-month run-in period on diet-only treatment, patients were randomized to repaglinide (2 mg) thrice daily followed by metformin (1 g) twice daily or vice versa each during 4 months with 1-month washout between interventions.

Methods: Postprandial metabolism was evaluated by a standard test meal (3515 kJ; 54% fat, 13% protein and 33% carbohydrate) with blood sampling 0–6 h postprandially.

Results: Fasting levels and total area under the curve (AUC) for plasma glucose, triglycerides and free fatty acids (FFA) changed equally between treatments. In contrast, fasting levels and AUC of total cholesterol, low-density lipoprotein (LDL) cholesterol, non-high-density lipoprotein (non-HDL) cholesterol and serum insulin were lower during metformin than repaglinide (mean (95% confidence intervals), LDL cholesterol difference metformin versus repaglinide: AUC: −0.17 mmol/l (−0.26; −0.08)). AUC differences remained significant after adjusting for fasting levels.

Conclusions: In non-obese T2DM patients, metformin reduced postprandial levels of glycaemia, triglycerides and FFA similarly compared to the prandial insulin secretagogue, repaglinide. Furthermore, metformin reduced fasting and postprandial cholesterolaemia and insulinaemia compared with repaglinide. These data support prescription of metformin as the preferred drug in non-obese patients with T2DM targeting fasting and postprandial glucose and lipid metabolism.

European Journal of Endocrinology 158 35–46

Introduction

Individuals with diabetes experience an excess risk of cardiovascular disease (CVD), which is not fully explained by conventional risk factors including fasting dyslipidaemia (1, 2). In individuals with and without diabetes, non-fasting abnormalities in glucose and lipid metabolism have been proposed as independent risk markers for CVD (3–5). In the postprandial state, patients with type 2 diabetes (T2DM) are characterized by more pronounced elevations in levels of glucose, insulin and triglycerides than those seen in non-diabetic individuals (6–9). In individuals with and without T2DM, levels of low-density lipoprotein (LDL) cholesterol have been demonstrated to decrease in the postprandial state (10, 11), and it has been suggested that LDL particles

have an increased atherogenic potential in the postprandial compared with the fasting state (12). Moreover, levels of glycaemia, insulinaemia and insulin sensitivity represent important determinants of postprandial lipidaemia (9, 13). Numerous studies have compared the effect of different antihyperglycaemic treatment regimens on postprandial glucose metabolism in patients with T2DM (14–18). So far, however, only relatively few among these studies included data on postprandial lipidaemia (17, 19–26) and most studies included mainly obese patients with T2DM (15, 16, 18–23, 26). However, among Caucasian patients with T2DM, ~15–20% are not obese (27) and compared with obese patients with T2DM, non-obese patients with T2DM have a similar risk of CVD (28), but are characterized by a more deficient insulin secretion and lesser degree of insulin

resistance (29). In obese patients with T2DM, enhancing insulin sensitivity with a treatment regimen of glitazone and pioglitazone was demonstrated to be more effective in lowering postprandial triglyceridaemia compared with a treatment regimen stimulating insulin secretion by an insulin secretagogue, glibenclamide (19). In non-obese patients with T2DM, it is unknown whether similar differences in postprandial responses apply between treatment regimens targeting insulin sensitivity or insulin secretion. Recently, in non-obese patients with T2DM, our group demonstrated similar overall antihyperglycaemic effect of metformin (a biguanide enhancing insulin sensitivity, lowering hepatic glucose output and improving cardiovascular risk (30)) versus the short-acting insulin secretagogue, repaglinide (a glibenclamide moiety meglitinide analogue—a so-called prandial glucose regulator (31)) (32). Interestingly, studies have suggested that the beneficial metabolic effects (including glycaemia) of metformin, to some unknown extent, may be more pronounced in the non-fasting than in the fasting state (21, 22, 33–37).

Here we report the results of a study evaluating the effects of an insulin-sensitizing treatment regimen using metformin versus an insulin-secretory treatment regimen using repaglinide on fasting and postprandial levels of glycaemia, insulinaemia, triglyceridaemia and cholesterolaemia in non-obese patients with T2DM.

Materials and methods

The study design and results according to the primary end point, HbA_{1c}, has been published in detail previously (32). Briefly, it was an investigator-initiated, single-centre, randomized, double-masked, double-dummy, crossover study of 96 non-obese (body mass index, BMI ≤ 27 kg/m²) insulin-naïve patients with T2DM. At enrolment, patients stopped previous oral hypoglycaemic agents (OHA) therapies and began a 1-month run-in period on diet-only treatment. Patients with HbA_{1c} $\geq 6.5\%$ after the run-in period were randomized to treatment sequences of either 2 mg repaglinide thrice daily followed by 1 g metformin twice daily, or vice versa, each for a period of 4 months with a 1 month washout between interventions. The secondary end points were additional glycaemic and non-glycaemic cardiovascular risk factors (32), including postprandial glucose and lipid metabolism, which have not been published previously. Fasting and postprandial metabolism were measured during the last week before the patient entered a treatment period and on the last day of each treatment period. Of the 96 randomized patients, 20 (21%) dropped out, leaving 76 (79%) patients who completed the trial (83 vs 82 patients completed a treatment period with metformin and repaglinide respectively). Ten (11%) and seven (8%) patients were excluded during metformin and repaglinide treatment respectively. Three (3%) more patients

dropped out during the washout period. Non-vital changes in concomitant medications were postponed until after the trial, and less than seven patients within each non-study medication category started and/or stopped this medication during the trial (32).

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Copenhagen County, Denmark.

Fasting levels of plasma glucose, insulin and C-peptide have been reported elsewhere (32).

