The Cardiovascular Effects of a Meal: J-T$_{peak}$ and T$_{peak}$-T$_{end}$ Assessment and Further Insights Into the Physiological Effects

Jörg Täubel, MD, FFPM[1,5], Georg Ferber, PhD[2], Leen Van Langenhoven, PhD[1], Teresa del Bianco, PhD[1], Sara Fernandes, PhD[1], Dilshat Djumanov, PhD[1], Jørgen K. Kanters, MD[3], Claus Graff, MSc, BME, PhD[4], and A. John Camm, MD[5]

Abstract

Meal intake leads to a significant and prolonged increase in cardiac output to supply the splanchnic vasculature. A meal is associated with sympathetic activation of the cardiovascular system, and food ingestion is correlated with an increase in heart rate, an increase in cardiac stroke volume, and QTc interval shortening for up to 7 hours. Given the complexity of the system, one or several of many mechanisms could explain this observation. The shortening of the QTc interval was correlated with a rise of C-peptide following food ingestion, but the mechanisms by which C-peptide may be involved in the modulation of cardiac repolarization are still unknown. This shortening of the myocardial action potential caused by the ingestion of food was further investigated in the present study by measuring the QRS, J-T$_{peak}$, and T$_{peak}$-T$_{end}$ intervals in search of further clues to better understand the underlying mechanisms. A retrospective analysis was conducted based on data collected in a formal thorough QT/QTc study in which 32 subjects received a carbohydrate-rich "continental" breakfast, moxifloxacin without food, and moxifloxacin with food. We assessed the effect of food on T-wave morphology using validated algorithms for measurement of J-T$_{peak}$ and T$_{peak}$-T$_{end}$ intervals. Our findings demonstrate that a standardized meal significantly shortened J-T$_{peak}$ for 4 hours after a meal and to a much lesser extent and shorter duration (up to 1 hour) prolonged the T$_{peak}$-T$_{end}$ and QRS intervals. This suggests that the QTc shortening occurs mainly during phase 2 of the cardiac action potential. As there was no corresponding effect on T$_{peak}$-T$_{end}$ beyond the first hour, we conclude that a meal does not interfere with the outward correcting potassium channels but possibly with Ca^{2+} currents. An effect on mainly Ca^{2+} aligns well with our understanding of physiology whereby an increase in stroke volume, as observed after a meal, is associated with changes in Ca^{2+} cycling in and out of the sarcoplasmic reticulum during cardiac myocyte contraction.

Keywords

calcium cycling, cardiac electrophysiology, cardiac repolarization, electrocardiogram after food, IKr, IKs, ion channel effects, J-T$_{peak}$, T$_{peak}$-T$_{end}$

The effect of food on QTc has been reported in different studies.[1–3] A standardized meal induces a decrease in the heart rate corrected QTcF of 6 to 8 milliseconds, 1.5 to 4 hours after food intake correlated with an increase in heart rate.[1] This effect was shown to be well reproducible in terms of both magnitude of the effect and time course.[4]

The effects of food leading to a change in QTc are the result of physiological effects on the heart and not the result of drug-induced ion channel blockade, which makes food challenge an attractive alternative to moxifloxacin as a positive control in thorough electrocardiographic studies. The effect of food on QTcF to demonstrate assay sensitivity in early-stage clinical studies has been recognized as a valuable tool to increase confidence in the results of exposure-response analyses of early phase I studies to request a waiver of the thorough QT/QTc study regulatory requirement.[5] However, the underlying mechanisms of such effect remain unclear.

C-peptide, which is released in equimolar amounts to insulin but stays in circulation for longer periods due to a longer circulatory elimination half-life has emerged as a potential effector for the QTc shortening observed following ingestion of a meal. It appears to antagonize

[1]Richmond Pharmacology Ltd., St George's University of London, Cranmer Terrace, London, UK
[2]Statistik Georg Ferber GmbH, Cagliostrostrasse, Riehen, Switzerland
[3]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[4]Department of Health Science and Technology, Aalborg University, Aalborg, Denmark
[5]Cardiovascular and Cell Sciences Research Institute, St George's University of London, London, UK

Corresponding Author:
Jörg Täubel, MD, FFPM, Richmond Pharmacology Ltd., St George's University of London, Cranmer Terrace SW17 0RE, London, United Kingdom
Email: j.taubel@richmondpharmacology.com

the QT prolonging effects of glucose,[6] while insulin at physiological levels was shown to have no effect on QTc.[7] In patients with type 1 diabetes with neuropathy, administration of C-peptide was also shown to shorten the QTc.[8]

The physiological response to a meal includes a significant increase in cardiac output (CO) to supply the splanchnic vasculature. Work by other groups suggests that the increase in CO is the result of a heart rate increase and an increase in stroke volume. The increase in CO results from an autonomic response, and elevated levels of norepinephrine have been observed during the postprandial period.[9] However, the QTc shortening effect of food goes well beyond the postprandial period, during which elevated levels of catecholamine are found. This suggests that other mechanisms may play a role in regulating repolarization.

Johannesen et al[10] hypothesized that the electrocardiogram (ECG) can differentiate the effects of ion channel drug blockade by separate analysis of early repolarization (global J-T_{peak}) and late repolarization (global T_{peak}-T_{end}). Results have shown that human Ether-à-go-go-Related Gene potassium channel block equally prolongs J-T_{peak} and T_{peak}-T_{end} intervals, whereas the addition of calcium or late sodium current block preferentially shortens the J-T_{peak} interval. Following a similar approach, in this study, we aim to better understand which ion channels may be involved in the meal response and better characterize the ECG signature and impact of food on T-wave morphology.

Materials and Methods

Study Design

The study was approved by the local National Health Service Ethics Committee (London Surrey-Borders, UK) and the Medicines and Healthcare Products Regulatory Authority and was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. The study population consisted of 32 healthy, nonsmoking, white (13; 7 males and 6 females) and Asian (19; 11 males and 8 females) subjects. The study was designed as a single-center, randomized, placebo- and positive-controlled, crossover study. Eligible subjects were randomized to 1 of 8 sequences of the treatments, insulin euglycemic clamp, a carbohydrate-rich "continental" breakfast, a calorie-reduced breakfast, a single dose of moxifloxacin 400 mg, a single dose of moxifloxacin 400 mg with a carbohydrate-rich continental breakfast, and placebo. The calorific content of the carbohydrate-rich continental breakfast and calorie-reduced breakfast, as well as the ECG assessments, are described in Täubel et al.[7] Breakfasts were provided 30 minutes prior to the scheduled dosing time (time zero) and were to be fully eaten by 10 minutes before dosing. Subjects were provided with lunch at 7 hours after time zero. The retrospective analysis reported here is based on the data from the following treatment sequences: a carbohydrate-rich continental breakfast, moxifloxacin 400 mg, moxifloxacin 400 mg administered with a carbohydrate-rich continental breakfast, and placebo; the other arms are described elsewhere.[7]

Subjects participating in the study attended for screening and 2 treatment periods (periods 1 and 2) separated by a 3-day washout interval. Each period consisted of a baseline ECG day (day –1) where no treatments were administered and only standardized meals were given, and treatment days (days 1, 2, and 3).[7] The ECG and samples for pharmacokinetics (PK)/pharmacodynamics analysis on the treatment days were taken at the corresponding clock time points as on the baseline day.

