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The impact of *CYP2C8* polymorphism and grapefruit juice on the pharmacokinetics of repaglinide

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Aims
The primary aim of the study was to investigate the possible effect of the *CYP2C8*3* allele and of grapefruit juice on the pharmacokinetics of repaglinide. Furthermore, the impact of a single dose of grapefruit juice on the pharmacokinetics of repaglinide in relation to dose.

Methods
Thirty-six healthy male subjects, genotyped for *CYP2C8*3* (11 genotyped as *CYP2C8*1/*3*, one as *CYP2C8*3/*3 and* 24 as *CYP2C8*1/*1*), participated in a randomized, cross-over trial. In the two phases, the subjects drank 300 mL water or 300 mL grapefruit juice, in randomized order, 2 h before administration of a single dose of either 0.25 mg or 2 mg repaglinide.

Results
Neither the mean $AUC_{0-\infty}$ (geometric mean ratio: 1.01; 95% CI: 0.93–1.1, $P = 0.88$) nor the mean C_{max} (geometric mean ratio: 1.05; 95% CI: 0.94–1.2, $P = 0.35$) of repaglinide were statistically significantly different in the group carrying the *CYP2C8*3* mutant allele compared with wild-types. Grapefruit juice caused a 19% decrease in the geometric mean ratio of the 3-hydroxyquinidine to quinidine ratio (difference: 0.81; 95% CI: 0.75–0.87, $P < 0.0001$), which was used as an index of CYP3A4 activity, and an increase in the mean $AUC_{0-\infty}$ of repaglinide (geometric mean ratio: 1.13; 95% CI: 1.04–1.2, $P = 0.0048$), but had no statistically significant effect on the $t_{1/2}$. There was no statistically significant difference in blood glucose concentration in subjects who had or had not ingested grapefruit juice. The effect was more pronounced at the low dose of repaglinide (0.25 mg) than at the therapeutic dose of 2 mg.

Conclusions
The pharmacokinetics of repaglinide in subjects carrying the *CYP2C8*3* mutant allele did not differ significantly from those in the wild-types. Grapefruit juice increased the bioavailability of repaglinide, suggesting significant intestinal elimination of the drug which was assumed to be primarily mediated by CYP3A4 in the gut.

Introduction

Repaglinide is a short-acting, oral, insulin secretagogue that is used in the treatment of type 2 diabetes mellitus. It is rapidly absorbed (t_{max} of 0.5–1.0 h), has an absolute bioavailability after oral administration of about 60% [1] and its $t_{1/2}$ is approximately 1 h [2]. Repaglinide is almost completely metabolized by cytochrome P450 enzymes and less than 2% of an oral dose is excreted unchanged in humans [3].

CYP2C8 and CYP3A4 have been found to contribute to the *in vitro* metabolism of repaglinide [4]. The CYP3A4 inhibitors ketoconazole and clarithromycin have shown small but statistically significant effects on repaglinide AUC (15% and 40% increase, respectively), with a more pronounced effect on C_{max}, whereas $t_{1/2}$ was relatively unaffected [5, 6]. The CYP3A4 and CYP2C inducer rifampicin has been shown to increase the human metabolism of repaglinide [7, 8]. Gemfibrozil and trimethoprim, both inhibitors of CYP2C8 [9, 10] decrease the metabolism of repaglinide (increasing its AUC by 8-fold and 61%, respectively), and prolong its $t_{1/2}$ by 2.4 h and 0.2 h, respectively [11, 12]. Furthermore, gemfibrozil in combination with the CYP3A4 inhibitor itraconazole increased the AUC of repaglinide by 19.4-fold and prolonged the blood glucose-lowering effect of the latter in healthy subjects [11]. Itraconazole alone had no significant effect on the $t_{1/2}$ and only a minor effect on the AUC of repaglinide (causing a 1.4-fold increase) [11]. Thus, these *in vivo* data suggest a relatively high contribution of CYP2C8 to the hepatic clearance of the drug, whereas the contribution of CYP3A4 seems to be most pronounced during first pass in the gut and liver, or possibly if CYP2C8 activity is decreased.