Postprandial investigation procedure and blood sampling

Patients were investigated at the Steno Diabetes Center, Gentofte, Denmark, after an overnight fast of at least 10 h. Patients were instructed not to drink alcohol for 3 days before the investigation and were asked not to make changes to their habitual diet and lifestyle for at least 1 month before the day of investigation. On the day of investigation, all patients were served a standard fat-rich breakfast meal (total energy content 3515 kJ) with 54% fat (139 mg cholesterol; 50.4 g fat including saturated 28.8 g, monounsaturated 15.0 g and polyunsaturated fatty acids 2.8 g), 13% protein and 33% carbohydrates (meal ingredients: dark bread 50 g, white bread 60 g, butter 20 g, cheese (60% fat) 40 g, sausage 20 g, jam 30 g, whole milk 200 ml). The morning dose of the study medication was taken immediately before or during the test meal and the pre-lunch dose was postponed until after the investigation. Only drinking water was allowed postprandially. Blood samples were drawn at 0 (fasting), 1.5, 3.0, 4.5 and 6.0 h (postprandial) from the moment when ingestion of breakfast was initiated. All blood samples were drawn with minimal venous occlusion from a dwelling catheter and after 25 min of supine rest. Time 0 was between 0800 and 0900 h.

Biochemical and other analyses

HbA_{1c} was measured by the ion-exchange HPLC method traceable to the Diabetes Control and Complication Trial standard (Bio-Rad VARIANT method, Bio-Rad Diagnostics Group, Hercules CA, USA; normal limits: 4.1–6.4%). Plasma glucose was measured in fresh venous blood samples by the glucose oxidase biosensor method (EuroFlash meter; LifeScan, Milpitas, CA, USA), calibrated for plasma glucose with the YSI Model 2300 Glucose Analyzer, YSI Inc., Yellow Springs, OH, USA. The coefficient of variation between test strips was <0.066 . Serum-specific insulin (excluding des-(31, 32)- and intact proinsulin) and serum C-peptide were measured by time-resolved fluoroimmunoassay (AutoDELFIA; Perkin-Elmer, Waltham, MA, USA). Homeostasis model assessment of β -cell function (HOMA- β) and insulin resistance (HOMA-IR) were calculated according to Matthews *et al.* (38). Plasma for measurement of lipoproteins was separated by

centrifugation at 2000 *g* for 10 min at 20 °C. Samples for measurement of plasma levels of total cholesterol (cholesterol oxidase para amino phenazone, CHOD PAP, Roche Diagnostics, Mannheim, Germany), high-density lipoprotein (HDL) cholesterol (HDL-Plus, homogenous assay, Roche Diagnostics, Mannheim, Germany) and triglycerides (triglycerides glycerol phosphate oxidase para amino phenazone, GPO PAP, Roche Diagnostics, Mannheim, Germany) were drawn in lithium-heparin vacuum tubes and analysed immediately. Plasma non-HDL cholesterol was calculated as the difference between plasma total cholesterol and plasma HDL cholesterol. Plasma LDL cholesterol was calculated using the Friedewald equation (LDL cholesterol (mmol/l) = total cholesterol – HDL cholesterol – triglycerides/2.2) (39). Samples for measurement of free fatty acids (FFA) were drawn in ice-cooled dry vacuum tubes, serum was immediately separated by centrifugation at 2000 *g* for 10 min at +4 °C and stored at –80 °C until analysis by an enzymatic colorimetric method (NEFA C test kit; Wako, Neuss, Germany). Body weight and height were measured with the patient standing upright wearing only underwear. BMI was calculated as body weight/height² (kg/m²). Macrovascular disease was defined as known atherosclerotic disease (e.g. stroke, ischaemic heart disease or peripheral arterial disease). Microalbuminuria and macroalbuminuria were defined as an urinary albumin excretion rate (UAER) 30–299 mg/day or ≥300 mg/day respectively in two out of three consecutive 24-h urine collections prior to enrolment. Neuropathy was defined as symptomatic or clinical signs of peripheral or autonomic neuropathy.

Statistical analysis

We evaluated the randomized population (*n* = 96 patients). However, for treatment effects, only patients who completed the first treatment period (*n* = 89 patients) were included in the analysis. Each outcome was evaluated after the run-in period (before initiating treatment in the first treatment period), referred to as the 'first-period baseline', and at the end of each treatment period, referred to as 'end of treatment'. The target parameter was the differences in treatment effects between interventions referred to as the 'between-treatment effect', evaluated by comparisons of end of treatment levels from both treatment periods with that of the first-period baseline (i.e. the 'change from first-period baseline').

Total area under the curve (AUC) during 0–6 h was estimated as the postprandial summary variable and calculated by the trapezoidal rule in units of concentration × hours, i.e. $1.5 \text{ h} \times (0.5 \times \text{concentration}_{t=1.5 \text{ h}} + \text{concentration}_{t=3.0 \text{ h}} + \text{concentration}_{t=4.5 \text{ h}} + 0.5 \times \text{concentration}_{t=6.0 \text{ h}})$ (40). To obtain comparable units between AUC and fasting levels, AUC values are presented as standardized by time, i.e. AUC divided by 6.0 h, yielding results for AUC given in units of concentration (e.g. mmol/l or equivalent units). When values between 0 and

6 h were missing, the AUC was not calculated (for LDL cholesterol, values of AUC were frequently missing due to the relatively frequent missing postprandial calculation of LDL cholesterol by the Friedewald formulae in the case of postprandial levels of triglycerides > 4.5 mmol/l (39)).