Statistical Analyses

ECG Recording and Data Processing. Twelve-lead ECGs were recorded at Richmond Pharmacology Ltd. using a MAC1200® (500-Hz sampling frequency, 4.88-μV amplitude least significant bit resolution, GE Healthcare, Milwaukee, Wisconsin) recorder connected via a fixed network connection to the MUSE® Cardiology Information System. ECG recordings were made at the following time points: before the dose (time zero) and 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, and 6.0 hours after the dose on all days 3 after the subjects had been resting in a supine position for at least 10 minutes. At each time point, the ECGs were recorded in triplicate and performed at approximately 1-minute intervals. Each ECG recording lasted 10 seconds. Repeat ECGs were performed until at least three 10-second ECG recordings per scheduled time point met the predefined quality criteria to enable reading and analyzing at least 5 complexes per derivation. All ECGs recorded during the study were stored electronically at the MUSE information system. Data were processed by the Department of Health Science and Technology of the Faculty of Medicine, University of Aalborg (Denmark), using the commercially available GE Healthcare Marquette 12SL ECG analysis program and the US Food and Drug Administration 510(k)-cleared GE research package QT GuardPlus, which uses validated algorithms for measurement.[11,12] This software uses the simultaneous vector magnitude of all 12 leads to determine the onset and offset of the QRS complex, as well as the offset of the T wave. The vector magnitude is a global single-lead representation of all 12 leads that did not display a biphasic (pos/neg or neg/pos) T wave. In cases with clear biphasic T waves in some of the 12 leads, the vector magnitude representation may have displayed 2 obvious positive peaks in the T wave. In these cases, the algorithm used

the second peak of the T wave, which is closest to the end of the T wave. The end of the T wave is determined by the method of small windows.[11,12]

Heart Rate Correction of J-T$_{peak}$ and Baseline Correction. For accounting for the influence of heart rate (HR) on the T wave, a correction to J-T$_{peak}$ was applied[10,13,14] as HR variation is expected only for early repolarization (J-T$_{peak}$). A previous study including a pooled analysis of subjects from 34 thorough QT studies, has shown that at resting HRs T$_{peak}$-T$_{end}$ exhibits minimal HR dependency and only J-T$_{peak}$, and QT showed a substantial (>10%) HR variation.[15]

In analogy to the situation with QTc, a study-specific correction was considered necessary, since both J-T$_{peak}$ and HR are affected.[16] The correction term β of 0.51 was derived from a linear mixed model regressing the logarithmic transformation of J-T$_{peak}$ on the logarithmic transformation of RR, using the predose samples only. This correction term is further validated by the small difference with the correction term reported by Johannesen et al.[10] Furthermore, the sum of the HR-corrected J-T$_{peak}$, QRS interval, and T$_{peak}$-T$_{end}$ gave an estimated QT interval that, on average, differed from QTcF by only 0.74 milliseconds, with an upper 1-sided 90% confidence interval (CI) of only 0.79 milliseconds.

The baseline-corrected variables (ΔQRS, ΔJ-T$_{peak}$cS, and ΔT$_{peak}$-T$_{end}$) were obtained by subtracting the predose value of QRS, the HR-corrected J-T$_{peak}$, and T$_{peak}$-T$_{end}$, calculated by time point, subject, and period, from the postdose corrected value, matched by subject, time point, and period.

Mixed Models. The time point analysis was based on the change of QRS, J-T$_{peak}$cS, and T$_{peak}$-T$_{end}$ from the time-matched baseline (outcome variables: ΔQRS, ΔJ-T$_{peak}$cS, and ΔT$_{peak}$-T$_{end}$).

The time point analysis involved 1 mixed model for each outcome variable (ΔQRS, ΔJ-T$_{peak}$cS, and ΔT$_{peak}$-T$_{end}$), with the interaction between treatment and time point as fixed effect and scaled baseline as covariate; the random term included correlated intercepts and slopes for the baseline. This analysis aimed to ascertain whether the time course of the ECG markers differed between treatment groups (moxifloxacin fasted, moxifloxacin combined with a high-carbohydrate breakfast, and a carbohydrate breakfast vs placebo).

The mixed models were fitted with the package lmerTest (version 3.0-0). Analyses were carried out using R (version 3.4.3; R Core Team, 2017).

Results

This study included 32 subjects. There were no unexpected treatment-related adverse events.

Confirmation of Assay Sensitivity

As previously described,[17] the largest QTcF change from baseline after a 400-mg oral dose of moxifloxacin in the fed state was observed at 4 hours with a peak value of 11.6 milliseconds (2-sided 90%CI, 9.1–14.1), while the largest QTcF change observed in the fasted state was 14.4 milliseconds (90%CI, 11.9–16.8) and occurred at 2.5 hours after the dose. For the continental breakfast, the maximum QTcF shortening was observed at 3.5 hours after the dose with a value of 7.9 milliseconds (2-sided 90%CI, –10.4 to –5.5).[7]

Time Point Analysis

The descriptive statistics of ΔQT, ΔQRS, ΔJ-T$_{peak}$cS, and ΔT$_{peak}$-T$_{end}$ (averages and 90%CIs) are presented in Figure 1. The mixed-model analysis by time point for each outcome variable is described below.

QRS Interval. ΔQRS did not significantly change from baseline in neither group (Table 1). A high-carbohydrate breakfast and moxifloxacin with a high-carbohydrate breakfast showed an immediate, small, short-lived positive difference from placebo 15 to 30 minutes after time zero, with a difference between the estimates of 2.72 milliseconds at 15 minutes ($P < .01$) and 2.40 milliseconds at 30 minutes ($P < .05$) for a high-carbohydrate breakfast, and a difference of 3.04 milliseconds at 15 minutes ($P < .001$) for moxifloxacin with a high-carbohydrate breakfast (Table 2). This suggests that the change in QRS is associated with the meal and not with moxifloxacin, as moxifloxacin has no effect when given fasted.

