Mechanisms of sex and age differences in ventricular repolarization in humans

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Introduction Corrected QT interval (QTc) is shorter in postpubertal men than in women; however, QTc lengthens as men age and testosterone levels decrease. Animal studies have demonstrated that testosterone decreases L-type calcium current and increases slow delayed rectifier potassium current; however, it is not known how these contribute to QTc differences by sex and age in humans. We separately analyzed early versus late repolarization duration and performed simulations of the effect of testosterone on the electrocardiogram (ECG) to examine the mechanism of sex and age differences in QTc in humans.

Methods Twelve-lead ECGs from 2,235 healthy subjects (41% women) in Thorough QT studies were analyzed to characterize sex- and age-dependent differences in depolarization (QRS), early repolarization (J-T_{peak}), and late repolarization (T_{peak}-T_{end}). In addition, we simulated the effects of testosterone on calcium current, slow delayed rectifier potassium current, and surface ECG intervals.

Results QTc was shorter in men than in women (394 ± 16 vs 408 ± 15 milliseconds, $P < .001$), which was due to shorter early repolarization (213 ± 16 vs 242 ± 16 milliseconds, $P < .001$), as men had longer depolarization (94 ± 7 vs 89 ± 7 milliseconds, $P < .001$) and longer late repolarization (87 ± 10 vs 78 ± 9 milliseconds, $P < .001$). Sex difference in QTc decreased with age and was due to changes in early repolarization. Simulations showed that the early repolarization changes were most influenced by testosterone's effect on calcium current.

Conclusion Shorter QTc in men compared to women is explained by shorter early repolarization, and this difference decreases with age. These sex and age differences in repolarization appear to be caused by testosterone effects on calcium current. (Am Heart J 2014;168:749-756.e3.)

Women are at higher risk than men for the ventricular arrhythmia torsade de pointes.[1,2] The reason for the increased risk is not clear, but sex differences in the electrophysiology of the heart might make women more susceptible to torsade. Women have a longer heart rate corrected QT interval (QTc) than men.[3] After puberty, QTc decreases in men, resulting in a sex difference in QTc.[4] Subsequently, male QTc values increase as men age; and elderly men and women have similar QTc values again. These changes are inversely related to testosterone levels in men.[5,6]

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Testosterone has been shown to shorten cardiac cell action potential duration in guinea pigs by inhibiting L-type calcium current (I_{CaL}; an inward depolarizing current) and enhancing the slow delayed rectifier potassium current (I_{Ks}; an outward repolarizing current).[7] However, significant differences in ion channel expression between species exist[8]; and it is unclear what the primary mechanism is that contributes to sex and age differences in QTc in humans.

Through analysis of preclinical and clinical data from 34 Thorough QT studies[9] submitted to the Food and Drug Administration (FDA), along with computer simulations, we demonstrated recently that drug-induced multi-ion channel block can be differentiated on the electrocardiogram (ECG).[10] One observation was that blocking I_{CaL} primarily shortens early repolarization (J-T_{peak}). In the present study, we use data from Thorough QT studies to characterize sex- and age-dependent differences in depolarization (QRS), early repolarization (J-T_{peak}), and late repolarization (T_{peak}-T_{end}) to elucidate the mechanisms for sex and age differences in QTc. In addition, we simulate the effect of decreasing testosterone levels on individual ion channel currents and early versus late repolarization on the ECG to understand the mechanism

behind sex and age differences in ventricular repolarization in humans.