Data were analysed with a linear normal mixed model with the patient as a random effect. Treatment type (metformin or repaglinide), treatment sequence (i.e. the combined effect of treatment sequence, the period by treatment interaction and carry-over), the period effect and the first-period baseline were included as fixed effects. This model enables information from incomplete blocks, i.e. those with dropouts, to be included when estimating treatment effects (i.e. both within- and between-subject information was included) (41). This was the default model. Due to the potential bias of postprandial triglycerides and HDL cholesterol affecting the calculation of LDL cholesterol by the Friedewald equation (39), all statistical analysis of postprandial levels of LDL cholesterol (including the LDL cholesterol analysis in the default model) included adjustment for the current (i.e. the first-period baseline and on-treatment) levels of triglycerides and HDL cholesterol. A subsequent analysis included adjustment for the current (i.e. the first-period baseline and on-treatment) fasting level of all postprandial-dependent variables. The adjustment for fasting levels was preferred to that of analysing unadjusted incremental excursions, e.g. incremental AUC (IAUC; i.e. IAUC obtained by subtraction of fasting levels from individual values at all postprandial times before calculation of AUC), since analysing the unadjusted IAUC does not take into account the potential confounding factor of differences in fasting levels of the dependent variable on the magnitude of IAUC (e.g. low fasting levels potentially tending to lower IAUC and high fasting levels potentially tending to increase IAUC). Such potential differences are accounted for by including the fasting level as a covariate in the between-treatment AUC analysis instead. An exploratory analysis (interaction analysis) was made in subgroups of patients defined by their ongoing statin therapy (i.e. on treatment during the entire study period, *n* = 20), pre-study antihyperglycaemic treatment regimen (i.e. diet only, OHA monotherapy or OHA combination therapy) or known macrovascular disease. Except for use of diuretics, which was more frequent among statin users, only the interaction between statin and study-drug therapy was considered of potential clinical relevance.

Data are presented as the mean (s.d.) or, for non-normally distributed variables, as the median (range) or geometric mean. Treatment effects are given as means (95% confidence intervals (CI)). End points with non-normally distributed residuals or random effects were logarithmically transformed before analysis. The effects for these end points are reported as percentage changes. All data are reported as raw values except for the differences between treatments and changes from first-period baseline, which are reported as derived by the model. The level of significance was 5% (two sided).

All statistical analyses were done with SPSS version 13 (Chicago, IL, USA).

Results

Subject characteristics

Details of patient characteristics at enrolment have been presented elsewhere (32). Briefly, the 96 randomized patients were all Caucasians and predominantly men (women, *n* = 23; men, *n* = 73). At enrolment, their mean age was 61.4 (9.3) years and median known duration of T2DM was 4 (0; 28) years. Mean BMI was 24.8 (2.0) kg/m² and mean HbA_{1c} was 7.45% (0.85) at pre-study antihyperglycaemic treatment (diet only, *n* = 16; OHA monotherapy, *n* = 65; OHA combination therapy, *n* = 15). A total of 30 (31%) patients presented with retinopathy and 27 (28%) patients with micro or macroalbuminuria. A total of 21 (22%) patients had known macrovascular disease and 70 (73%) patients presented with neuropathy. At enrolment, 29 (30%) patients received lipid-lowering treatment as concomitant non-study medication (statin therapy, *n* = 22 (23%) – hereof 20 patients (21%) during the entire study period received fish oil, *n* = 8 (8%); acipimox, *n* = 1 (1%); none of the patients received fibrates). β-Blockers, diuretics or thyroxine were taken by 8, 24 and 2 patients respectively (32). First-period baseline data are summarized in Table 1. At first-period baseline levels of fasting and postprandial, total LDL and non-HDL cholesterol were ~0.5 mmol/l lower in patients with ongoing statin therapy than in those without ongoing statin therapy (*P* < 0.05). A greater proportion of patients received diuretics among statin users (diuretics plus statin therapy: *n* = 9 (41%); diuretics without statin therapy: *n* = 14 (19%); *P* = 0.047). At first-period baseline, other clinical or demographic variables did not differ significantly between patients who were or were not receiving ongoing statin therapy (data not shown).

Fasting and postprandial metabolic variables (without fasting adjustments)

Investigated by the meal test, fasting and postprandial levels (including AUC) of plasma glucose decreased by ~2–4 mmol/l during both treatments (*P* < 0.05), with no significant difference between them (Table 2; Fig. 1). Also, except for slightly higher levels of FFA at 6.0 h during metformin versus repaglinide, fasting and postprandial levels (including AUC levels) of plasma HDL cholesterol, triglycerides and serum FFA changed equally with both treatments (mean (95% CI) difference in FFA at 6.0 h during metformin versus repaglinide: 45 μmol/l (0; 89, *P* = 0.049) (Table 3; Fig. 2). In contrast, except for levels of total cholesterol at 3.0 h, fasting as well as postprandial levels (including AUC) of plasma total cholesterol, LDL cholesterol, non-HDL

Table 1 Fasting and postprandial variables at first-period baseline during a standardized fatty meal test in non-obese patients with type 2 diabetes (data were obtained after a 1-month washout with diet-only treatment after stopping pre-study antihyperglycaemic treatment).

Endpoint	First-period baseline mean (s.d.) or median (range)
Plasma glucose	
Fasting (mmol/l; <i>n</i> = 96)	13.94 (4.07)
AUC (mmol/l; <i>n</i> = 91)	16.67 (4.96)
Serum insulin	
Fasting (pmol/l; <i>n</i> = 96)	29 (3; 137)
AUC (pmol/l; <i>n</i> = 96)	70 (16; 268)
Serum C-peptide	
Fasting (pmol/l; <i>n</i> = 96)	577 (227; 1150)
AUC (pmol/l; <i>n</i> = 96)	1052 (405; 2209)
Plasma total cholesterol	
Fasting (mmol/l; <i>n</i> = 96)	4.95 (0.96)
AUC (mmol/l; <i>n</i> = 93)	4.89 (0.92)
Plasma LDL cholesterol	
Fasting (mmol/l; <i>n</i> = 94)	3.05 (0.74)
AUC (mmol/l; <i>n</i> = 79)	2.76 (0.71)
Plasma non-HDL cholesterol	
Fasting (mmol/l; <i>n</i> = 96)	3.84 (0.98)
AUC (mmol/l; <i>n</i> = 93)	3.85 (0.93)
Plasma HDL cholesterol	
Fasting (mmol/l; <i>n</i> = 96)	1.11 (0.27)
AUC (mmol/l; <i>n</i> = 93)	1.04 (0.26)
Plasma triglycerides	
Fasting (mmol/l; <i>n</i> = 96)	1.45 (0.50; 12.60)
AUC (mmol/l; <i>n</i> = 93)	2.13 (0.70; 11.99)
Serum free fatty acids	
Fasting (μmol/l; <i>n</i> = 96)	541 (203)
AUC (μmol/l; <i>n</i> = 93)	436 (167)
HOMA-β (%) (<i>n</i> = 96)	8.9 (0.6; 46.7)
HOMA-IR (<i>n</i> = 96)	2.2 (0.3; 11.9)

