Shortening of the QT Interval After Food Can Be Used to Demonstrate Assay Sensitivity in Thorough QT Studies

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The effect of food was investigated under conditions of a thorough QT (TQT) study and with confirmation of assay sensitivity by the use of a positive control (400 mg of moxifloxacin). Fifty-five healthy subjects were randomized to treatment and a sequence of fasted and fed baseline electrocardiography days. Subjects received standard breakfast 30 to 10 minutes prior to dosing. Measurement of QT interval was performed automatically with subsequent manual onscreen overreading using electronic calipers. A profound increase in heart rate of 9.4 bpm was observed in the fed condition compared with the fasted condition at 1.5 hours after dose with a corresponding shortening of QT (27 milliseconds); (baseline data). When corrected, QTcF interval was shortened significantly with the maximal effect observed at 2 hours after dose of 8.2 (95% confidence interval, 6-10) milliseconds. A concurrent shortening of the PR interval with a maximum value of 5.6 milliseconds was also observed. The findings of this study demonstrate that food alters the QT–RR relationship and shortens QTc and PR for at least 4 hours after a carbohydrate-rich meal. The findings are of regulatory interest as this study shows that normal physiological causes can shorten QTc significantly and that it could be used as a method to demonstrate assay sensitivity.

The challenge of conducting and analyzing a thorough QT study (TQT) is that one cannot readily distinguish between the effects of heart rate (HR) changes, autonomic effects, and the effects caused by ion channel blockade. The International Conference on Harmonization (ICH) E14 guideline has set narrow limits for QTc changes that will not raise any regulatory safety concern, and it follows that it is desirable to remove all factors that may lead to a false signal in these studies. Bazett's[1] initial investigations in the 1920s concluded that the QT varies with the HR, and various correction methods have been proposed to normalize this effect to define the underlying intrinsic QT–RR relationship. Nonetheless, the autonomic nervous system effects on cardiac repolarization are still poorly understood, which complicates the interpretation of QT measurements in the context of TQT studies.

The QT–RR relationship is influenced by the autonomic nervous system, with various publications in the past 3 decades showing that the intrinsic QT–RR relationship changes in response to changes in sympathetic and vagal tones. The present study shows a significant effect of food of a magnitude that might be viewed as being of regulatory concern had it been elicited by a drug.[2]

The effects of food on the QT interval have been described in a number of publications, which appear at first to contradict the findings of our study. There are several reports of prolonged QT resulting from low-calorie meals and starvation.[3-5] Long QT interval was also reported in anorexia nervosa,[6] in healthy subjects undergoing experimental starvation,[7] in

patients following gastroplasty or ileojejunal bypass,[8-10] in dieters using the "liquid protein modified fast" diet,[11] and in patients with untreated celiac disease.[12] QTc lengthening can be a result of insulin and adrenaline, possibly mediated by hypokalemia and direct ion-channel effects.[13-15] Physiological hyperinsulinemia causes prolongation both directly and through sympathetic activation, transferring potassium ions from the extracellular to the intracellular space.[16] Withdrawal of the vagal tone using atropine was reported to prolong corrected QT interval (QTc) 5 minutes after atropine, which remained prolonged for the entire study period (60 minutes).[17]

Unspecified postprandial QT shortening in TQT studies has been reported by Hulhoven et al[18] and Bloomfield et al.[19] Nagy et al[5] reported QT prolongation. Scott et al[20] described postprandial increases in HR as a result of sympathetic stimulation, whereas Lu et al[21] concluded that postprandial changes in HR were due to vagal withdrawal.

Direct proof of the effects of food on QTc in TQT studies is lacking. The data presented in this article originate from a secondary analysis of data acquired in a TQT study measuring the effect of food on the QTc in healthy subjects in the resting state.

METHODS

This article reports the results of a secondary analysis of data obtained during a TQT double-blind, randomized, placebo-controlled, 4-way crossover study that evaluated the effect of a therapeutic and supratherapeutic single dose of an investigational drug given with and without food on the QT/QTc interval of the electrocardiograph (ECG) using a single 400 mg dose of moxifloxacin as a positive control in healthy male and female volunteers. The study was compliant with the ICH E14 guideline.[22]

Subjects

A total of 55 subjects were randomized to 1 of 2 sequences: either fasted followed by fed or fed followed by fasted conditions on the 2 consecutive ECG days that served as baseline for the main TQT study, which will not be presented in this article.