Methods

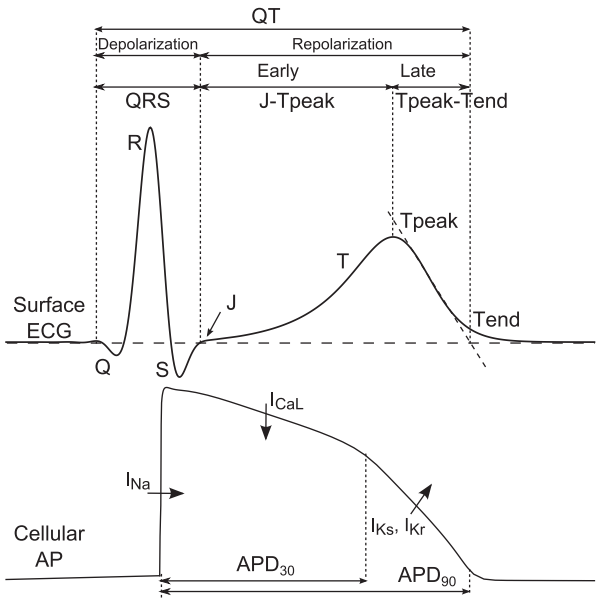
This study was approved by the Research Involving Human Subjects Committee of the US FDA. For each of the individual clinical studies included in this analysis, the studies were approved by the local institutional review boards; and all subjects gave informed consent. This project was supported in part by FDA's Critical Path Initiative, FDA's Office of Women's Health, and appointments to the Research Participation Program at the Center for Devices and Radiological Health administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the US Department of Energy and the US Food and Drug Administration. The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper, and its final contents.

ECG analysis

We analyzed resting 12-lead ECG recordings from 2,235 healthy subjects aged 18 to 78 years from 30 Thorough QT studies. Inclusion criteria for a typical Thorough QT study include healthy men or women without any clinically significant abnormalities; taking no medication (except oral contraceptives); and, for women, not pregnant or lactating before enrollment. Exclusion criteria include use of drugs, tobacco products, or alcohol consumption, as well as ECG thresholds such as QTc >450 milliseconds for men and >470 milliseconds for women, PR >220 milliseconds, QRS >110 milliseconds, and other cardiovascular abnormalities. Other inclusion and exclusion criteria limited weight, body mass index, and vital signs measurements to ensure that all included subjects were considered healthy when enrolled in the study.

Every study protocol specified a set of time points at which multiple 10-second ECG recordings (ECG replicates) were extracted and individual cardiac beats were either manually or semiautomatically annotated by the sponsor ECG core laboratories. Information and details on the consistency of the quality of ECGs in Thorough QT studies have been described previously.[11] Subsequently, we used the same preprocessing and measurement methodology as described previously.[10] Briefly, ECGs with missing leads or excessive noise were excluded. The sponsors' provided ECG measurements were projected onto a median QRST waveform (or cardiac beat, Figure 1). Then, in the median cardiac beat, the peak of the T wave was located automatically using the vector magnitude lead (from the vectorcardiogram constructed using Guldenring's transform[12]). Lastly, we computed early repolarization (sponsor-provided QRS_{offset} [J point] to T_{peak}) and late repolarization durations (T_{peak} to sponsor provided T-wave offset [T_{end}]) in addition to sponsor-provided QRS and QT. We computed the mean values of each ECG measurement for each time point from the 24,345 analyzed ECG replicates. Finally, the average daily value for every single ECG biomarker was computed for each of the 2,235 subjects.

Figure 1



The QT interval of the surface ECG reflects the entire ventricular electrical activity during depolarization and repolarization. Figure illustrates the relationship between a representative ventricular action potential and the ECG. Wheras early repolarization is mainly regulated by the inward L-type calcium current (I_{CaL}) during the plateau phase of the ventricular action potential, late repolarization is regulated by outward potassium currents (I_{Ks} and hERG potassium current [I_{Kr}]). This is a simplified figure for illustration purposes only, and additional overlapping ion currents contribute to the different phases and morphology of the cellular action potential.

The primary analysis was performed with QTc calculated with Fridericia's[13] (QTcF) correction formula; however, QTc with Bazett's[3] is also reported. The J-T_{peak} interval was corrected for heart rate using a previously published correction (J-T_{peak}c = J-T_{peak}/$RR^{0.58}$ with RR in seconds).[10] Although T_{peak}-T_{end} has been shown to be rate dependent,[14] in this study, we did not correct for heart rate because prior analysis demonstrated that T_{peak}-T_{end} has minimal heart rate dependency within the limited range of heart rates included in this study where all ECGs were recorded with subjects in the resting supine state.[10,14]

Simulations of the effect of testosterone on the ECG

To study the relationship between the effects of testosterone on I_{CaL} and I_{Ks}, along with early (J-T_{peak}) and late repolarization (T_{peak}-T_{end}) on the human surface ECG, we combined the O'Hara-Rudy ventricular cell