Total area under the curve (AUC) values are expressed per unit time of investigation, i.e. AUC for 360 min divided by 360 min. LDL, low-density lipoprotein; HDL, high-density lipoprotein; Non-HDL, non-high-density lipoprotein; HOMA-β, Homeostasis model assessment of β-cell function; HOMA-IR, Homeostasis model assessment of insulin resistance.

cholesterol, serum insulin and C-peptide were significantly lower during metformin than repaglinide treatments (mean (95% CI) difference in LDL cholesterol during metformin versus repaglinide: AUC: −0.17 mmol/l (−0.26; −0.08), *P* = 0.001; Tables 2 and 3 and Figs 1 and 2). At all individual times, the absolute plasma levels of postprandial LDL cholesterol decreased significantly during metformin treatment when evaluated as a change from first-period baseline (Fig. 2). HOMA-β and HOMA-IR were significantly lower during metformin versus repaglinide treatments.

Postprandial metabolic variables after fasting adjustments

After adjustment for the fasting level of each dependent variable, the following were observed compared with the analyses without fasting adjustment: the between-treatment effects in AUC levels of all postprandial variables did not change substantially (Tables 2 and 3). Also, at individual postprandial times, levels of serum insulin and

Table 2 Comparison of effects of metformin and repaglinide on fasting and postprandial glycaemic variables during a standardized fatty meal test in non-obese patients with type 2 diabetes.

End point	End-of-treatment mean (s.d.) or median (range)		Change from first-period baseline mean (95% confidence interval)		Between-treatment effect mean (95% confidence interval)	
	Metformin	Repaglinide	Δ Metformin	Δ Repaglinide	Metformin versus repaglinide	P
Plasma glucose						
Fasting (mmol/l; $n=89$) ^a	11.24 (3.09)	11.30 (3.44)	-2.15 (-2.65; -1.64)	-2.07 (-2.59; -1.56)	-0.07 (-0.71; 0.57)	0.825
AUC (mmol/l; $n=84$) ^a	12.40 (3.74)	12.50 (4.34)	-3.69 (-4.30; -3.07)	-3.58 (-4.22; -2.95)	-0.10 (-0.86; 0.66)	0.790
AUC fasting adjusted (mmol/l; $n=84$) ^a	—	—	-3.77 (-4.08; -3.47)	-3.67 (-3.98; -3.36)	-0.11 (-0.49; 0.28)	0.585
Serum insulin						
Fasting (pmol/l) ^b ($n=88$) ^a	34 (6; 139)	39 (10; 114)	12 (2; 22) ^c	29 (17; 42) ^c	-13 (-20; -6) ^d	0.001
AUC (pmol/l) ^b ($n=88$) ^a	75 (29; 228)	106 (41; 330)	2 (-5; 10) ^c	41 (31; 52) ^c	-27 (-32; -22) ^d	<0.001
AUC fasting adjusted (pmol/l) ^b ($n=88$) ^a	—	—	6 (-0; 12) ^c	36 (28; 44) ^c	-22 (-27; -16) ^d	<0.001
Serum C-peptide						
Fasting (pmol/l) ^b ($n=88$) ^a	637 (253; 1.340)	753 (253; 2.015)	4 (-1; 10) ^c	20 (14; 26) ^c	-13 (-17; -8) ^d	<0.001
AUC (pmol/l) ^b ($n=88$) ^a	1184 (524; 2.621)	1566 (587; 2.626)	6 (2; 11) ^c	35 (29; 41) ^c	-21 (-25; -17) ^d	<0.001
AUC fasting adjusted (pmol/l) ^b ($n=88$) ^a	—	—	10 (6; 14) ^c	29 (25; 34) ^c	-15 (-19; -11) ^d	<0.001
HOMA- β (%) ^b ($n=88$) ^a	13.7 (2.3; 74.5)	14.2 (2.4; 95.4)	41 (27; 57) ^c	64 (47; 82) ^c	-14 (-24; -3) ^d	<0.015
HOMA-IR ^b ($n=88$) ^a	2.2 (0.3; 7.5)	2.7 (0.6; 7.8)	-5 (-15; 6) ^c	9 (-2; 22) ^c	-13 (-22; -4) ^d	<0.007

Total area under the curve (AUC) values are expressed per unit time of investigation, i.e. AUC for 360 min divided by 360 min. Fasting-adjusted results include the current level at time 0 as a covariate in the analysis. End-of-treatment levels represent raw absolute values, whereas change from first-period baseline and between-treatment effects represents estimates derived from the model. HOMA- β , Homeostasis model assessment of β -cell function; HOMA-IR, Homeostasis model assessment of insulin resistance.

^aTotal number of patients with available data included in the default model (see Statistical analysis section of Material and methods for details). Numbers of patients in each of the two treatment groups at end of treatment and as intrinsic effects are not shown.