Several mutations of the *CYP2C8* gene have been characterized, namely *CYP2C8*2* (Ile296Phe, with a frequency of 0.18 in African-Americans [13]), *CYP2C8*3* (Arg139Lys, Lys399Arg, with a frequency of approximately 0.13 in Caucasians [13]), C*YP2C8*4* (Ile264Met, with frequency of 0.075 in a Caucasian population [14]) and C*YP2C8*5* (471delA, frameshift early stop codon, identified in one Japanese individual [15]). *CYP2C8*3* has showed a decreased *in vitro* biotransformation of paclitaxel and arachidonic acid. However, a previous *in vivo* study has shown that subjects receiving a subtherapeutic (0.25 mg) dose of repaglinide and who carry the *CYP2C8*3* mutant allele, had a 45% lower mean AUC and a 39% lower C_{max}, compared with subjects carrying two *CYP2C8*1* alleles [16].

Ingestion of grapefruit juice causes inhibition of intestinal CYP3A4 activity [17]. Different furanocoumarin derivatives in grapefruit juice are probably responsible for this effect [17–19], because they cause a mechanism-based inactivation of CYP3A4 [17] mainly in the gut wall [20]. Grapefruit juice inhibits the activities of CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2E1, CYP2D6 *in vitro* [17]. However, its effect *in vivo* is limited [21, 22], probably due to the lower abundance of these CYPs in the gut wall [23].

The primary aim of the present study was to investigate the effect of the *CYP2C8*3* single nucleotide polymorphism on the pharmacokinetics of repaglinide given at a therapeutic dose. A secondary aim was to characterize the effect of a single dose of grapefruit juice on the pharmacokinetics of repaglinide given at different doses.

Methods

This was an open-labelled, randomized, cross-over trial and subjects were divided into two groups, based on *CYP2C8* genotype. Group 1 comprised 12 subjects carrying the *CYP2C8*3* mutant allele (11 with the *CYP2C8*1/*3* genotype and one with the *CYP2C8*3/*3* genotype). Group 2 comprised 24 subjects with the *CYP2C8*1/*1* wild-type genotype. The grapefruit juice used in the study was from the same production batch (Rynkeby Foods A/S, Denmark, L4823BD2/19NOV04).

The subjects were to drink water or grapefruit juice 2 h before administration of a single dose of either 0.25 mg or 2 mg repaglinide (see Table 1).

Subjects

Thirty-six nonsmoking, healthy male subjects aged 22–30 years were studied. All subjects gave written informed consent and the Regional Ethical Committee of Vejle and Funen Counties and the Danish National Board of Health approved the study, which was performed according to the Declaration of Helsinki.

Before inclusion, physical examination, laboratory testing and electrocardiography were performed (visit 1). Consumption of caffeine-containing food/beverages or alcohol was not allowed on study days. Subjects were asked not to consume grapefruit or grapefruit juice (except for the purpose of the study) during the whole experimental period.

Study procedure and sample collection

At visit 2, a 200-mg single oral dose of quinidine sulphate was administered with 150 mL water following an overnight fast. A single spot blood sample (10 mL) was collected after 4 h to obtain baseline measurements of the 3-hydroxyquinidine/quinidine ratio, used as a measure of CYP3A4 activity [24]. At visit 3, the subjects (following an overnight fast) drank 300 mL grapefruit juice, 2 h before taking the quinidine capsule with 150 mL water, and again a single blood sample (10 mL) was collected after 4 h. Plasma was separated by centrifugation (10 min at 7000 *g*) and frozen at −20 °C until analysis.

At visit 2, the 12 subjects in group 1 were randomized to follow either schedule 1 or 2 and the 24 subjects in group 2 were randomized to follow either schedule 1, 2, 3 or 4.