J-T$_{peak}$cS Interval. A pronounced shortening of ΔJ-T$_{peak}$cS was observed with the ingestion of a standardized carbohydrate-rich breakfast. The estimated changes of ΔJ-T$_{peak}$cS with food were consistently and significantly different from baseline starting 15 minutes after time zero (–9.52 milliseconds; 90%CI, –14.93 to –3.34) up to 4 hours after the dose (–9.39 milliseconds; 90%CI, –15.33 to –4.04), denoting 45 minutes to 4.5 hours after starting a meal, as the meal was started 30 minutes before dosing/time zero (Table 3). The estimate of ΔJ-T$_{peak}$cS with moxifloxacin combined with food also significantly shortened until 2.5 hours after the dose (–6.55 milliseconds; 90%CI, –12.89 to –0.14), started to lengthen up to 6 hours, and exceeded placebo only 6 hours after the dose, showing a different tendency of the one observed for breakfast alone (Table 4). It is important to note that the PK of moxifloxacin is affected by a predose meal, leading to lower concentrations of moxifloxacin in plasma for the first 3 hours. The difference becomes small from the 4-hour time point onward.
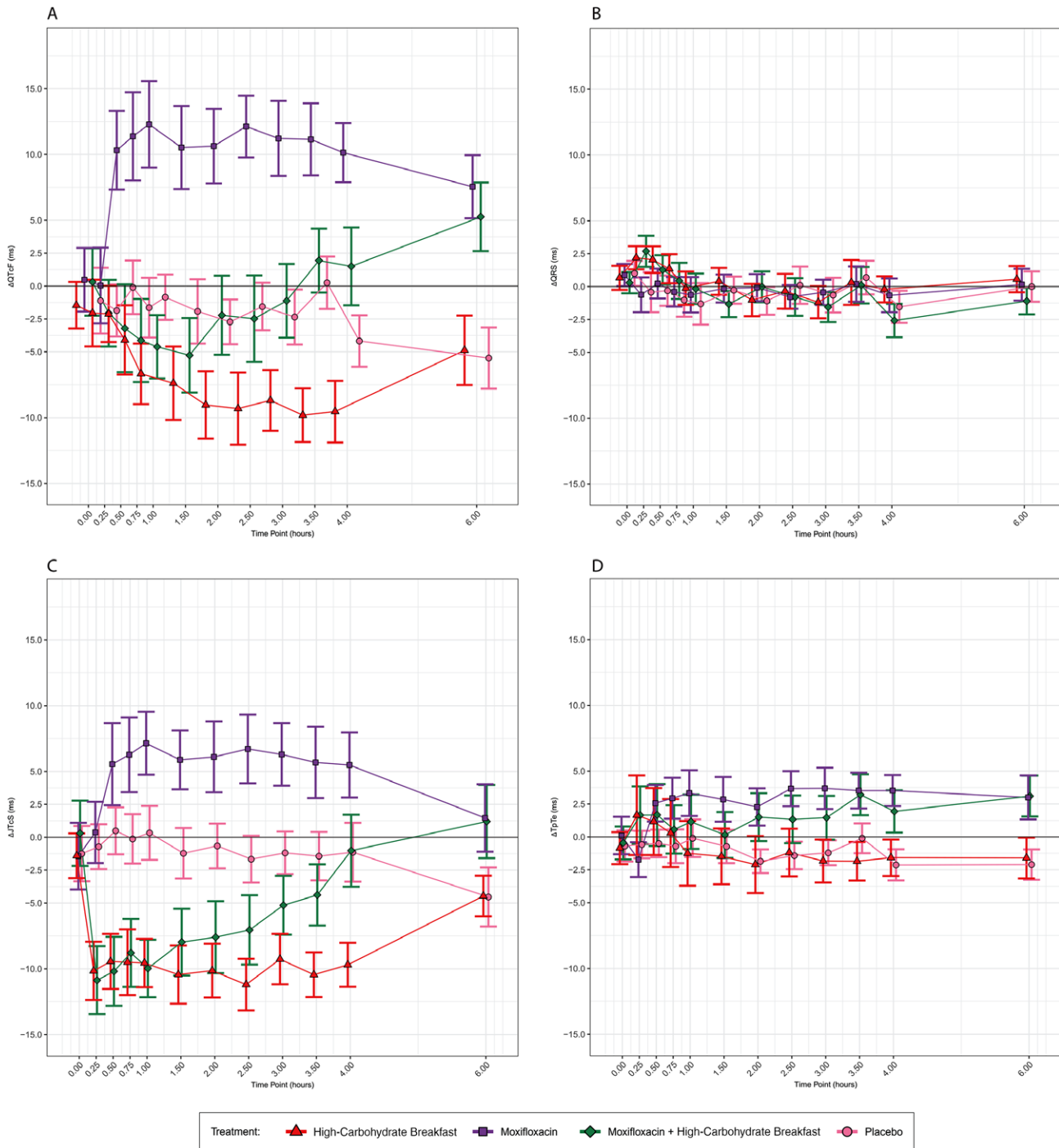
Figure 1. Averages by time point and treatment group of the observed values of ΔQT (A), ΔQRS (B), $\Delta J\text{-}T_{peak}cS$ (C), and $\Delta T_{peak}\text{-}T_{end}$ (D), as measured from the participants. The 90% confidence intervals are shown as vertical lines.

Following administration of moxifloxacin in the fasted state, $\Delta J\text{-}T_{peak}cS$ increased steadily over time, with significant changes starting from 30 minutes (5.80 milliseconds; 90%CI, 0.26–11.28). The estimated changes of $\Delta J\text{-}T_{peak}cS$ with moxifloxacin were consistently and significantly different from placebo from 45 minutes after dosing up to 6 hours (Table 4).

$T_{peak}\text{-}T_{end}$ Interval. Moxifloxacin administered in a fasting condition clearly prolonged $\Delta T_{peak}\text{-}T_{end}$. The increase of $\Delta T_{peak}\text{-}T_{end}$ was significantly higher than baseline from 2.5 hours (5.36 milliseconds; 90%CI, 1.02–9.49) up to 6 hours (7.50 milliseconds; 90%CI, 2.82–11.46). However, if given after breakfast, it was not associated with $\Delta T_{peak}\text{-}T_{end}$ prolongation until

Table 1. Output of the Mixed Model on $\triangle$QRS (Contrasts on Baseline)

Parameter		Estimate	Standard Error	Degrees of Freedom (df)	T Value	P Value	5%CI	95%CI
Baseline		−5.93	0.28	34.04	−21.24	< .001	−6.44	−5.52
Treatment	Time point							
Placebo	H 0.25	−0.63	1.24	41.28	−0.51	.61	−2.71	1.54
	H 0.5	−0.27	1.24	41.18	−0.22	.83	−2.19	1.99
	H 0.75	−0.2	1.24	41.19	−0.16	.87	−1.85	1.74
	H 1	−0.59	1.24	41.19	−0.47	.64	−2.5	1.55
	H 1.5	−1.18	1.24	41.12	−0.95	.35	−3.33	0.92
	H 2	−1.07	1.24	41.24	−0.86	.40	−3.07	1.15
	H 2.5	−0.67	1.24	41.29	−0.54	.59	−2.65	1.75
	H 3	−0.82	1.24	41.11	−0.66	.51	−2.67	1.06
	H 3.5	−0.12	1.24	41.12	−0.10	.92	−1.96	2.13
	H 4	−1.15	1.24	41.01	−0.92	.36	−3.29	1.18
	H 6	0.55	1.24	41.29	0.44	.66	−1.43	2.48
Moxifloxacin	H 0.25	−1.25	1.24	41.41	−1.00	.32	−3.26	0.86
	H 0.5	−0.15	1.24	41.21	−0.12	.91	−2.04	1.94
	H 0.75	−0.17	1.24	41.38	−0.14	.89	−2.14	2.40
	H 1	−0.70	1.25	41.52	−0.56	.58	−2.66	1.41
	H 1.5	−1.33	1.24	41.41	−1.07	.29	−3.43	0.69
	H 2	−0.20	1.24	41.34	−0.16	.87	−2.57	2.11
	H 2.5	−1.52	1.24	41.34	−1.23	.23	−3.70	0.57
	H 3	−0.39	1.24	41.31	−0.31	.76	−2.41	1.62
	H 3.5	−0.93	1.24	41.32	−0.75	.46	−2.86	0.94
	H 4	−1.37	1.24	41.43	−1.10	.28	−3.06	0.74
	H 6	0.34	1.24	41.3	0.27	.79	−2.05	2.34
High-carbohydrate breakfast	H 0.25	1.50	1.24	41.29	1.20	.24	−0.38	3.33
	H 0.5	1.53	1.24	41.17	1.24	.22	−0.16	3.72
	H 0.75	1.34	1.24	41.36	1.07	.29	−0.48	3.40
	H 1	−0.31	1.24	41.35	−0.25	.80	−2.48	2.16
	H 1.5	−0.10	1.24	41.37	−0.08	.93	−2.03	2.33
	H 2	−1.15	1.24	41.36	−0.92	.36	−3.01	1.24
	H 2.5	−1.12	1.24	41.24	−0.90	.37	−3.57	0.82
	H 3	−0.92	1.24	41.49	−0.74	.46	−3.09	1.00
	H 3.5	−0.90	1.24	41.39	−0.72	.48	−2.87	1.91
	H 4	−0.89	1.24	41.46	−0.72	.48	−3.05	1.26
	H 6	0.62	1.24	41.21	0.50	.62	−1.5	3.05
Moxifloxacin + high-carbohydrate breakfast	H 0.25	2.41	1.24	41.17	1.94	.06	0.47	4.73
	H 0.5	1.18	1.24	41.14	0.95	.35	−1.02	3.60
	H 0.75	1.02	1.24	41.15	0.82	.42	−1.07	3.28
	H 1	0.44	1.24	41.03	0.36	.72	−1.36	3.09
	H 1.5	−1.55	1.24	41.08	−1.25	.22	−3.59	0.65
	H 2	−0.02	1.24	41.27	−0.01	.99	−2.09	2.12
	H 2.5	−1.60	1.24	41.18	−1.29	.20	−3.89	0.88
	H 3	−1.52	1.24	41.28	−1.22	.23	−3.69	0.20
	H 3.5	−0.80	1.24	41.20	−0.65	.52	−2.93	1.86
	H 4	−2.17	1.24	41.03	−1.75	.09	−4.41	0.01
	H 6	−0.69	1.24	41.20	−0.55	.58	−2.54	1.35