Table. Population summary and ECG measurements

	Age group											
	All		**20s**		**30s**		**40s**		**50s**		**60+**	
	Men	**Women**	**Men**	**Women**	**Men**	**Women**	**Men**	**Women**	**Men**	**Women**	**Men**	**Women**
n	1322	913	610	402	385	231	254	203	62	58	11	19
Age (y)	32 ± 10	34 ± 11	24 ± 3	24 ± 3	34 ± 3	35 ± 3	44 ± 3	44 ± 3	53 ± 2	53 ± 2	65 ± 4	66 ± 6
QTcF (ms)	394 ± 16	408 ± 15*	392 ± 17	408 ± 15*	393 ± 15	407 ± 15*	397 ± 15	410 ± 14*	401 ± 14	411 ± 14*	409 ± 19	414 ± 16§
QTcB (ms)	395 ± 17	415 ± 16*	392 ± 17	408 ± 15*	395 ± 15	415 ± 16*	399 ± 17	415 ± 15*	402 ± 14	417 ± 16*	406 ± 23	416 ± 18‡
QRS (ms)	94 ± 8	89 ± 7*	95 ± 8	89 ± 7*	93 ± 7	88 ± 7*	93 ± 7	88 ± 8*	94 ± 8	88 ± 6*	96 ± 10	87 ± 7†
J-T_{peak}c (ms)	213 ± 16	242 ± 16*	210 ± 17	241 ± 16*	213 ± 15	241 ± 16*	217 ± 15	243 ± 14*	219 ± 16	246 ± 16*	225 ± 19	244 ± 16†
T_{peak}-T_{end} (ms)	87 ± 10	78 ± 9*	87 ± 10	78 ± 9*	87 ± 10	78 ± 8*	87 ± 10	79 ± 9*	87 ± 9	78 ± 10*	89 ± 5	84 ± 11‖

Results are presented as mean ± SD. *QTcB*, QTc Bazett.
Differences in ECG measurements between men and women within group:
* $P < .001$.
† $P < .05$.
‡ $P = .209$.
§ $P = .419$.
‖ $P = .105$.

model[15] with the van Oosteron and Oostendorp action potential-to-body surface ECG model (ECGSIM),[16] as described in a prior study.[10] To obtain approximate steady-state behavior, the ventricular cell model was paced at 1 Hz for 1000 cycles.[15] The testosterone effects on I_{CaL} and I_{Ks} were modeled by multiplying their conductance in the O'Hara-Rudy model by the corresponding scaling factors previously reported in isolated guinea pig ventricular myocytes.[7] Simulations were performed for testosterone's effects on I_{CaL} block and I_{Ks} enhancement alone and in combination. Action potential duration was measured at 30% (APD30), 60% (APD60), and 90% (APD90) of repolarization in the simulated action potential for both endocardial and epicardial cells. To compute the effects of decreasing levels of testosterone as men age, we selected the highest testosterone concentration (20-year-old male group) as the baseline and then computed the relative action potential duration changes from this baseline because testosterone levels in men decrease as they age. In addition, we simulated the average value of testosterone in women. These testosterone values were taken from prior reports in the literature.[17,18]

ECGSIM is based on the equivalent double-layer source model.[16] We used ECGSIM's 22-year-old healthy male[19] example case as baseline and simulated ECGs for different testosterone levels by changing the repolarization time and slope in all endocardial and epicardial action potentials to match the corresponding relative changes in APD30, APD60, and APD90 measured in the O'Hara-Rudy model. Simulated ECGs were semiautomatically analyzed with a wavelet-based delineation algorithm[20,21] in ECGlab.[22]

Statistical analysis

Unpaired Student *t* tests were computed to assess differences in each ECG measurement by sex in the overall population and between age groups. We used a linear model for each sex to assess the effects of age on the ECG parameters separately in men and women. An additional linear model with sex and age as covariates and an interaction term between them was used to assess whether the age-ECG parameter relationship was different between men and women. *P* values <.05 were considered statistically significant. All statistical analysis was performed using R version 2.15.3 (Vienna, Austria).[23]