Table 1
Summary of trial design

Visit	Group 1: Mutant allele (12 subjects)		Group 2: Wild-type (24 subjects)			
1 2 3	Screening Baseline quinidine 200 mg Grapefruit juice 300 mL/quinidine 200 mg		Screening Baseline quinidine 200 mg Grapefruit juice 300 mL/quinidine 200 mg			
	Repaglinide 2 mg		Repaglinide 2 mg		Repaglinide 0.25 mg	
	Sch. 1 (6 subj.)	Sch. 2 (6 subj.)	Sch. 1 (6 subj.)	Sch. 2 (6 subj.)	Sch. 3 (6 subj.)	Sch. 4 (6 subj.)
4 5	Water/repaglinide 2 mg Grapefruit juice/ repaglinide 2 mg	Grapefruit juice/ repaglinide 2 mg Water/repaglinide 2 mg	Water/repaglinide 2 mg Grapefruit juice/ repaglinide 2 mg	Grapefruit juice/ repaglinide 2 mg Water/repaglinide 2 mg	Water/repaglinide 0.25 mg Grapefruit juice/ repaglinide 0.25 mg	Grapefruit juice/ repaglinide 0.25 mg Water/repaglinide 0.25 mg
6	Follow-up visit		Follow-up visit			

Sch. = schedule, subj. = subject.

In schedule 1, the subjects drank 300 mL water at visit 4, after an overnight fast. Thirty minutes later, they were given an individualized standard breakfast. This was chosen by the subject, but was identical to that eaten at visit 4 and 5 and did not contain grapefruit/grapefruit juice or caffeine-containing food/beverages or alcohol. After a further 1.5 h, 2 mg of repaglinide was administered with 150 mL of water. Blood samples (4 mL) were collected from venous catheter at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6 and 7 h. Samples were cooled to 0 °C and after 20–30 min, serum was obtained by centrifugation (10 min at 7000 *g*, 4 °C) and stored at −20 °C until analysis. After a washout period of a minimum of 7 days, the subjects attended visit 5. Following an overnight fast, they drank 300 mL grapefruit juice, and 30 min later, they ate their standard breakfast (containing the same as at visit 4). After a further 1.5 h, 2 mg repaglinide was administered with 150 mL of water. Blood samples were taken as described above.

Schedules 2, 3, and 4 were performed in exactly the same way as schedule 1, with the following exceptions (Table 1).

In schedule 2, subjects drank 300 mL grapefruit juice at visit 4 and 300 mL water at visit 5. In schedule 3 the repaglinide dose was 0.25 mg. In schedule 4 subjects drank 300 mL grapefruit juice at visit 4 and 300 mL water at visit 5 and the repaglinide doses were 0.25 mg.

Blood glucose measurements were performed 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6 and 7 h after repaglinide administration, and glucose containing fluids and fruit (not grapefruit) was given when glucose concentrations fell below 3.6 mmol L^{-1}. Adverse events were recorded on the days of medication. At the follow-up visit control, physical examination and laboratory testing were performed.

CYP2C8 genotyping

Genotyping for the *CYP2C8*3* variant was performed according to a previously described procedure [25].

Drug and metabolite analysis

Serum repaglinide concentrations were measured by a previously described LC-MS/MS assay [26]. The limit of quantification was 0.55 nM (0.25 ng mL^{-1}). The assay was validated in the concentration range 0.55–552.25 nM (0.250–250 ng mL^{-1}) and the interassay coefficients of variation were <10%.

Quinidine and 3-hydroxyquinidine in plasma were analysed by a previously described HPLC method [27]. The limit of quantification was 5 nM (1.62 ng mL^{-1}) for quinidine and 3 nM (1.02 ng mL^{-1}) for 3-hydroxyquinidine, and the coefficient of variation was <5% determined at concentrations between 0.25 and 10.0 μM for

both compounds. The metabolic ratio was defined as the 3-hydroxyquinidine/quinidine ratio in plasma.