Eligible subjects had normal ECG, vital signs, physical examination, and laboratory tests; had no clinically significant medical history; and were taking no medication that would interfere with the procedures, compromise subject safety, or influence any of the ECG parameters. All subjects provided written informed consent prior to any study-specific procedures being undertaken. The study was approved by the local ethics committee (Yorkshire Independent Research Ethics Committee, Leeds, UK) and the Medicines and Healthcare Products Regulatory Authority. It was conducted in accordance with the principles of the current version of the Declaration of Helsinki, current UK law, and in accordance with the Good Clinical Practice (GCP) regulations as set forth in the ICH Harmonized Tripartite Guideline for GCP (ICH Topic E6).

Study Design

Subjects received placebo after an overnight fast of at least 10 hours or 30 minutes after starting a standardized breakfast that had to be consumed within 20 minutes. A predose baseline ECG was taken before dose and breakfast, whereby breakfast was consumed 30 to 10 minutes before a dose of placebo at time 0. Therefore, all postdose ECG times occurred at an additional 30 minutes later when comparing the food effects with other studies, where time 0 usually corresponds to the start of feeding rather than a dose of placebo, as is the case in this study. Subjects remained on bed rest and fasted for a minimum of 4 hours after dosing. Food and fluid intake were strictly controlled.

The breakfast was rich in carbohydrates (68%) and had 617 kcal (Table I); it contained 30 g of cornflakes, 150 mL of semi-skimmed milk, a 79 g whole-meal hoagie, 20 g of jam, 10 g of butter, 200 mL of apple juice, and 10 g of sugar.

The sensitivity of the study and its ability to detect differences of clinical significance were assessed by comparing moxifloxacin 400 mg (single dose) to placebo on change from average baseline and the change from time-matched baseline (under the same conditions as for the main comparison), showing an effect on QTcF of 12 milliseconds (95% confidence interval [CI], 10-14 milliseconds).

Electrocardiogram Assessments and QTc Evaluation

Twelve-lead ECG recordings were collected using a digital format GE Marquette 12-Lead Digital Recorder equipment (GE Marquette, Milwaukee, Wisconsin) until 24 hours after dosing with placebo. ECG recordings were taken at 9 time points within 1 hour before dosing and then at 0.5, 1, 1.5, 2, 2.5, 3, 4, 8, and 24 hours post dose. Triplicate recordings were taken approximately 1 minute apart at each of the assessment time points. All ECGs were recorded after the subject had been resting in supine position for a minimum of 10 minutes. All subjects remained in their bed for the first 4 hours post dose.

Table I   Contents of Breakfast

	Serving	Calories	Protein		Carbohydrate		Fat		Fiber	
			g	kcal	g	kcal	g	kcal	g	kcal
Kellogg's Cornflakes	30 g	111.9	2.1	8.4	25.2	100.8	0.3	2.7	0.9	0.0
Milk	150 mL	72.5	5.0	20.0	7.5	30.0	2.5	22.5	0.0	0.0
Sugar	10 g	40.0	0.0	0.0	10.0	40.0	0.0	0.0	0.0	0.0
Whole-meal hoagie (1 roll)	79 g	178.1	8.6	34.4	27.6	110.4	3.7	33.3	5.5	0.0
Jam	20 g	50.0	0.0	0.0	12.5	50.0	0.0	0.0	0.2	0.0
Butter	10 g	72.9	0.0	0.0	0.0	0.0	8.1	72.9	0.0	0.0
Apple juice (pure)	200 mL	93.1	0.3	1.2	22.3	89.2	0.1	0.9	0.1	0.0
Total		616.7	16.0	64.0	105.1	420.4	14.7	132.3	6.7	0.0
				10%		68%		21%		0%

The digital ECG recordings were transmitted electronically to the ECG core laboratory where the computer-analyzed ECG results were all manually verified using the MUSE Interval Editor manufactured by GE Medical. The algorithm used is a recent version of SL12 using a threshold method assessing a computer-derived global beat. All ECGs for a given subject were shuffled and then read by the same person in blinded fashion.