6 hours after the dose (6.47 milliseconds; 90%CI, 2.32–10.05), indicating that the addition of food reduced the effects of moxifloxacin (Table 5). The linear contrasts with placebo estimates confirm this pattern, showing consistently higher estimates of ΔT_{peak}-T_{end} with moxifloxacin than placebo up to 6 hours and nonsignificant differences when food is added until 2 hours (Table 6). This could be the result of changes in the PK profile or a true increase of the moxifloxacin-induced QT prolongation.

Discussion

In this study, we retrospectively assessed the effects of food on the QRS, J-T_{peak}, and T_{peak}-T_{end} intervals by using data from a previously published thorough QT/QTc study in which moxifloxacin was administered as a positive control confirming assay sensitivity of the study.[7,17]

Moxifloxacin is known as a blocker of the inward rectifying potassium channels Ikr and Iks.[18] After a

Table 2. Linear Contrasts Between Placebo and Treatment Groups Derived From the Mixed Model on ΔQRS

Parameters Contrast	Time Point	Group	Estimate	Standard Error	T Value	P Value
Placebo	H 0.25	High-carbohydrate breakfast	2.72	0.70	3.90	<.01
		Moxifloxacin	−0.02	0.70	−0.04	>0.99
		Moxifloxacin + high-carbohydrate breakfast	3.04	0.70	4.38	<.001
	H 0.5	High-carbohydrate breakfast	2.40	0.70	3.45	<.05
		Moxifloxacin	0.72	0.70	1.03	>0.99
		Moxifloxacin + high-carbohydrate breakfast	1.45	0.69	2.09	.66
	H 0.75	High-carbohydrate breakfast	2.13	0.70	3.06	.06
		Moxifloxacin	0.62	0.70	0.89	>0.99
		Moxifloxacin + high-carbohydrate breakfast	1.81	0.69	2.61	.24
	H 1	High-carbohydrate breakfast	0.87	0.70	1.24	.99
		Moxifloxacin	0.48	0.70	0.69	>0.99
		Moxifloxacin + high-carbohydrate breakfast	1.03	0.70	1.48	.98
	H 1.5	High-carbohydrate breakfast	0.48	0.70	0.69	>0.99
		Moxifloxacin	−0.15	0.70	−0.22	>0.99
		Moxifloxacin + high-carbohydrate breakfast	−0.97	0.70	−1.39	.99
	H 2	High-carbohydrate breakfast	−0.08	0.70	−0.12	>0.99
		Moxifloxacin	0.87	0.70	1.24	.99
		Moxifloxacin + high-carbohydrate breakfast	1.05	0.69	1.51	.97
	H 2.5	High-carbohydrate breakfast	−0.45	0.70	−0.64	>0.99
		Moxifloxacin	−0.85	0.70	−1.22	.99
		Moxifloxacin + high-carbohydrate breakfast	−0.93	0.70	−1.34	.99
	H 3	High-carbohydrate breakfast	−0.69	0.70	−0.98	>0.99
		Moxifloxacin	−0.16	0.70	−0.23	>0.99
		Moxifloxacin + high-carbohydrate breakfast	−0.7	0.70	−1.00	>0.99
	H 3.5	High-carbohydrate breakfast	−0.18	0.70	−0.26	>0.99
		Moxifloxacin	−0.8	0.70	−1.15	.99
		Moxifloxacin + high-carbohydrate breakfast	−0.68	0.70	−0.97	>0.99
	H 4	High-carbohydrate breakfast	1.44	0.70	2.05	.69
		Moxifloxacin	0.96	0.70	1.37	.99
		Moxifloxacin + high-carbohydrate breakfast	−1.02	0.70	−1.47	.98
	H 6	High-carbohydrate breakfast	0.66	0.70	0.95	>0.99
		Moxifloxacin	0.38	0.70	0.55	>0.99
		Moxifloxacin + high-carbohydrate breakfast	−1.24	0.70	−1.78	.89

single 400-mg oral dose has previously been reported to cause a modest (10–14 milliseconds) prolongation of the QT interval, J-T_{peak}, and T_{peak}-T_{end} intervals.[19] Our results from this study are aligned with this ECG signature.

Interactions of feeding and moxifloxacin-induced QTcF prolongation not secondary to changes in the PK profile were first reported by Bloomfield et al.[20] Our previous analysis of the QTcF interval had shown an apparent reduction in QTcF prolongation when moxifloxacin was given after breakfast,[17] but it is unclear how much of that effect is attributable to the altered PK profile that occurs when moxifloxacin is given after a meal. An interaction between the QT shortening effects of food on the cardiac action potential and the QT prolonging block of Ikr/Iks after moxifloxacin may exist even if the underlying mechanisms were different.

The main objective of this study was to further investigate the effects of a meal on the ECG utilizing an automated algorithm allowing the assessments of change to QRS, J-T_{peak}, and T_{peak}-T_{end}. This analysis revealed that a meal leads to a prompt, lasting, and significant shortening of J-T_{peak} in both male and female volunteers. We did not see effects on QRS and T_{peak}-T_{end} beyond a small prolongation of both intervals during the first hour after starting a meal.