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated by non-compartmental methods using the software package WinNonLin Pro Node PAL (Pharsight, Mountain View, CA, USA). The area under the serum concentration-time curve (AUC) of repaglinide was calculated using the linear trapezoidal method with extrapolation to infinity. Values for C_{max} (maximum serum concentration) and t_{max} (time to maximum serum concentration) were obtained directly from the data. The terminal elimination half-life ($t_{1/2}$) of repaglinide was calculated from the expression: $t_{1/2} = \ln 2/\lambda$, where λ is the terminal slope of the log serum concentration-time profile.

Statistical analyses

Sample size calculations were based on the primary outcome, represented by differences in repaglinide $AUC_{0-\infty}$ in carriers of *CYP2C8*3* and wild-types. It was estimated that a true difference of at least 40% in AUC could be detected, given a two-sided α of 0.05 and a β of 80%, using an unbalanced design with 12 subjects in the mutant group and 24 in the wild-type group.

An unbalanced design was used as twice as many wild-types as carriers of *CYP2C8*3* would enter the study. The pharmacokinetics of repaglinide in the wild-type group was studied at different doses in a randomized, parallel design. Proportionality with regard to repaglinide AUC has been established within the dose-range (0.25–2 mg) studied [28], and all 24 subjects were included in the mutant/wild-type analysis using a dose-corrected AUC.

The data are presented as median values with 90% interpercentile range. Before statistical analysis, all data except t_{max} were transformed to their natural logarithms to create a Gaussian distribution.

Analysis of the effects of *CYP2C8*3* were performed using unpaired *t*-tests. Geometric ratios of mean data (baseline wild-types/baseline mutant allele) with 95% confidence intervals (CI) and *P*-values are presented.

Analysis of the effects of grapefruit juice was performed using paired *t*-tests. Geometric ratio of mean data (grapefruit juice/baseline) with 95% confidence intervals and *P*-values are presented. The effects on t_{max} are Hodges-Lehmann estimates of median differences with exact 95% confidence intervals. Statistical analyses were performed using GraphPad Prism™ v 3.03 and GraphPad QuickCalcs (http://graphpad.com/quickcalcs/index.cfm; GraphPad Software, Inc., San Diego, California USA), StatXact-3 (Cytel Software Corporation, Cambridge, Mass., USA) and Microsoft Excel.

Results

All subjects completed the study. No side-effects were noted except for one case of headache probably due to caffeine abstinence, and one of dysuria, probably not connected to the intake of study drugs. Blood glucose concentrations ranged from 2.2 to 7.7 mmol L^{-1} during days with repaglinide administration without symptoms of hypoglycaemia, because glucose was given when measurements fell below 3.6 mmol L^{-1}. All control laboratory tests were within normal values.

The results of the effects of the *CYP2C8*3* polymorphisms on repaglinide pharmacokinetics are summarized in Table 2 and Figure 1. Neither the mean $AUC_{0-\infty}$ (geometric mean ratio: 1.01; 95% CI: 0.93–1.1, $P = 0.88$) nor the mean C_{max} (geometric mean ratio: 1.05; 95% CI: 0.94–1.2, $P = 0.35$) of repaglinide were significantly different in the group carrying the *CYP2C8*3* mutant allele (group A, Table 2) compared with wild-type subjects who received 2 mg repaglinide (group B, Table 2). Repaglinide $AUC_{0-\infty}$ was also not significantly different in group A compared with the dose-corrected $AUC_{0-\infty}$ in all 24 wild-type subjects (group B + C, Table 2) (geometric mean ratio: 1.04; 95% CI: 0.95–1.1, $P = 0.37$).

The results of the effects of grapefruit juice are summarized in Tables 3 and 4 and illustrated in Figure 1. Grapefruit juice decreased the geometric mean of 3-hydroxyquinidine to quinidine ratio in the whole group of 36 subjects by approximately 19% (geometric mean ratio: 0.81; 95% CI: 0.75–0.87, $P < 0.0001$).