Data Analysis and Statistical Methods

Primary correction was done using QTcIP (individual) as well as QTcF (Fridericia correction) and QTcB (Bazett correction). For each subject, at each time point, QTc interval values were then calculated from the median of triplicate measures. The primary end point for this analysis was prospectively defined as QTcIP, with QTcF and QTcB being secondary end points. The food effect was calculated as time-matched difference from baseline using the formula $\Delta QTc = (QTcF^{fed} - QTcF^{fasted})$ and $(QTcIP^{fed} - QTcIP^{fasted})$. Point estimates and corresponding 95% CIs were calculated for the difference at each time point. Scatter plots of manually read, uncorrected QT, QTcF, and QTcB against RR were produced to check the efficiency of each of the correction factors in correcting for HR. All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary North Carolina).

The food effect is shown as $\Delta QTcF = (QTcF_{fed} - QTcF_{fasted})$ and $\Delta QTcIP = (QTcIP_{fed} - QTcIP_{fasted})$.

RESULTS

The analysis set for the evaluation of QTc included all 55 subjects who were dosed and had at least 2 complete sets of evaluable ECGs.

Table II   Summary of Demographic Data

Parameter	Overall
Age, y, mean ± SD	28.6 ± 5.3
Weight, kg, mean ± SD	65.84 ± 9.74
Body mass index, kg/m², mean ± SD	22.29 ± 1.95
Male, n (%)	31 (56.4)
Female, n (%)	24 (43.6)
N (%)	55 (100)

Subject Demographics and Disposition

Fifty-five subjects were enrolled (31 male and 24 female) with a mean age of 28.6 years and were randomized to fed or fasting conditions during the baseline days (Table II). None of the subjects took concomitant medication that could have confounded the interpretation of the ECG data.

Food Effect on Heart Rate, QT/QTc, and PR

We observed a steep increase in mean HR of 9.4 beats per minute (bpm) at 1.5 hours following time 0, gradually returning to baseline at the 4-hour time point. A reduction of mean QT duration occurred that was inversely related to the change in HR. The food effect on the uncorrected QT interval reached its maximum of 27 milliseconds at 1.5 hours after dose or 2 hours from the start of breakfast (Figures 1 and 2), which was statistically significant when compared with the baseline data obtained in the fasted state. However, at the 4-hour time point, QT was still significantly prolonged by 10 milliseconds.

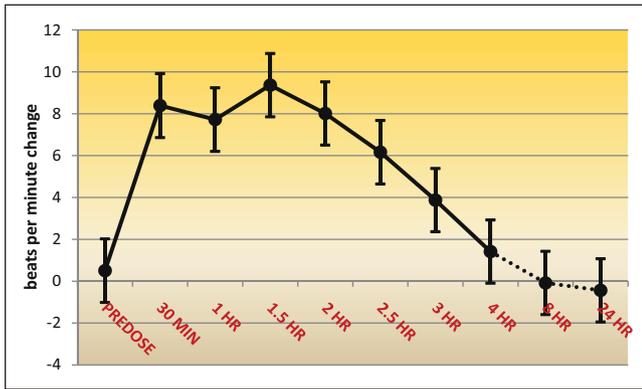We applied different HR correction methods. The QTcF interval showed a maximum shortening at 2 hours

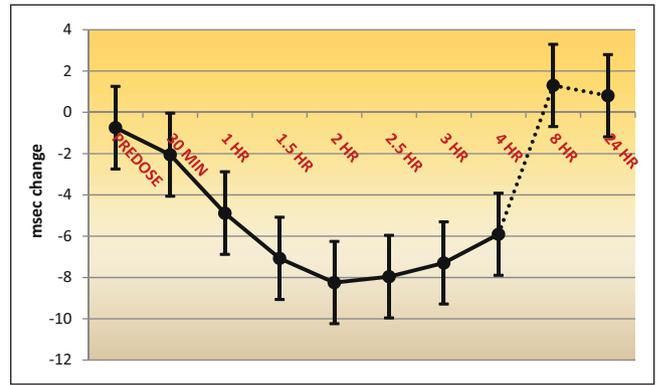*Figure 1. Food effect on heart rate with confidence interval of 95%.*