It is well recognized that eating a meal is associated with changes in the cardiovascular system due to the increase in blood flow to the splanchnic vascular bed. The increase in CO is delivered by an increase of HR and stroke volume. In our studies, we typically find HR increases of 8 beats/min, which gradually return to baseline over 4 hours after a meal.[1,7] In healthy subjects, the CO showed increases depending on type of meal, age, and sex.[21,22] The composition of a meal may play an important role, as high-carbohydrate meals were shown to be accompanied by a prolonged rise of CO: 32% compared with a rise of 22% after ingestion of high-fat meals.[21] In addition to the differing effects of meal composition on CO, there are different patterns of change in peripheral hemodynamics. High-fat

Table 3. Output of the Mixed Model of ΔJ-T$_{peak}$ (Contrasts on Baseline)

Parameter	Time point	Estimate	Standard Error	Degrees of Freedom (df)	T Value	P Value	5%CI	95%CI
Baseline		−16.41	1.32	31.41	−12.43	<.001	−18.81	−14.02
Treatment	Time point							
Placebo	H 0.25	−0.35	3.33	33.37	−0.11	.92	−5.85	5.68
	H 0.5	−0.1	3.34	33.49	−0.03	.98	−5.68	5.72
	H 0.75	−0.29	3.34	33.75	−0.09	.93	−6.13	5.53
	H 1	0.47	3.34	33.52	0.14	.89	−5.13	6.58
	H 1.5	−0.34	3.34	33.5	−0.1	.92	−5.9	5.05
	H 2	−0.37	3.34	33.58	−0.11	.91	−5.91	5.46
	H 2.5	−1.27	3.34	33.74	−0.38	.71	−6.87	4.98
	H 3	−1.25	3.34	33.56	−0.38	.71	−6.87	3.68
	H 3.5	−2.07	3.33	33.44	−0.62	.54	−6.87	4.08
	H 4	−1.58	3.33	33.47	−0.47	.64	−7.41	4.81
	H 6	−4.69	3.34	33.48	−1.41	.17	−10.09	1.14
Moxifloxacin	H 0.25	1.16	3.33	33.41	0.35	.73	−4.49	6.9
	H 0.5	5.8	3.34	33.64	1.74	.09	0.26	11.28
	H 0.75	6.7	3.35	33.9	2	.05	0.91	13.07
	H 1	8.01	3.34	33.75	2.4	<.05	2.00	13.87
	H 1.5	7.23	3.34	33.83	2.16	<.05	1.41	13.68
	H 2	6.62	3.34	33.6	1.98	.06	0.33	12.24
	H 2.5	7.53	3.34	33.81	2.25	<.05	1.44	14.15
	H 3	6.3	3.34	33.7	1.89	.07	0.50	11.46
	H 3.5	5.69	3.34	33.56	1.71	.1	0.63	11.35
	H 4	5.94	3.34	33.6	1.78	.08	0.26	11.31
	H 6	1.4	3.34	33.71	0.42	.68	−4.73	6.93
High-carbohydrate breakfast	H 0.25	−9.52	3.34	33.6	−2.85	<.01	−14.93	−3.34
	H 0.5	−9.48	3.34	33.61	−2.84	<.01	−14.35	−3.27
	H 0.75	−9.78	3.34	33.6	−2.93	<.01	−15.67	−4.19
	H 1	−8.58	3.34	33.82	−2.57	<.05	−14.14	−2.19
	H 1.5	−9.38	3.34	33.76	−2.81	<.01	−15.21	−3.4
	H 2	−9.32	3.34	33.62	−2.79	<.01	−14.45	−3.36
	H 2.5	−10.28	3.34	33.65	−3.08	<.01	−16.6	−5.27
	H 3	−9.1	3.34	33.62	−2.73	<.05	−15.18	−3.82
	H 3.5	−10.11	3.34	33.69	−3.03	<.01	−15.42	−3.16
	H 4	−9.39	3.34	33.63	−2.81	<.01	−15.33	−4.04
	H 6	−3.85	3.34	33.68	−1.15	.26	−9.98	2.82
Moxifloxacin + high-carbohydrate breakfast	H 0.25	−10.65	3.34	33.55	−3.19	<.01	−16.32	−5.01
	H 0.5	−11.07	3.33	33.46	−3.32	<.01	−17.7	−5.21
	H 0.75	−9.67	3.33	33.45	−2.9	<.01	−15.33	−3.74
	H 1	−9.72	3.34	33.59	−2.91	<.01	−15.04	−3.71
	H 1.5	−7.38	3.33	33.44	−2.21	<.05	−12.74	−1.28
	H 2	−7.01	3.34	33.6	−2.1	<.05	−12.56	−1.04
	H 2.5	−6.55	3.34	33.58	−1.96	.06	−12.89	−0.14
	H 3	−5.08	3.33	33.48	−1.52	.14	−11.07	−0.13
	H 3.5	−4.69	3.34	33.57	−1.4	.17	−11.02	1.64
	H 4	−1.61	3.34	33.5	−0.48	.63	−7.39	3.76
	H 6	1.72	3.33	33.45	0.52	.61	−3.8	6.64

meals increased the postprandial superior mesenteric blood flow of 121%, compared with 87% for the high-carbohydrate meal.[23]

Plasma noradrenaline levels were shown to increase after a high-carbohydrate meal, while levels were virtually unchanged after a high-fat meal,[24] although we found little difference on QTcF between carbohydrate meals and meals with a higher fat content.[7] The ingestion of a meal was associated with an increase in cardiac sympathetic activity in young subjects,[25,26] while healthy elderly subjects do not increase cardiac sympathetic activity after meal ingestion.[27] Activation of the sympathetic nervous system plays a major role in maintaining cardiovascular homeostasis by increasing inotropy, chronotropy, and lusitropy. These changes are mediated by activation of the β-adrenergic receptor signaling pathway, leading to protein kinase A activation and phosphorylation of intracellular proteins. A key target of protein kinase A is the sarcolemmal L-type Ca^{2+} channel, which, when

Table 4. Linear Contrasts Between Placebo and Treatment Groups Derived From the Mixed Model on ΔJ-T$_{peak}$