Grapefruit juice caused a statistically significant increase in the mean $AUC_{0-\infty}$ and dose-corrected mean value in the entire group of 36 subjects (geometric mean ratio: 1.13; 95% CI: 1.04–1.2, $P = 0.0048$). There was no statistical significant difference in blood glucose concentrations (lowest value recorded between 0 and 2 h following repaglinide administration) between days with and without grapefruit juice (mean difference (grapefruit juice minus baseline): –0.17; 95% CI: –0.39–0.055, $P = 0.14$, paired *t*-test).

Stratified by genotype and repaglinide dose as described above (Table 4), only a significant increase in mean repaglinide $AUC_{0-\infty}$ in group A (geometric mean ratio: 1.11; 95% CI: 1.01–1.2, $P = 0.028$) and a significant increase in mean C_{max} in group C (geometric mean ratio: 1.3; 95% CI: 1.04–1.7, $P = 0.028$) were found after grapefruit juice intake. A statistically significant

Table 2
The effect of of *CYP2C8*3* mutation on repaglinide pharmacokinetics in 36 healthy male subjects divided into group (A) (B) and (C) after a single dose of either 2 mg or 0.25 mg repaglinide. (A) Carriers of *CYP2C8*3* (2 mg repaglinide) ($n = 12$); (B) Wild-type (2 mg repaglinide) ($n = 12$); (C) Wild-type (0.25 mg repaglinide) ($n = 12$)

Group	Median of Baseline results and 90% interpercentile range			Geometric mean ratio, 95% confidence interval and *P* value	
Parameter	A	B	B + C	(Baseline B/Baseline A)	(Baseline B + C/Baseline A)
C_{max} (ng/mL)	19 (9.3–31)	19 (15–34)	–	1.05 [0.94; 1.2] $P = 0.35$	–
AUC(0→∞) (ng*h/mL)	26 (22–66)	30 (25–52)	33** (24–83)	1.01 [0.93; 1.1] $P = 0.88$	1.04** [0.95; 1.1] $P = 0.37$

***Dose-corrected AUC*$_{(0-\infty)}$.

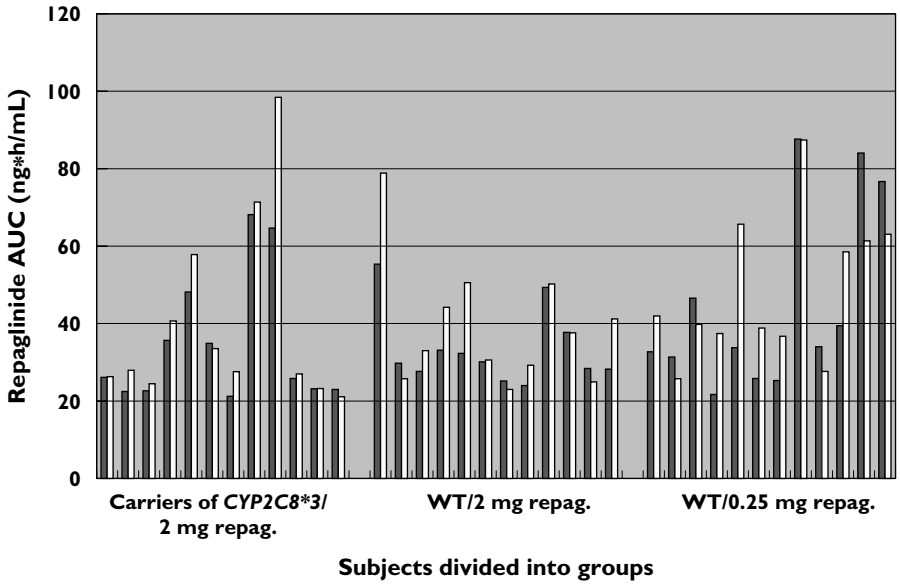

Figure 1
Repaglinide (repag.) $AUC_{0-\infty}$ (area under the serum concentration-time curve) following a 2-mg oral dose of the drug and dose-corrected $AUC_{0-\infty}$ following a 0.25-mg oral dose in 36 healthy male subjects before (baseline) and after a single dose of 300 mL grapefruit juice (GFJ). WT = *CYP2C8* wild-types. Baseline (■); after GFJ (□)