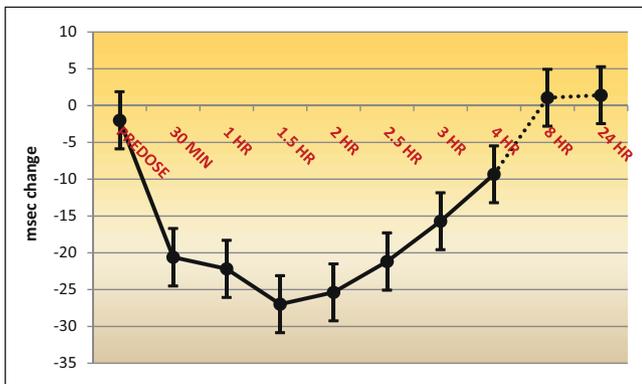*Figure 2. Food effect on QT with confidence interval of 95%.*

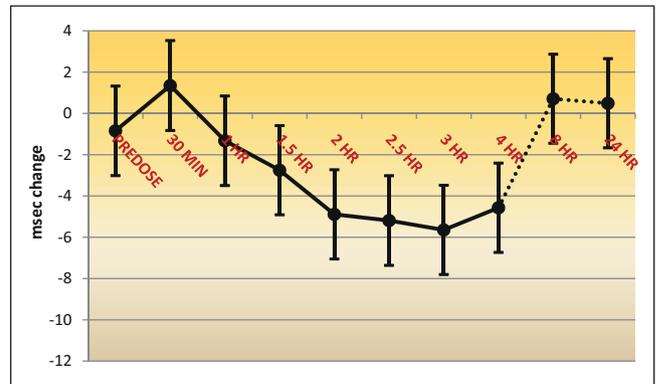*Figure 3. Food effect on QTcF with confidence interval of 95%.*

Figure 4. Food effect on QTcIP with confidence interval of 95%.

after dose or 2.5 hours from the start of breakfast of 8.2 milliseconds (95% CI, 6-10) (Figure 3). QTcIP interval showed a maximum shortening at 3 hours after dose or 3.5 hours from the start of breakfast of 5.6 milliseconds (95% CI, 3-8) (Figure 4). QTcB showed a biphasic pattern in that there was a temporary increase in QTcB with a maximal effect at 0.5 hours after dose of about +7 milliseconds (Figure 6) with a subsequent shortening of QTcB returning to baseline after 2 hours after dose with a maximum shortening at 4 hours after dose of about 4 milliseconds. It is noteworthy that the maximum HR effect and the maximum shortening of QT occur 1:30 hours after the placebo dose, whereas the maximum shortening of QTcF occurs 2:00 hours after dose. The maximum effect on QTcIp and PR (Figure 5) occurs after 3 hours and a shortening of QTcB after 4 hours. At that time the HR has returned to baseline, whereas QT remains shortened relative to baseline and RR.

The categorical analyses show that the majority of subjects had either slight decreases or increases from
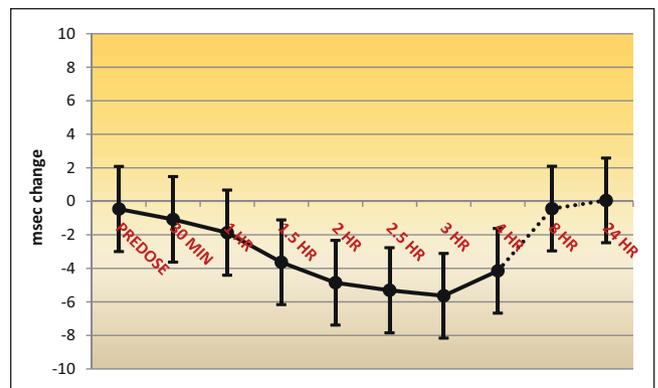

Figure 5. Food effect on PR with confidence interval of 95%.

baseline in QTcIP that were less than 30 milliseconds for both study regimens. All of the subjects were found to have all their uncorrected QT and corrected QTc values 500 milliseconds or less.
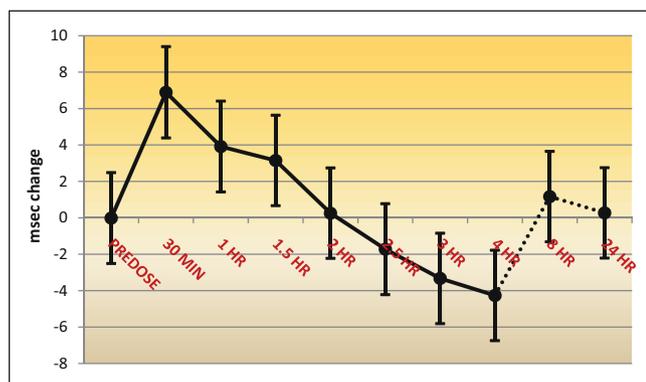
Figure 6. Food effect on QTcB with confidence interval of 95%.