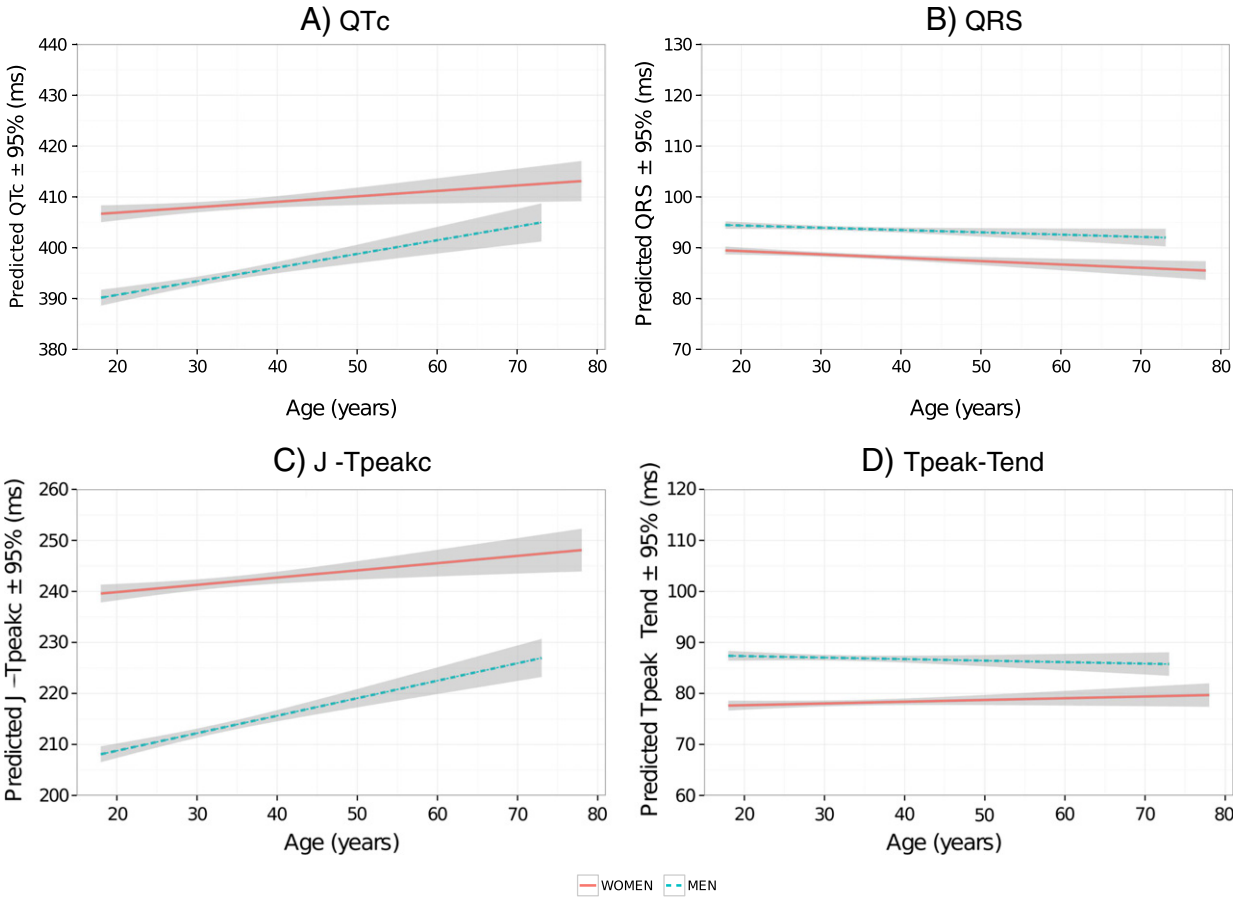
Results

Women represented 41% of the 2,235 subjects in the study population. The Table summarizes the population characteristics and the ECG measurements by sex for the whole population as well as for different age groups by decade.

In the overall population, QTc was shorter in men than in women (QTcF: [mean ± SD] 394 ± 16 vs 408 ± 15 milliseconds, $P < .001$). QTc increased more with age in men (QTcF: 2.7 milliseconds per decade, 95% CI 1.8-3.6 milliseconds per decade, $P < .001$) than in women (QTcF: 1.1 [0.2-1.9] milliseconds per decade, $P = .015$) (interaction QTcF: $P = .012$), which resulted in a decreasing QTc difference between sexes as age increased (Table and Figure 2, *A*).