decrease in median t_{max} was observed in group C (median difference: –0.50; 95% CI: –1.4 to –0.38). The half-life was not significantly affected by grapefruit juice in any of the groups.

Discussion

In this study, the pharmacokinetics of repaglinide was compared between subjects carrying *CYP2C8*3* and wild-type subjects. A statistically significant difference between the two groups was not found. *CYP2C8*3* is the most common *CYP2C8* mutant allele present in Caucasian populations, where it has been found at frequencies of 0.13–0.17 [13, 14, 29].

*CYP2C8*3* has been reported to exhibit decreased activity, *in vitro* toward CYP2C8 substrates paclitaxel and arachidonic acid [13, 14, 30], but not toward the *N*-deethylation of the antiarrhythmic drug amiodarone [31]. There are limited data on the *in vivo* effect of the *CYP2C8*3* variant on drug metabolism. One study showed that heterozygotes of *CYP2C8*3* had a significantly increased metabolism of repaglinide compared with homozygotes of *CYP2C8*1* [16]. In another report significantly decreased metabolism of (R)-ibuprofen in carriers of the *CYP2C8*3* allele compared with homozygotes of *CYP2C8*1* was observed [29].

Table 3
The effect of a single dose of 300 mL grapefruit juice (GFJ) on repaglinide pharmacokinetics and the ratio of 3-OH-quinidine to quinidine in 36 healthy male subjects as a whole (ALL) and in group (A) (B) and (C) after a single dose of either 2 mg or 0.25 mg repaglinide. (ALL) All 36 subjects; (A) Carriers of *CYP2C8*3* (2 mg repaglinide) (n = 12); (B) Wild-type (2 mg repaglinide) (n = 12); (C) Wild-type (0.25 mg repaglinide) (n = 12)

	Median and 90% Interpercentile range							
Group	ALL		A		B		C	
Parameter	Baseline	After GFJ	Baseline	After GFJ	Baseline	After GFJ	Baseline	After GFJ
Age (years)	–	–	25.5 (24–27.9)	–	25 (22.6–26)	–	26 (24–29.5)	–
Weight (kg)	–	–	78.5 (68.7–89.5)	–	82.5 (68.1–98)	–	79.5 (73.7–103.8)	–
C_{max} (ng/mL)	–	–	19 (9.3–31)	18 (14–41)	19 (15–34)	22 (17–33)	2.3 (1.2–3.9)	3.0 (2.0–4.0)
t_{max} (h)	–	–	1.5 (0.75–2.5)	1.3 (0.62–1.7)	1.0 (0.5–2.0)	1.0 (0.64–2.5)	1.5 (0.5–2.7)	1.0 (0.63–1.5)
$t_{1/2}$ (h)	–	–	1.1 (0.72–1.5)	1.1 (0.95–1.9)	1.3 (1.0–1.8)	1.5 (0.91–2.9)	1.0 (0.64–1.4)	0.8 (0.56–2.5)
$AUC_{(0\rightarrow\infty)}$ (ng*h/mL)	32** (22–79)	38** (23–81)	26 (22–66)	28 (22–84)	30 (25–52)	35 (24–63)	34** (24–86)	41** (27–75)
3-OH-quinidine/ quinidine	0.11 (0.067–0.17)	0.085 (0.056–0.15)	0.13 (0.059–0.17)	0.085 (0.047–0.13)	0.11 (0.092–0.18)	0.089 (0.072–0.15)	0.11 (0.067–0.13)	0.080 (0.066–0.14)