Safety and Tolerability Results

There were no clinically significant changes in clinical parameters and no adverse events that would have had any bearing on the results reported.

DISCUSSION

To the best of our knowledge, this is the first published study to report the relationship between ingestion of a standardized meal conducted under the rigorous conditions of a TQT study until 4 hours after a meal. It is also the first study to describe a definitive shortening of the QTc interval using a population-based correction method as well as Fridericia correction.

The marked effect on HR of 9.4 bpm is likely to be due to redistribution of blood to the gastrointestinal system leading to an increased workload to the heart[23] attributable to an increased blood flow to the superior mesenteric artery and total splanchnic blood flow[16] via a central reflex as described by Pan et al.[24] The gastrovascular reflex leads to an increase in muscle sympathetic nerve activity and blood pressure in response to a distension of the stomach,[25] which also prevents a decrease in systolic blood pressure by increasing local arterial resistance in muscles. This sympathetic response appears to be a direct (neural) effect of stomach distension mediated by tension receptors in the stomach wall through vagal afferent fibers.[26,27] However, Scott et al[20] described an increase of sympathetic tone in the peripheral vasculature as a direct result of increases in glucose and insulin.

Widerlov et al[28] described a statistically significant increase in HR of 12 bpm and a reduction of area under the T-wave, after food intake (500 mL of sour milk, 200 mL of fruit muesli, 3 cheese sandwiches,

and 1 apple) peaking 1:30 hours and lasting for at least 3 hours, which was of similar magnitude at different times of the day, thereby excluding underlying circadian effects. These effects on the HR were combined with a reduction of the area under the T-wave due to flattening, a shortening of QT, or both.

A shortening of QT (27 milliseconds) was also observed in our study, which corresponds to the increase in HR. However, the effect on QT appears to be greater than proportional, particularly between 2 and 4 hours after dose, thereby shifting the relationship between RR and QT interval. This becomes apparent when applying a HR correction factors such as QTcF and even more so when calculating QTcIP.

Nagy et al[5] reported a prolongation of QTcB after a meal. This is consistent with findings in this study in that those investigators observed an increase in HR of 10 bpm, a shortening of QT of about 30 milliseconds, and an increase of QTcB during the 1 hour that the experiment lasted. Bloomfield et al[19] reported a maximal QTcF shortening 11 milliseconds at 1 to 2 hours post meal lasting for more than 3 hours. Again this is consistent with our findings.

A change in autonomic balance between the 2 limbs of the autonomic system is most likely to be responsible for the changed QT–RR relationship leading to the shortening of the QTc demonstrated in the fed group. It has been shown that QT shortens as HR increases in response to pacing[29-33] and that changes of the QT–RR relationship occur with sympathetic stimulation versus vagal blockade, which both lead to an increase in HR: the use of isoprenaline results in more prolongation of the QTc interval in comparison to atropine or exercise.[34] It was deduced that the autonomic system changes the QT interval through direct effects on the myocardium via effects on the Ca^{++} and K^+ ion channels, thereby changing the intrinsic QT–RR relationship. Sympathetic stimulation increases HR, pumping force, and conduction velocity, whereas parasympathetic stimulation of the heart has opposite effects. Sympathetic stimuli are mediated by β-adrenoceptors ($β_1$) and parasympathetic effects via muscarinic receptors (M_2). Sympathetic stimulation is general and non-specific to an end organ. In normal resting conditions, the HR is predominantly regulated by vagal tone; the intrinsic firing rate of the SA node of approximately 110 beats per minute is downregulated to the normal resting HR of an individual. Vagal action also is organ specific,[35] meaning that there may be opposing effects at different end organs. It is not clear whether the observed increases in HR are due to sympathetic effects following insulinemia, as proposed by Scott et al,[20] or

due to vagal withdrawal, as proposed by Lu et al.[21] In either case, this mechanism would suggest that there ought to be an increase in QTc as a result of sympathetic stimulation. This may well be the case during the first 1:30 hours after dosing, but would not fully explain the extended QTc shortening effect of up to 4:00 hours post dose.