^bData are natural logarithmically transformed before analysis of intrinsic and between-treatment effects.

^cPercentage change in the absolute levels at end of treatment versus at first-period baseline.

^dPercentage difference in the absolute change versus first-period baseline between metformin (Δ Met) and repaglinide (Δ Rep): $((\Delta\text{Met}/\Delta\text{Rep}) - 1) \times 100$.

Figure 1 Circulating levels of fasting and postprandial glycaemic variables during a standardized fatty meal test in non-obese patients with type 2 diabetes. Data are included for a maximum of $n=89$ patients with available measurements after the first treatment period. First-period baseline: open squares. After metformin: closed triangles. After repaglinide: closed circles. Values on the abscissa represent time in hours since initiation of ingestion of the test meal (time 0 = fasting). Data are raw values presented as means (s.e.m.), or geometric means for non-normally distributed variables. $^{\dagger}P<0.05$ versus first-period baseline unadjusted. $^{\S}P<0.05$ versus first-period baseline adjusted for the fasting levels of the dependent variable. $^{*}P<0.05$ metformin versus repaglinide unadjusted. $^{\dagger\S}P<0.05$ metformin versus repaglinide adjusted for the fasting levels of the dependent variable.

C-peptide remained significantly different at all postprandial times during metformin versus repaglinide treatments (Fig. 1). Levels of plasma LDL cholesterol remained significantly lower at 6.0 h, whereas levels of plasma total cholesterol and non-HDL cholesterol remained significantly lower at 4.5 and 6.0 h during metformin than repaglinide treatments (Fig. 2). In contrast, levels of plasma glucose at 3.0 h and triglycerides at 1.5 h became slightly lower, whereas levels of plasma HDL cholesterol at 3.0 h became slightly higher during metformin than repaglinide treatments (mean (95% CI) fasting-adjusted difference during metformin versus repaglinide: HDL cholesterol at 3.0 h: 0.02 mmol/l (0.00; 0.03); $P=0.011$; plasma glucose at 3.0 h: -0.6 mmol/l (-1.2 ; -0.0), $P=0.041$; triglycerides at 1.5 h: -5% (-9 ; -2 ; $P=0.003$; Figs 1 and 2). At other postprandial times, the changes in plasma glucose and lipoprotein fractions were of similar magnitude between treatments after adjustment for fasting levels (Figs 1 and 2).

Subgroups of patients according to ongoing statin treatment, pre-study antihyperglycaemic treatment regimens or known macrovascular disease

After fasting adjustment, the test for heterogeneity (interaction) of study-drug treatment by statin therapy was significant at 6.0 h postprandial for plasma total cholesterol ($P=0.024$) and plasma LDL cholesterol ($P=0.023$) (mean (95% CI) plasma LDL cholesterol difference at 6.0 h postprandial with metformin versus repaglinide: Without fasting adjustment: statin treatment ($n=18$): -0.39 mmol/l (-0.62 ; -0.17), $P<0.001$; no statin treatment ($n=55$): -0.16 mmol/l (-0.29 ;

-0.02), $P=0.021$; Fasting adjusted: statin treatment ($n=18$): -0.25 mmol/l (-0.38 ; -0.12), $P=0.001$; no statin treatment ($n=54$): -0.08 mmol/l (-0.15 ; -0.00), $P=0.041$). At fasting or other postprandial times (including AUC), the test for heterogeneity (interaction) between study-drug treatment by statin therapy was not significant for plasma levels of total, LDL or non-HDL cholesterol (data not shown). Also, after fasting adjustment at 6.0 h, the interactions of study drug by diuretic therapy or study drug by statin by diuretic therapy were not significant, whereas the interaction of study drug by statin therapy remained significant for total and LDL cholesterol when all three interaction terms plus the interaction of statin by diuretic therapy were included in the analysis (data not shown).

For all fasting, AUC and fasting-adjusted AUC variables, the test for heterogeneity (interaction) with study-drug treatment by pre-study antihyperglycaemic treatment regimen or known macrovascular disease was not significant (data not shown).

Discussion

Fasting and integrated 6-h postprandial measures of plasma glucose, triglycerides and serum FFA decreased equally after 4 months of metformin versus repaglinide treatment in 96 non-obese patients with T2DM. Furthermore, we found lower fasting and postprandial levels of total, LDL and non-HDL cholesterol, as well as lower serum insulin levels, in those receiving metformin compared with repaglinide. The between-treatment

Table 3 Comparison of effects of metformin and repaglinide on fasting and postprandial lipid variables during a standardized fatty meal test in non-obese patients with type 2 diabetes.