***Dose-corrected* $AUC_{(0\rightarrow\infty)}$.

One explanation for the results in the present study, where no effect of *CYP2C8* genotype on repaglinide metabolism was observed, could be that the wild-type group also contained carriers of other *CYP2C8* mutant alleles such as *CYP2C8*4* [13]. However, as the frequency of these alleles in Caucasian populations has been reported to be quite low, other mechanisms may be more important. Another possibility is that CYP3A4 may make a greater contribution to the *in vivo* biotransformation of repaglinide in subjects with a low level of CYP2C8 activity. However, baseline indices of CYP3A4 activity did not differ between groups (Table 3). Alternatively, the *CYP2C8*3* allele may not affect the kinetic parameters for all substrates to an equal extent. Whereas the pharmacokinetics of paclitaxel and arachidonic acid are altered [13, 14, 30], those of repaglinide (present work) and amiodarone [31] are not.

In the previous study showing that the presence of *CYP2C8*3* was associated with increased repaglinide metabolism, a low dose of repaglinide (0.25 mg) was used [16]. In the present work, subjects received a clinically relevant dose of 2 mg repaglinide, and no effect of the *CYP2C8*3* was found. It could be speculated that at low doses levels of repaglinide, the relative contribution of CYP2C8 and CYP3A4 might be different than at clinically relevant doses.

The decrease in the quinidine metabolic ratio following ingestion of grapefruit juice is consistent with inhibition of CYP3A4. The relatively small magnitude of this decrease (19%) supports the hypothesis that only the intestinal fraction of CYP3A4 is affected.

Repaglinide AUC and dose-corrected AUC were increased overall by 13% after grapefruit juice intake in a statistically significant fashion. This observation together with a lack of influence on $t_{1/2}$ suggests that the presystemic metabolism of repaglinide is most likely to be mediated by intestinal CYP3A4.

The extent of the increase in mean repaglinide AUC after grapefruit juice observed in this study was relatively small compared with that caused by CYP2C8 inhibitors gemfibrozil (8.1-fold) and trimethoprim (61%), and CYP3A4 inhibitor clarithromycin (40%). This is a likely to be a consequence of grapefruit juice only inhibiting the intestinal fraction of CYP3A4, and the fact that the oral bioavailability of repaglinide is

Table 4
Statistical analysis of 300 mL grapefruit juice (GFJ) on repaglinide pharmacokinetics and the ratio of 3-OH-quinidine to quinidine in 36 healthy male subjects as a whole (ALL) and divided into group (A) (B) and (C) after a single dose of either 2 mg or 0.25 mg repaglinide. (ALL) All 36 subjects; (A) Carriers of *CYP2C8*3* (2 mg repaglinide) (n = 12); (B) Wild-type (2 mg repaglinide) (n = 12); (C) Wild-type (0.25 mg repaglinide) (n = 12)

Group		Statistical Inference		
Parameter	ALL	A	B	C
C_{max} (ng/mL)†	–	1.2 [0.94; 1.5] P = 0.13	1.1 [0.94; 1.3] P = 0.22	1.3 [1.04; 1.7]* P = 0.028
t_{max} (h)‡	–	–0.48 [–0.76; 0.09]	0.25 [–0.53; 1.0]	–0.50 [–1.4; –0.38]*
$t_{1/2}$ (h)†	–	1.2 [0.95; 1.5] P = 0.12	1.1 [0.9; 1.4] P = 0.27	0.95 [0.69; 1.32] P = 0.75
$AUC_{(0\rightarrow\infty)}$ (ng*h/mL)†	1.13** [1.04; 1.2] P = 0.0048	1.11 [1.01; 1.2]* P = 0.028	1.13 [0.99; 1.3] P = 0.061	1.14** [0.92; 1.4] P = 0.22
3-OH-quinidine/quinidine†	0.81 [0.75; 0.87]* P < 0.0001	0.74 [0.65; 0.85]* P = 0.0005	0.79 [0.7; 0.89]* P = 0.0013	0.89 [0.78; 1.02] P = 0.092