Meals of high carbohydrate content such as in this study have been associated with a transient endogenous physiological insulinemia, which increases sympathetic activity through a central neural action, crossing the blood–brain barrier.[20,36] Scott et al[20] showed a 10 bpm increase in HR combined with peak increases in blood glucose at 0:40 hours and peak insulin levels at 1:20 hours following carbohydrate ingestion. Gastaldelli et al[15] showed an effect of insulin in a euglycemic clamp experiment on HR and QTcB. However, the study by Gastaldelli et al failed to demonstrate an effect on the uncorrected QT. Furthermore, the modest effects on HR of only 5 bpm were decreased during the infusion of insulin, whereas levels in plasma were of a similar magnitude of those reported by Scott et al.[20] Neither study measured the levels of C-peptide, which is excreted in equimolar amounts to insulin and has been associated with a reduction of QTc by Johansson et al.[37] It is conceivable that the effects of C-peptide are responsible for the extended shortening of QT beyond the immediate postprandial period.

If postprandial insulinemia plays a significant role in the observed effects of food on the ECG in this study, then meals with high levels of carbohydrates would be expected to show a greater effect. The breakfast meal in this study consisted of 68% carbohydrates, similar to the studies by Widerlov et al[28] (57%) and Nagy et al[5] (53%). These are distinctly different in that they have a much higher carbohydrate and lower fat content than, for example, a Food and Drug Administration (FDA) standard breakfast, which delivers 950 kcal and contains 58% of fat with a much lower carbohydrate content; this may not elicit a similar effect but there are no data to suggest that this is indeed the case.

Most TQT studies reported in the literature have used moxifloxacin 400 mg as positive control to demonstrate its well-known effect of QTc prolongation in comparison to placebo, thereby complying with the ICH E14 guideline.[22] The guideline states that an active control should be included to ensure that the clinical trial study demonstrates approximately a 6-millisecond change in QTc; however, moxifloxacin produces an effect far greater than that. This study demonstrates an effect on QTc much closer to the effect suggested in the ICH E14 guideline, albeit a negative one.

However, the direction of that effect is unimportant given that the assay control is used to ensure that a study is capable of detecting a small change.

The effects of food leading to a change in QTc are the result of physiological effects on the heart and not the result of ion channel blockade, as is the case with moxifloxacin. However, the purpose of the positive control is to show that a study is capable of detecting a small change in QTc; this is clearly the case if a study is capable of detecting a 5- to 10-millisecond change in QTcF.

A food arm could be easily included as confirmation of ECG assay sensitivity in early phase studies such as multiple ascending dose studies and could be used for hypothesis generation and early characterization of cardiac on- or off-target effects. Furthermore, food could be used in oncology and pediatric TQT studies, where the use of moxifloxacin has been proven to be problematic.

The extent of effect on QTc may vary with foods of higher or lower carbohydrate content, and further studies may be warranted to investigate the effects of different types of food, particularly to define the effect on the magnitude of effect. Both the filling of the stomach by itself and the increase in blood glucose lead to an increase in HR. However, the study by Scott et al[20] appeared to suggest that the increase in blood glucose by itself leads to an increase in HR and insulin and C-peptide and may well be the dominant driver. However, there is sufficient evidence in the literature to suggest that the food effect we have observed is well reproducible in terms of magnitude of effect and time course and is robust against short and temporary changes in posture: significant HR increases of approximately 11 bpm over 2 hours[20] and 10 bpm over 4 hours[28] and QT change of approximately 30 milliseconds within 15 minutes, which was maintained for 1 hour[5] and beyond.

CONCLUSION

A standardized food arm could be used as an alternative method to demonstrate assay sensitivity in thorough ECG studies. However, the fact that other groups have found similar effects suggests that the method would be reproducible and robust even in relatively small study populations. It should be used in all instances where a pharmacological assay control is not feasible, given that the proposed method is easy to implement: it is simple to include a fasted and fed ECG day in most trials and this would not be objectionable to ethics committees. Additional studies will help further quantify the effects observed in our study,

which would allow consideration whether a well-defined food arm may allow the replacement of moxifloxacin in formal thorough QT studies.

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