Endpoint	End-of-treatment mean (s.d.) or median (range)		Change from first-period baseline mean (95% confidence interval)		Between-treatment effect mean (95% confidence interval)	
	Metformin	Repaglinide	ΔMetformin	ΔRepaglinide	Metformin versus repaglinide	P
Plasma total cholesterol						
Fasting (mmol/l; n=89) ^a	4.73 (0.86)	4.89 (0.85)	−0.23 (−0.34; −0.12)	−0.10 (−0.21; 0.01)	−0.13 (−0.25; −0.02)	0.026
AUC (mmol/l; n=85) ^a	4.68 (0.83)	4.85 (0.85)	−0.24 (−0.34; −0.14)	−0.08 (−0.18; 0.02)	−0.16 (−0.27; −0.05)	0.006
AUC fasting adjusted (mmol/l; n=85) ^a	—	—	−0.20 (−0.24; −0.17)	−0.15 (−0.18; −0.11)	−0.06 (−0.10; −0.01)	0.010
Plasma LDL cholesterol						
Fasting (mmol/l; n=87) ^a	2.81 (0.68)	3.04 (0.76)	−0.25 (−0.34; −0.15)	−0.06 (−0.16; 0.04)	0.19 (−0.29; −0.08)	<0.001
AUC (mmol/l; n=69) ^a	2.56 (0.70)	2.78 (0.71)	−0.23 (−0.32; −0.13)	−0.06 (−0.15; 0.04)	−0.17 (−0.26; −0.08)	<0.001
AUC fasting adjusted (mmol/l; n=69) ^a	—	—	−0.18 (−0.21; −0.15)	−0.13 (−0.16; −0.10)	−0.05 (−0.10; −0.01)	0.027
Plasma non-HDL cholesterol						
Fasting (mmol/l; n=89) ^a	3.55 (0.88)	3.73 (0.89)	−0.29 (−0.40; −0.18)	−0.14 (−0.25; −0.03)	−0.15 (−0.27; −0.04)	0.011
AUC (mmol/l; n=84) ^a	3.53 (0.85)	3.74 (0.88)	−0.32 (−0.42; −0.22)	−0.14 (−0.24; −0.04)	−0.18 (−0.29; −0.07)	0.001
AUC fasting adjusted (mmol/l; n=84) ^a	—	—	−0.27 (−0.30; −0.24)	−0.21 (−0.25; −0.18)	−0.06 (−0.10; −0.02)	0.005
Plasma HDL cholesterol						
Fasting (mmol/l; n=89) ^a	1.18 (0.28)	1.16 (0.33)	0.06 (0.03; 0.10)	0.04 (0.01; 0.08)	0.02 (−0.02; 0.06)	0.276
AUC (mmol/l; n=84) ^a	1.13 (0.27)	1.11 (0.31)	0.07 (0.03; 0.11)	0.06 (0.02; 0.10)	0.01 (−0.02; 0.05)	0.474
AUC fasting adjusted (mmol/l; n=84) ^a	—	—	0.06 (0.05; 0.07)	0.06 (0.05; 0.07)	0.00 (−0.01; 0.01)	0.925
Plasma triglycerides						
Fasting (mmol/l) ^b (n=89) ^a	1.24 (0.40; 8.68)	1.46 (0.40; 7.68)	−7 (−14; 1) ^c	−9 (−17; −1) ^c	3 (−5; 12) ^d	0.478
AUC (mmol/l) ^b (n=85) ^a	2.03 (0.69; 8.28)	1.98 (0.66; 8.85)	−6 (−12; 1) ^c	−10 (−17; −3) ^c	5 (−2; 12) ^d	0.164
AUC fasting adjusted (mmol/l) ^b (n=85) ^a	—	—	−9 (−12; −5) ^c	−8 (−11; −5) ^c	−1 (−5; 4) ^d	0.751
Serum-free fatty acids						
Fasting (μmol/l; n=88) ^a	537 (199)	519 (231)	4 (−34; 42)	−14 (−52; 25)	18 (−26; 62)	0.418
AUC (μmol/l; n=84) ^a	366 (116)	344 (122)	−50 (−69; −32)	−66 (−85; −46)	16 (−5; 36)	0.143
AUC fasting adjusted (μmol/l; n=84) ^a	—	—	−52 (−69; −36)	−63 (−80; −46)	11 (−8; 30)	0.259

Total area under the curve (AUC) values are expressed per unit time of investigation, i.e. AUC for 360 min divided by 360 min. Fasting-adjusted results include the current level at time 0 as a covariate in the analysis. End of treatment levels represent raw absolute values, whereas change from first-period baseline and between-treatment effects represent estimates derived from the model. LDL, low-density lipoprotein; HDL, high-density lipoprotein; Non-HDL, non-high-density lipoprotein.

^aTotal number of patients with available data included in the default model (see statistical section of Materials and methods for details). Numbers of patients in each of the two treatment groups at end of treatment and as change from first-period baseline are not shown.

^bData are natural logarithmically transformed before analysis of change from first-period baseline and between-treatment effects.

^cPercentage change in the absolute levels at end of treatment versus the absolute levels at first-period baseline.

^dPercentage difference in the absolute change from first-period baseline between metformin (ΔMet) and repaglinide (ΔRep): ((ΔMet/ΔRep) − 1) × 100.

Figure 2 Circulating levels of fasting and postprandial lipids during a standardized fatty meal test in non-obese patients with type 2 diabetes. Data are included for a maximum of $n=89$ patients with available measurements after the first treatment period. First-period baseline: open squares. After metformin: closed triangles. After repaglinide: closed circles. Values along the abscissa represent time in hours since initiation of ingestion of the test meal (time 0 = fasting). Except for free fatty acids, the values on the ordinate represent an interval of exactly 2.0 mmol/l. Hence, despite the level of 0 not being included on the ordinate for all variables, postprandial changes can be readily compared between variables. Postprandial analyses of LDL cholesterol are adjusted for the current levels of triglycerides and HDL cholesterol. Data are raw values presented as means (S.E.M.), or geometric means for non-normally distributed variables. $^{\dagger}P < 0.05$ versus first-period baseline unadjusted. $^{\ddagger}P < 0.05$ versus first-period baseline adjusted for the fasting levels of the dependent variable. $^{\ast}P < 0.05$ metformin versus repaglinide unadjusted. $^{\ast\ast}P < 0.05$ metformin versus repaglinide adjusted for the fasting levels of the dependent variable. a Mean (95% CI) LDL cholesterol difference at 3.0 h, repaglinide versus first-period baseline: unadjusted: 0.01 mmol/l (−0.09; 0.10); fasting adjusted: −0.06 mmol/l (−0.10; −0.01). AUC, total area under the curve; LDL, low-density lipoprotein; HDL, high-density lipoprotein; non-HDL, non-high-density lipoprotein.

differences in integrated postprandial measures were independent of those in the fasting state.