When dividing the QTc into its subintervals, depolarization (QRS duration) was 5 milliseconds longer in men than in women (94 ± 8 vs 89 ± 7 milliseconds, $P < .001$). A small decrease in QRS duration was observed with age in both men (−0.4 [−0.9 to 0.0] milliseconds per decade, $P = .035$) and women (−0.7 [−1.1 to −0.3] milliseconds per decade, $P = .001$). There was no significant difference in the age-QRS relationship between men and women (interaction $P = .48$) (Figure 2, *B*). Early repolarization duration, measured as the heart rate corrected J-T_{peak} interval (J-T_{peak}c), was 29 milliseconds shorter in men than in women (213 ± 16 vs 242 ± 16 milliseconds, $P < .001$) and

Figure 2



Linear model predictions for men (dashed lines) and women (solid lines) and 95% CIs (gray area) of changes in QTcF (**A**), QRS (**B**), J-T_{peak}c (**C**), and T_{peak}-T_{end} (**D**) with age by sex. Vertical axes are scaled to the same range to facilitate visual comparison.

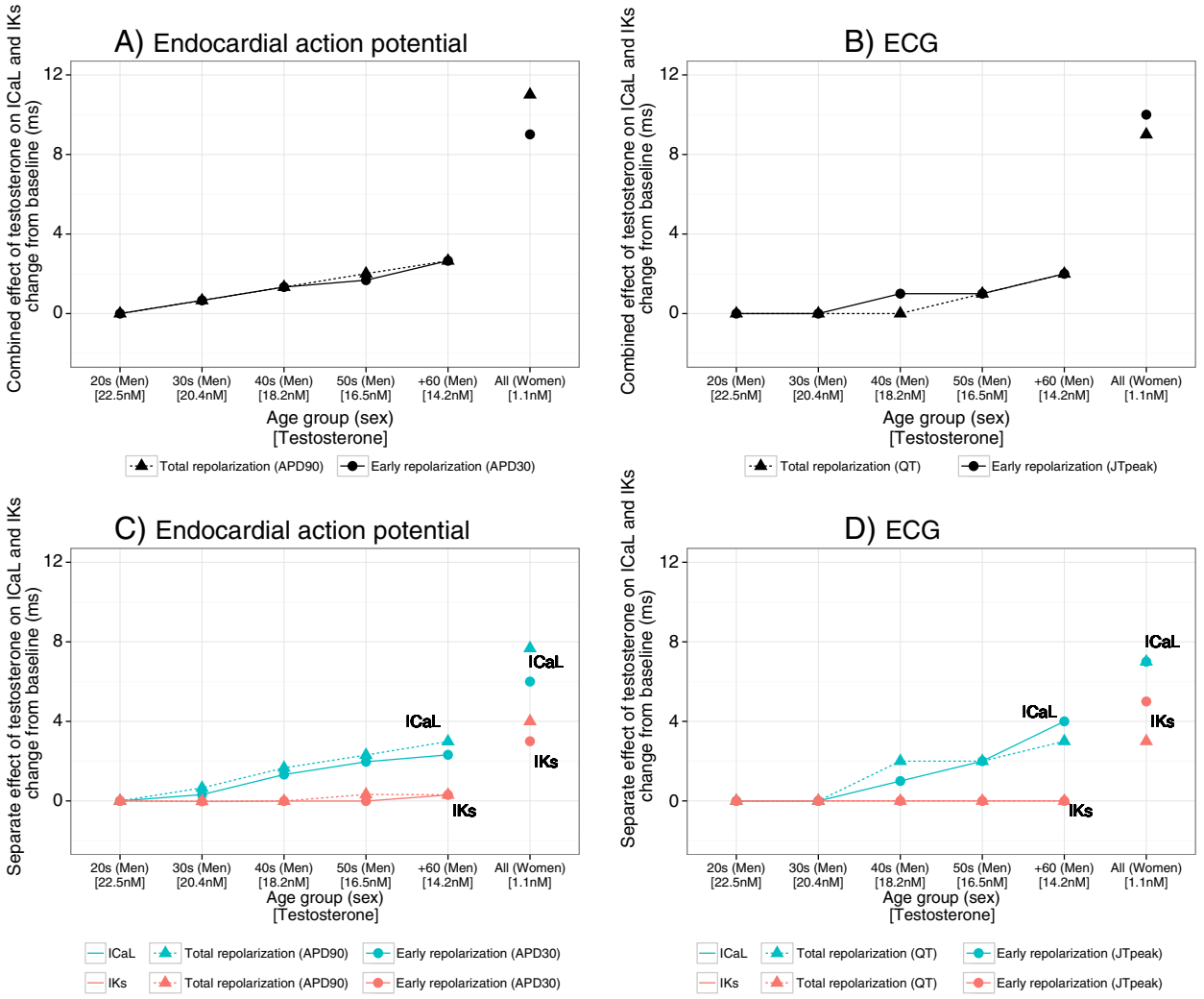
prolonged with age more in men (3.4 [2.5-4.3] milliseconds per decade, $P < .001$) than in women (1.4 [0.5-2.3] milliseconds per decade, $P = .002$) (interaction $P = .002$). Consequently, the difference in early repolarization between men and women diminished with age (Table and Figure 2, *C*). Late repolarization duration, measured as T_{peak}-T_{end}, was 9 milliseconds longer in men than in women (87 ± 10 milliseconds vs 78 ± 9 milliseconds, $P < .001$). There was no significant relationship between age and late repolarization (T_{peak}-T_{end}) in either men ($P = .30$) or women ($P = .18$) (Figure 2, *D*).