Parameters Contrast	Timepoint	Group	Estimate	Standard Error	T Value	P Value
Placebo	H 0.25	High-carbohydrate breakfast	−9.16	1.36	−6.74	<.001
		Moxifloxacin	1.51	1.35	1.11	.9998
		Moxifloxacin + high-carbohydrate breakfast	−10.29	1.35	−7.6	<.001
	H 0.5	High-carbohydrate breakfast	−9.38	1.36	−6.89	<.001
		Moxifloxacin	4.26	1.36	3.13	.0534
		Moxifloxacin + high-carbohydrate breakfast	−10.97	1.35	−8.1	<.001
	H 0.75	High-carbohydrate breakfast	−9.48	1.36	−6.97	<.001
		Moxifloxacin	6.99	1.35	5.17	<.001
		Moxifloxacin + high-carbohydrate breakfast	−9.37	1.35	−6.92	<.001
	H 1	High-carbohydrate breakfast	−10.69	1.37	−7.82	<.001
		Moxifloxacin	7.54	1.36	5.55	<.001
		Moxifloxacin + high-carbohydrate breakfast	−10.19	1.35	−7.53	<.001
	H 1.5	High-carbohydrate breakfast	−9.05	1.36	−6.66	<.001
		Moxifloxacin	7.57	1.35	5.59	<.001
		Moxifloxacin + high-carbohydrate breakfast	−7.05	1.35	−5.21	<.001
	H 2	High-carbohydrate breakfast	−8.95	1.36	−6.58	<.001
		Moxifloxacin	6.99	1.36	5.16	<.001
		Moxifloxacin + high-carbohydrate breakfast	−6.64	1.35	−4.9	<.001
	H 2.5	High-carbohydrate breakfast	−9	1.37	−6.59	<.001
		Moxifloxacin	8.8	1.35	6.5	<.001
		Moxifloxacin + high-carbohydrate breakfast	−5.28	1.36	−3.88	<.01
	H 3	High-carbohydrate breakfast	−7.85	1.36	−5.77	<.001
		Moxifloxacin	7.55	1.35	5.58	<.001
		Moxifloxacin + high-carbohydrate breakfast	−3.83	1.35	−2.83	.135
	H 3.5	High-carbohydrate breakfast	−9.68	1.36	−7.11	<.001
		Moxifloxacin	7.77	1.35	5.75	<.001
		Moxifloxacin + high-carbohydrate breakfast	−2.61	1.35	−1.93	.7936
	H 4	High-carbohydrate breakfast	−9.46	1.37	−6.92	<.001
		Moxifloxacin	5.88	1.36	4.32	<.001
		Moxifloxacin + high-carbohydrate breakfast	−0.04	1.36	−0.03	>0.9999
	H 6	High-carbohydrate breakfast	0.84	1.37	0.61	>0.9999
		Moxifloxacin	6.09	1.36	4.49	<.001
		Moxifloxacin + high-carbohydrate breakfast	6.41	1.36	4.72	<.001

phosphorylated, enhances Ca^{2+} entry into the cell.[28] It was suggested that norepinephrine mediates Ca^{2+} release by a rapid increase in the free Ca^{2+} concentration near the sarcoplasmic reticulum.[29] As a result of the Ca^{2+} cycling between the cytoplasm and the sarcoplasmic reticulum, contractility is increased, and a greater volume of blood is ejected during systole. It is important to note that the increases in epinephrine and norepinephrine plasma levels observed after a meal are short lasting (90 minutes),[24] whereas the QTcF shortening is consistently present for up to 7 hours after the start of a meal.[30]

The main effect of a meal on cardiac repolarization seen in this study was a shortening of J-T$_{peak}$, which corresponds to phase 2 and the early part of phase 3 of the cardiac myocyte action potential. Phase 2 of the action potential is characterized by a number of ion channels closing and opening. The duration of phase 2 is influenced in a significant way by the balance of Ca^{2+} influx and K^+ outflow. We therefore suggest that the mechanism by which food affects J-T$_{peak}$ may be associated with the signaling pathways of calcium cycling. This is consistent with the increase in cardiac stroke volume after a meal, which involves an increase in Ca^{2+} release and uptake to and from the sarcoplasmic reticulum associated with increased inotropy.

In our previous work, we have shown that a meal shortens the QTcF interval in the context of a formal thorough QT study using moxifloxacin as a positive control to assess assay sensitivity.[1] We could reconcile our findings with previous literature reports suggesting that meals prolong QTcB: First, these studies looked only at the first 1 to 2 hours after a meal, when there is a significant HR increase, and they used Bazett's HR correction, which is sensitive to HR changes. We could replicate these findings from our data. We conducted a further research study[17] to investigate claims by Gastaldelli et al[31] that insulin prolongs QTc. We found that C-peptide and glucose had antagonistic effects on the QTc interval. In a healthy individual, the presence of C-peptide shortens the QTc, and it appears that this

Table 5. Output of the Mixed Model of ΔT_{peak}-T_{end} (Contrasts on Baseline)

Parameter		Estimate	Standard Error	Degrees of Freedom (df)	T Value	P Value	5%CI	95%CI
Baseline		−9.72	0.58	30.12	−16.88	<.001	−10.75	−8.55
Treatment	Time point							
Placebo	H 0.25	0.28	2.53	32.16	0.11	.91	−3.76	4.79
	H 0.5	0.16	2.53	32.08	0.06	.95	−3.78	4.63
	H 0.75	1.08	2.53	32.04	0.43	.67	−2.89	5.58
	H 1	1.24	2.53	32.1	0.49	.63	−3.03	5.45
	H 1.5	0.93	2.53	32.1	0.37	.71	−3.31	5.48
	H 2	0.13	2.53	32.08	0.05	.96	−4.02	4.63
	H 2.5	0.07	2.53	32.11	0.03	.98	−3.97	4.67
	H 3	0.33	2.53	32.12	0.13	.90	−3.54	4.09
	H 3.5	1.71	2.53	32.17	0.68	.50	−2.35	6.80
	H 4	0.89	2.53	32.13	0.35	.73	−3.38	5.10
	H 6	2.79	2.53	32.25	1.10	.28	−1.18	7.07
Moxifloxacin	H 0.25	−0.54	2.53	32.18	−0.21	.83	−4.75	3.49
	H 0.5	2.76	2.53	32.14	1.09	.28	−1.24	7.40
	H 0.75	4.39	2.53	32.06	1.74	.09	0.52	9.00
	H 1	4.33	2.53	32.13	1.71	.10	−0.13	8.47
	H 1.5	4.22	2.53	32.1	1.67	.10	−0.21	8.05
	H 2	4.11	2.53	32.12	1.63	.11	−0.53	8.46
	H 2.5	5.36	2.53	32.12	2.12	<.05	1.02	9.49
	H 3	4.96	2.53	32.16	1.96	.06	0.69	9.04
	H 3.5	5.29	2.53	32.2	2.09	<.05	1.4	9.06
	H 4	5.85	2.53	32.12	2.32	<.05	1.72	9.71
	H 6	7.5	2.53	32.25	2.96	<.01	2.82	11.46
High-carbohydrate Breakfast	H 0.25	2.72	2.53	32.24	1.07	.29	−1.33	6.61
	H 0.5	1.55	2.53	32.27	0.61	.55	−2.17	6.06
	H 0.75	1.74	2.53	32.18	0.69	.50	−2.47	6.07
	H 1	0.05	2.53	32.2	0.02	.99	−4.22	5.02
	H 1.5	−0.1	2.53	32.16	−0.04	.97	−4.26	4.42
	H 2	−0.92	2.53	32.16	−0.36	.72	−4.94	3.99
	H 2.5	0.01	2.53	32.16	0	>.99	−4.62	4.04
	H 3	−0.85	2.53	32.14	−0.34	.74	−5.18	2.84
	H 3.5	−0.97	2.53	32.16	−0.38	.70	−4.85	4.15
	H 4	0.01	2.53	32.15	0	>.99	−4.39	4.69
	H 6	1.36	2.53	32.16	0.54	.59	−3.06	6.12
Moxifloxacin + high-carbohydrate breakfast	H 0.25	2.39	2.53	32.23	0.94	.35	−1.46	6.88
	H 0.5	2.51	2.53	32.2	0.99	.33	−2.34	6.89
	H 0.75	2.35	2.53	32.15	0.93	.36	−1.93	7.15
	H 1	2.79	2.53	32.17	1.11	.28	−0.89	7.82
	H 1.5	1.83	2.53	32.15	0.72	.47	−2.14	6.63
	H 2	2.83	2.53	32.11	1.12	.27	−1.22	7.48
	H 2.5	2.33	2.53	32.15	0.92	.36	−2.27	6.98
	H 3	2.7	2.53	32.1	1.07	.29	−1.62	6.51
	H 3.5	4.14	2.53	32.13	1.64	.11	−0.40	9.22
	H 4	4.21	2.53	32.16	1.66	.11	−0.47	8.25
	H 6	6.47	2.53	32.15	2.56	<.05	2.32	10.05

is offsetting the effect of glucose, which by itself appears to prolong the QTc interval; this has been described by others.[32] We hypothesized that the net effect of the 2 antagonistic effects leads to an approximate 8-millisecond QTc shortening with a peak occurring 2 to 4 hours after starting a meal in a normal individual. Our analysis also confirmed that insulin in a euglycemic clamp has no effect on QTcF.[7]