This parallel reduction of meal-test plasma glucose levels between treatments is consistent with our previous report of similar reductions in HbA_{1c} and the mean of seven-point, home-monitored plasma glucose levels during both treatments (only pre-dinner plasma glucose was significantly lower by 0.85 mmol/l during repaglinide versus metformin treatments) (32). In an open-labelled study, in predominantly non-obese patients with T2DM, Derosa *et al.* observed favourable post-lunch antihyperglycaemic effect with repaglinide versus

metformin. Both drugs were administered thrice daily (repaglinide, pre-meal; metformin, post-meal) and non-fasting plasma glucose was measured only 2.0 h post-lunch (14). Hence, differences in dosing schedules and/or the number of daily plasma glucose measurements might explain the discrepancies between studies.

In contrast, in obese patients with T2DM, Furlong *et al.* demonstrated favourable fasting and postprandial plasma glucose levels (seven-point profile) and HbA_{1c} with metformin compared with repaglinide, both in combination with insulin (15). Compared with obese patients with T2DM, non-obese patients with T2DM are

characterized by disproportional reduced insulin secretion (29). Nevertheless, our present study in non-obese patients with T2DM showed that treatment with a prandial insulin secretagogue was not more efficient than metformin in reducing postprandial glucose excursions. This notion, which also extends to the observed similarity between-treatments effects on triglycerides and FFA, is remarkable, although not completely unexpected since other studies, in predominantly obese patients with T2DM, suggested metformin to have enhanced efficacy in the non-fasting versus the fasting state (21, 22, 33–37). Indeed, our data suggest that, in non-obese patients with T2DM, increasing insulin sensitivity could be equally – or even more – effective by targeting postprandial metabolism than by stimulating insulin secretion. In fact, much similar conclusions have also been suggested in obese patients with T2DM treated with pioglitazone or glibenclamide (19). Thus, our data suggest that the fasting and postprandial beneficial effects of metformin therapy are not restricted to obese subjects, but also are relevant for non-obese patients with T2DM.

Measures of insulinaemia and insulin resistance have been demonstrated as independent predictors of CVD (42, 43). Hence, our observation of decreased levels of insulinaemia and improved insulin action (as estimated by the HOMA model) could have beneficial cardiovascular effects during metformin versus repaglinide therapy. This should be taken into account when choosing among present and future glucose-lowering drugs. However, our findings must be interpreted cautiously since the HOMA-model assumptions are not necessarily fulfilled under conditions of pharmacologically altered insulin secretion/action (44).

Also, the lower fasting and postprandial levels of plasma total, LDL and non-HDL cholesterol during metformin versus repaglinide treatment might have significant clinical implications. Firstly, fasting and postprandial levels of plasma total, LDL and non-HDL cholesterol represent independent risk factors for the development of CVD (45, 46) and in the United Kingdom Prospective Diabetes Study, levels of LDL cholesterol was the single most powerful predictor of CVD (47). As noted in the recent National Cholesterol Education Programme report, data from clinical trials suggest that a 1% lowering of LDL cholesterol equals a 1% lowering of cardiovascular events with no lower threshold of LDL cholesterol below which no further reduction in risk occurs (48). Accordingly, data from the National Health and Nutrition Examination Surveys from 1976 to 1991 demonstrated a decline in levels of LDL cholesterol in the US population of 0.21 mmol/l (i.e. 5–10% decrease), which was suggested to account for 8–17% of the concomitant decline in CVD (49, 50). Hence, in non-obese patients with T2DM, our observation of a mean ~ 0.2 mmol/l (i.e. 5–10%) reduction in plasma LDL cholesterol with metformin versus repaglinide (i.e. a magnitude of reduction in LDL cholesterol comparable to the effect of doubling the dose of a statin (51)) might potentially reduce the relative risk for

future CVD by 5–10%. Secondly, the lowering of cholesterolaemia with metformin was achieved despite 20–25% of patients receiving statin treatment during the trial. In fact, interaction analysis at single postprandial times suggested that the decrease in postprandial levels of cholesterolaemia could even be enhanced during metformin treatment in patients receiving statin therapy compared with those who did not. This is important since guidelines now recommend statin therapy for almost all patients with diabetes (52). However, despite ongoing statin therapy, many patients with diabetes do not attain the recommended goals of cholesterolaemia (53). Hence, in non-obese patients with T2DM, additional lipid-lowering therapy in conjunction with statins, e.g. by metformin, could be beneficial.

In patients with T2DM, previous studies suggested that metformin lowered fasting levels of total and LDL cholesterol by ~ 0.2 – 0.3 mmol/l (54–56). Although many previous studies of the effect of metformin on fasting FFA and triglycerides were inconclusive (54, 55), a recent meta-analysis suggested a triglyceride-rich lowering effect of ~ 0.11 – 0.34 mmol/l of either metformin or repaglinide (56). Otherwise, there are no equivalent conclusive data for repaglinide (55–57). Data concerning the effects of either drug on postprandial lipids in patients with T2DM suggested a decrease in remnant lipoprotein cholesterol levels, FFA and triglycerides during metformin monotherapy in obese patients with T2DM (21, 22, 36, 37). Tentolouris *et al.* did not find a significant effect on postprandial lipids of a 2 mg single dose of repaglinide (57), whereas Rizzo *et al.* demonstrated significantly higher levels of postprandial HDL cholesterol during treatment with repaglinide compared with glimepiride in patients with T2DM (25).