†*Geometric mean ratio (After GFJ/Baseline), 95% confidence interval and* P *value.*
‡*Hodges-Lehmann estimates of median difference and exact 95% confidence interval.*
**95% CI does not include 1 for Geometric Mean ratio or 0 for Hodges-Lehmann estimates.*
***Dose-corrected* $AUC_{(0\rightarrow\infty)}$.

about 60% [1]. These previous studies used a much lower dose of repaglinide (0.25 mg), which also confounds a direct comparison with our data. Studies using a clinically relevant dose of 2 mg have shown an increase in repaglinide AUC of only approximately 15% by ketoconazole, a potent CYP3A4 inhibitor, and 8% by simvastatin, a CYP3A4 substrate [5] and an inhibitor of CYP2C8 *in vitro* [32]. It is possible that other enzymes could be involved to a greater extent in the metabolism of repaglinide at this higher dose. Stratified analysis of the influence of grapefruit juice revealed that statistically significant effects were only observed for C_{max} and t_{max} in subjects carrying the wild-type allele, and who received the low dose of the drug. All these data indicate a larger potential for drug interactions with repaglinide following low dose (0.25 mg) administration. As inhibitors of CYP2C8 [11, 12] generally seem to have a larger effect on the $t_{1/2}$ of repaglinide compared with inhibitors of CYP3A4 [5, 6], CYP2C8 might contribute mainly to the hepatic clearance of the drug, whereas the contribution of CYP3A4 might be more pronounced during the first-pass metabolism in the gut and liver or if CYP2C8 activity is decreased.

As grapefruit juice has been found to inhibit other CYP enzymes including CYP2C9 and CYP2C19 [17], it is possible that CYP2C8-mediated repaglinide biotransformation could be inhibited by grapefruit juice as well. However, as only very limited amounts of *CYP2C8* mRNA and no expression of the protein have been detected in human duodenum and small intestine [33], the presence of CYP2C8 in the intestine is probably very limited, and significant *in vivo* effects of grapefruit juice on this enzyme seems unlikely. The *in vivo* effects of grapefruit juice on the efflux transporter P-glycoprotein, which is also present in the gut wall, have been somewhat contradictory [22, 34–36]. However, repaglinide is not a substrate of P-glycoprotein (personal communication, September 2003, data on file at Novo Nordisk A/S), any inhibition of P-glycoprotein would have no influence on the pharmacokinetics of repaglinide.

The family of human organic anion transporting polypeptides (OATPs) contains members that are exclusively located in the basolateral membrane of hepatocytes, for example OATP1B1 (previously OATP-C) and OATP8, as well as members that are expressed in other

tissues including placenta, intestine, kidney and lungs, for example OATP-A and OATP-B [37, 38]. These transporters actively mediate the cellular uptake of several endogenous and exogenous compounds. Grapefruit juice has been shown to decrease the activity of OATPs *in vitro* [39] and several fruit juices including grapefruit decrease the *in vivo* uptake of the antihistamine fexofenadine, which is mediated by OATPs in humans [39]. If the uptake of repaglinide into enterocytes is also mediated by OATPs, inhibition of these transporters by grapefruit juice could counteract the increase in bioavailability caused by inhibition of CYP3A4. This could represent another explanation of the relatively small effect of grapefruit juice on repaglinide bioavailability observed in the present study. A recent study strongly indicates that the cellular uptake of repaglinide is mediated by OATPs [37].

We conclude that the *CYP2C8*3* mutant allele does not influence the pharmacokinetics of repaglinide to a clinically relevant degree. Grapefruit juice increased the bioavailability of repaglinide, suggesting intestinal elimination of repaglinide by CYP3A4 in the gut. Concomitant administration of repaglinide and grapefruit juice has no effect on blood glucose concentration, and thus the interaction is unlikely to be of clinical relevance.

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