It has been demonstrated that physiological concentrations of C-peptide increase the expression of erythrocyte Na^+/K^+-adenosine triphosphatase[33] and induce intracellular Ca^{2+} increases in human renal tubular cells,[34] making it a reasonable hypothesis that the same occurrence could be observable in cardiomyocytes. C-peptide was also shown to increase the release of nitric oxide from endothelial nitric oxide synthase in bovine aortic endothelial cells in a concentration- and time-dependent manner. The data indicated that C-peptide is likely to stimulate the activity of the Ca^{2+}-sensitive endothelial nitric oxide synthase by increasing

Table 6. Linear Contrasts Derived From the Mixed Model on ΔT_{peak}-T_{end}

Parameters Contrast	Time Point	Group	Estimate	Standard Error	T Value	P Value
Placebo	0.25	High-carbohydrate breakfast	2.43	0.97	2.51	.31
		Moxifloxacin	−0.82	0.97	−0.85	>.99
		Moxifloxacin + high-carbohydrate breakfast	2.11	0.97	2.18	.58
	0.5	High-carbohydrate breakfast	2.36	0.97	2.43	.36
		Moxifloxacin	2.6	0.97	2.69	.19
		Moxifloxacin + high-carbohydrate breakfast	2.35	0.97	2.43	.37
	0.75	High-carbohydrate breakfast	1.63	0.97	1.68	.94
		Moxifloxacin	3.31	0.96	3.43	<.05
		Moxifloxacin + high-carbohydrate breakfast	2.24	0.97	2.32	.46
	1	High-carbohydrate breakfast	−1.19	0.97	−1.23	>.99
		Moxifloxacin	3.09	0.96	3.2	<.05
		Moxifloxacin + high-carbohydrate breakfast	1.56	0.97	1.61	.96
	1.5	High-carbohydrate breakfast	−0.06	0.97	−0.06	>.99
		Moxifloxacin	3.28	0.96	3.4	<.05
		Moxifloxacin + high-carbohydrate breakfast	0.9	0.97	0.93	>.99
	2	High-carbohydrate breakfast	−0.08	0.97	−0.08	>.99
		Moxifloxacin	3.97	0.97	4.12	<.01
		Moxifloxacin + high-carbohydrate breakfast	3.67	0.97	3.8	<.01
	2.5	High-carbohydrate breakfast	−0.06	0.97	−0.07	>.99
		Moxifloxacin	5.28	0.97	5.47	<.001
		Moxifloxacin + high-carbohydrate breakfast	3.23	0.96	3.35	<.05
	3	High-carbohydrate breakfast	−0.21	0.97	−0.22	>.99
		Moxifloxacin	4.63	0.97	4.8	<.001
		Moxifloxacin + high-carbohydrate breakfast	2.37	0.97	2.46	.34
	3.5	High-carbohydrate breakfast	−1.71	0.97	−1.77	.90
		Moxifloxacin	3.58	0.96	3.71	<.01
		Moxifloxacin + high-carbohydrate breakfast	3.4	0.97	3.52	<.05
	4	High-carbohydrate breakfast	0.09	0.97	0.09	>.99
		Moxifloxacin	5.94	0.97	6.15	<.001
		Moxifloxacin + high-carbohydrate breakfast	4.29	0.97	4.44	<.001
	6	High-carbohydrate breakfast	0.52	0.97	0.53	>.99
		Moxifloxacin	4.71	0.96	4.88	<.001
		Moxifloxacin + high-carbohydrate breakfast	4.65	0.97	4.81	<.001

the influx of Ca^{2+} into endothelial cells.[35] Combined, data suggest that C-peptide may elicit changes in cardiac repolarization through a potential interaction with Ca^{2+} cycling in the cardiac myocyte as one possible explanation for QTc shortening after a meal. Although C-peptide has been shown to bind specifically to human cell membranes[36,37] and G-protein coupled receptor 146 was identified as an essential contributor for the C-peptide signaling complex,[38] the receptor of C-peptide remains unknown. Further investigations are required to understand the role of C-peptide and determine the underlying mechanisms of the impact on cardiac repolarization during the postprandial period.

In summary, this study demonstrates that food exerts an effect on early repolarization and that an initial shortening of the J-T_{peak} interval was seen with moxifloxacin in the fed state even when the differences in plasma concentrations are taken into account (data not shown).

From the analysis presented in this paper, it could be postulated that some type of relationship might exist between shortening of J-T_{peak} and C-peptide concentrations having an effect on Ca^{2+} cycling in the cardiomyocyte. Further work will be necessary to unravel the precise mechanism(s) by which this interplay could occur.

Limitations

This study has some limitations. This was a retrospective analysis and exploratory in nature, as the technical methods utilized to measure the subintervals of QTc were unavailable at the time of conducting the study. However, the analysis is sufficiently robust regarding the J-T_{peak} interval shortening following a meal, and the results align well with previous published work. The sample size is relatively small and contains both females and males. Matsukura et al[39] recently published a report suggesting that males and females show a different response regarding the J-T_{peak} interval. This has not been explored with this data.

Declaration of Conflicting Interests

Data Sharing

Requests for access to data should be addressed to the corresponding author.

References

1. Täubel J, Wong AH, Naseem A, Ferber G, Camm AJ. Shortening of the QT interval after food can be used to demonstrate assay sensitivity in thorough QT studies. *J Clin Pharmacol*. 2012;52(10):1558–1565.

2. Täubel J, Ferber G, Lorch U, Wang D, Sust M, Camm AJ. Single doses up to 800 mg of E-52862 do not prolong the QTc interval—a retrospective validation by pharmacokinetic-pharmacodynamic modelling of electrocardiography data utilising the effects of a meal on QTc to demonstrate ECG assay sensitivity. *PLoS One*. 2015;10(8):e0136369.

3. Cirincione B, Sager PT, Mager DE. Influence of meals and glycemic changes on QT interval dynamics. *J Clin Pharmacol*. 2017;57(8):966–976.

4. Täubel J, Fernandes S, Ferber G. Stability of the effect of a standardized meal on QTc. *Ann Noninvasive Electrocardiol*. 2017;22(1), https://doi.org/10.1111/anec.12371.

5. Mohamed MF, Zeng J, Jiang P, Hosmane B, Othman AA. Use of early clinical trial data to support thorough QT study waiver for upadacitinib and utility of food effect to demonstrate ECG assay sensitivity. *Clin Pharmacol Ther*. 2018;103(5):836–842.

6. Gordin D, Forsblom C, Rönnback M, Groop PH. Acute hyperglycaemia disturbs cardiac repolarization in type 1 diabetes. *Diabet Med*. 2008;25(1):101–105.