Hence, to our knowledge, the present study is the first to report differential effects between two pharmaceutical compounds on postprandial levels of proatherogenic cholesterol-rich lipoprotein fractions (i.e. plasma levels of total, LDL and non-HDL cholesterol), independently of fasting levels. Also, there was no significant interaction of macrovascular disease by study-drug treatment for any of the variables – in support of an independent lipid-lowering effect of metformin treatment. In individuals with or without T2DM, pharmacological lowering of postprandial glycaemia or cholesterolaemia has been associated with a decrease in CVD and surrogate markers of CVD (18, 58, 59) for glycaemia apparently not explained by differences in fasting glycaemia or HbA_{1c} (18, 59). However, in contrast to plasma glucose which is only quantitatively changed, triglyceridaemia and cholesterolaemia differ both quantitatively (10, 11) as well as qualitatively (e.g. size and lipid composition of lipoprotein particles) (60–62) between the fasting and the postprandial states. These lipoprotein particle changes have been linked to CVD (62). Thus, the additional lowering of postprandial cholesterolaemia during metformin therapy might contribute to the mechanisms by which metformin treatment improves cardiovascular risk.

We used a high-fat, low-carbohydrate test meal with the purpose of investigating primarily postprandial lipid metabolism. High-fat test meals are known to increase postprandial triglyceride levels more than low-fat meals (63, 64). Our time of postprandial investigation was 6 h, which is sufficient to detect the most acute changes after a single meal in individuals with T2DM (65).

From the present trial, we recently demonstrated decreased seven-point home-monitored plasma glucose profiles within the first 1–2 months after study-drug initiation. Hereafter, plasma glucose profiles stabilized for the remaining 2–3 months with both treatments (32). In our opinion, this suggests sufficient metabolic stability for reliable evaluation of glucose and lipid variables after 4-month intervention.

The changes in levels of AUC for triglycerides and HDL cholesterol did not differ significantly between treatments. Very importantly, the differences in all postprandial measures of plasma LDL cholesterol were demonstrated despite statistical adjustments were made for any between-treatment differences in the current levels of postprandial triglycerides and HDL cholesterol. Hence, in our opinion, the overall between-treatment effects on postprandial plasma LDL cholesterol levels seem valid, despite the potential confounding factors of the triglycerides (or chylomicrons) and HDL cholesterol used in the Friedewald equation (39). We did not measure apolipoproteins (e.g. ApoB and ApoA1). However, levels of total cholesterol, like the ApoB/ApoA1 ratio, represent direct plasma measurements of predominantly proatherogenic cholesterolaemia. We therefore expect that apolipoprotein data, had they been available, would have shown much similar (favourable) effects during metformin treatment as obtained for levels of total cholesterol.

Non-vital changes in concomitant medications were postponed until after the trial. Also, despite that the more frequent use of diuretics among statin users, this did not affect the study drug by statin interaction on levels of postprandial cholesterolaemia. We therefore do not expect potential effects of concomitant non-statin medications with potential influence on lipid metabolism (e.g. β -blockers, diuretics or thyroxine) to explain the differences in lipid variables between treatments.

In conclusion, in non-obese patients with T2DM, levels of postprandial glycaemia, triglycerides and FFA, as determined during a fatty meal test, decreased to a similar extent after 4-month treatment with metformin or the prandial insulin secretagogue, repaglinide. However, independent of fasting levels, postprandial cholesterolaemia and insulinaemia were lower with metformin than with repaglinide. Using previous epidemiologic and clinical trial data, the between-treatment differences in cholesterolaemia with metformin compared with repaglinide may be estimated to potentially decrease the risk of CVD by ~ 5 –10%. Therefore, improving postprandial glucose and lipid metabolism might, in non-obese patients with T2DM, be achieved equally or more effectively by targeting insulin sensitivity rather than insulin secretion.

In non-obese patients with T2DM, metformin therapy may have additional cardiovascular protective potentials and may with these data be included among prandial glucose regulators.

Acknowledgements

We would like to thank the following people and companies for their great help and support in carrying out this study: Novo Nordisk A/S, Bagsværd, Denmark, for financial support and provision of study medication; The Clinical Development Foundation at the Steno Diabetes Center for financial support; Hexal A/S (GEA Ltd), Hvidovre, Denmark, for supplying study medication and LifeScan Johnson & Johnson for technical provision; Mari-Anne Gall and Kirstine Brown Frandsen for their enthusiastic support in initiating and carrying out the trial; Bente Blaaholm Nielsen, the trial nurse; Birgitte Vilsbøl Hansen, Tina Ragnholm Juhl, Berit Ruud Jensen, Lotte Pietraszek and Ingelise Rossing, the laboratory technicians; Ellis Tauber-Lassen, the dietician; Annalise Klausen and all staff employed in the kitchen of Steno Diabetes Center for preparing test meals. Novo Nordisk A/S and the Clinical Development Foundation at the Steno Diabetes Center co-sponsored the study financially. Novo Nordisk A/S supplied repaglinide and repaglinide placebo tablets. Hexal A/S (GEA Ltd) supplied metformin and metformin placebo tablets. LifeScan Johnson & Johnson supplied blood glucose hand devices and test strips. The sponsors took no part in the study design, the collection, analysis and interpretation of the data, the production of the report or the decision to submit the paper for publication.

Søren Sogaard Lund, Lise Tarnow, Merete Frandsen, Ulla Meng Smidt, Oluf Pedersen, Hans-Henrik Parving and Allan Vaag have reported equity in Novo Nordisk A/S. Hans-Henrik Parving and Allan Vaag have received funds from Novo Nordisk A/S for research. Søren Sogaard Lund and Allan Vaag have received fees from Novo Nordisk A/S for speaking and Allan Vaag has received fees from Novo Nordisk A/S for organizing education. Allan Vaag is a member of the editorial board for European Journal of Endocrinology.

Søren Sogaard Lund, Lise Tarnow, Merete Frandsen, Ulla Meng Smidt, Oluf Pedersen, Hans-Henrik Parving and Allan Vaag are present or former employees at Steno Diabetes Center, Gentofte, Denmark. Steno Diabetes Center is an independent academic institution owned by Novo Nordisk A/S and The Novo Nordisk Foundation.

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Received 2 October 2007

Accepted 17 October 2007