7. Täubel J, Lorch U, Ferber G, et al. Insulin at normal physiological levels does not prolong QT(c) interval in thorough QT studies performed in healthy volunteers. *Br J Clin Pharmacol*. 2013;75(2):392–403.

8. Wahren J, Ekbery K, Jornvall H. C-peptide and neuropathy in type 1 diabetes. *Immunol Endocr Metab Agents Med Chem*. 2007;7:69–77.

9. Bo S, Broglio F, Settanni F, et al. Effects of meal timing on changes in circulating epinephrine, norepinephrine, and acylated ghrelin concentrations: a pilot study. *Nutr Diabetes*. 2017;7:303.

10. Johannesen L, Vicente J, Mason JW, et al. Differentiating drug-induced multichannel block on the electrocardiogram: randomized study of dofetilide, quinidine, ranolazine, and verapamil. *Clin Pharmacol Ther*. 2014;96(5):549–558.

11. Xue JX, Gao W, Chen Y, Han X. Study of repolarization heterogeneity and electrocardiographic morphology with a modeling approach. *J Electrocardiol*. 2008;41:581–587.

12. Xue JX. Robust QT interval estimation: from algorithm to validation. *Ann Noninvasive Electrocardiol*. 2009;14:S35–S41.

13. Tornoe CW, Garnett CE, Wang Y, Florian J, Li M, Gobburu JV. Creation of a knowledge management system for QT analyses. *J Clin Pharmacol*. 2011;51(7):1035–1042.

14. Smetana P, Batchvarov V, Hnatkova K, John Camm A, Malik M. Sex differences in the rate dependence of the T wave descending limb. *Cardiovasc Res*. 2003;58:549–554.

15. Johannesen L, Vicente J, Gray RA, et al. Improving the assessment of heart toxicity for all new drugs through translational regulatory science. *Clin Pharmacol Ther*. 2014;95:501–508.

16. Garnett CE, Zhu H, Malik M, et al. Methodologies to characterize the QT/corrected QT interval in the presence of drug-induced heart rate changes or other autonomic effects. *Am Heart J*. 2012;163(6):912–930.

17. Täubel J, Ferber G, Lorch U, Batchvarov V, Savelieva I, Camm AJ. Thorough QT study of the effect of oral moxifloxacin on QTc interval in the fed and fasted state in healthy Japanese and Caucasian subjects. *Br J Clin Pharmacol*. 2014;77(1): 170–179.

18. Johannesen L, Vicente J, Mason JW, et al. Late sodium current block for drug-induced long QT syndrome: results from a prospective clinical trial. *Clin Pharmacol Ther*. 2016;99(2):214–223.

19. Vicente J, Johannesen L, Mason JW, et al. Comprehensive T wave morphology assessment in a randomized clinical study of dofetilide, quinidine, ranolazine, and verapamil. *J Am Heart Assoc*. 2015;4(4):pii: e001615.

20. Bloomfield DM, Kost JT, Ghosh K, et al. The effect of moxifloxacin on QTc and implications for the design of thorough QT studies. *Clin Pharmacol Ther*. 2008;84(4):475–480.

21. Kearney MT, Cowley AJ, Macdonald IA. The cardiovascular responses to feeding in man. *Exp Physiol*. 1995;80(5):683–700.

22. Waaler BA, Eriksen M, Toska K. The effect of meal size on postprandial increase in cardiac output. *Acta Physiol Scand*. 1991;142(1):33–39.

23. Sidery MB, Macdonald IA, Cowley AJ, Fullwood LJ. Cardiovascular responses to high-fat and high-carbohydrate meals in young subjects. *Am J Physiol*. 1991;261(5 Pt 2):H1430–436.

24. Heseltine D, Potter JF, Hartley G, MacDonald IA, James OFW. Blood pressure, heart rate and neuroendocrine responses to a high carbohydrate and a high fat meal in healthy young subjects. *Clin Sci (Lond)*. 1990;79(5):517–522.

25. Hayano J, Sakakibara Y, Yamada M, et al. Diurnal variations in vagal and sympathetic cardiac control. *Am J Physiol*. 1990;258(3 Pt 2):H642–646.

26. Ryan SM, Goldberger AL, Ruthazer R, Mietus J, Lipsitz LA. Spectral analysis of heart rate dynamics in elderly persons with postprandial hypotension. *Am J Cardiol*. 1992;69:201–205.

27. Lipsitz LA, Ryan SM, Parker JA, Freeman R, Wei JY, Goldberger AL. Hemodynamic and autonomic nervous system responses to mixed meal ingestion in healthy young and old subjects and dysautonomic patients with postprandial hypotension. *Circulation* 1993;87:391–400.

28. Bers DM. Calcium cycling and signaling in cardiac myocytes. *Annu Rev Physiol*. 2008;70:23–49.

29. Leijten P, Saida K, van Breemen C. Norepinephrine-induced intracellular Ca^{2+} release from vascular smooth muscle. *J Cardiovasc Pharmacol*. 1985;7(suppl 6):S38–42.

30. Täubel J, Ferber G, Fernandes S, Camm AJ. Diurnal profile of the QTc interval following moxifloxacin administration. *J Clin Pharmacol*. 2019;59(1):35–44.

31. Gastaldelli A, Emdin M, Conforti F, Camastra S, Ferrannini E. Insulin prolongs the QTc interval in humans. *Am J Physiol Regul Integr Comp Physiol*. 2000;279(6):R2022–2025.

32. Gordin D, Forsblom C, Rönnback M, Groop PH. Acute hyperglycaemia disturbs cardiac repolarization in type 1 diabetes. *Diabet Med*. 2008;25(1):101–105.

33. Galuska D, Pirkmajer S, Barres R, Ekberg K, Wahren J, Chibalin AV. C-Peptide increases Na, K-ATPase expression via PKC- and MAP kinase-dependent activation of transcription factor ZEB in human renal tubular cells. *PLoS ONE* 2011;6:e28294.

34. Shafqat J, Juntti-Berggren L, Zhong Z, et al. Proinsulin C-peptide and its analogues induce intracellular Ca2+ increases in human renal tubular cells. *Cell Mol Life Sci*. 2002;59(7):1185–1189.

35. Wallerath T, Kunt T, Forst T, et al. Stimulation of endothelial nitric oxide synthase by proinsulin C-peptide. *Nitric Oxide*. 2003;9(2):95–102.

36. Rigler R, Pramanik A, Jonasson P, et al. Specific binding of proinsulin C-peptide to human cell membranes. *Proc Natl Acad Sci U S A*. 1999;96(23):13318–13323.

37. Luppi P, Cifarelli V, Wahren J. C-peptide and long-term complications of diabetes. *Pediatr Diabetes*. 2011;12(3 Pt 2):276–292.

38. Yosten GL, Kolar GR, Redlinger LJ, Samson WK. Evidence for an interaction between proinsulin C-peptide and GPR146. *J Endocrinol*. 2013;218(2):B1–B8.

39. Matsukura S, Nakamura Y, Hoshiai K. Effects of moxifloxacin on the proarrhythmic surrogate markers in healthy Filipino subjects: exposure-response modeling using ECG data of thorough QT/QTc study. *J Pharmacol Sci*. 2018;136(4):234–241.