Testosterone induced-effect simulations

Decreasing levels of testosterone as men age resulted in action potential prolongation (endocardial cells: Figure 3, *A*; epicardial cells: online Appendix Supplementary Figure 1, *A*) that was due to testosterone's effects on I_{CaL}, but not on I_{Ks} (endocardial cells: Figure 3, *C*; epicardial cells: online Appendix Supplementary Figure 1, *B*). This action potential duration prolongation was entirely due to prolongation of early repolarization (APD30), as there was no additional prolongation in APD90 compared to APD30 (Figure 3, *A*; online Appendix Supplementary Figure 1, *A*). On the ECG, decreased testosterone levels prolonged early repolarization (J-T_{peak}), with no additional effect on total QT (Figure 3, *B*). These age-related changes in early repolarization were entirely due to testosterone effect's on I_{CaL} (Figure 3, *D*).

For women, the simulated level of testosterone was 1.1 nmol/L, compared to a range of 14.2 to 22.5 nmol/L in men. This lower level of testosterone caused an increased action potential duration (endocardial cells: Figure 3, *A*; epicardial cells: online Appendix Supplementary Figure 1, *A*) due to testosterone's effects on both I_{CaL} and I_{Ks} (endocardial cells: Figure 3, *C*; epicardial cells: online Appendix Supplementary Figure 1, *B*); however, I_{CaL} had a larger

Figure 3



Combined effects of testosterone on total (triangles) and early (circles) repolarization on (**A**) endocardial action potential and (**B**) surface ECG in different age groups in men and in all women. Separate effects of testosterone on I_{CaL} (blue) and I_{Ks} (red) and their contribution to total (triangles) and early (circles) repolarization on (**C**) endocardial action potential and (**D**) surface ECG. Changes in milliseconds from reference group (men in their 20s). Horizontal axis labels show each group's mean testosterone level from literature.[17,18]

effect than I_{Ks}. Similar to the clinical ECG data, the shorter QT in men compared to women was due to men having shorter early repolarization duration (J-T_{peak}) (Figure 3, *B*).

Discussion

This study demonstrated that, in healthy adult subjects at rest, shorter QTc in men than in women is entirely explained by shorter early repolarization and that this difference diminishes with increasing age. Simulations of testosterone's effects on I_{CaL} and I_{Ks} confirmed this finding and revealed that testosterone's effects on I_{CaL} play a larger role than its effects on I_{Ks} in shortening early repolarization. In the context of drug-induced arrhythmias, the decreased I_{CaL} from testosterone may lower risk of torsade de pointes by preventing early afterdepolarizations,[24,25] which are the trigger for initiating torsade de pointes. This deserves further study. Whereas testosterone's effects on I_{Ks} do not seem to play a large role in regulating QTc at rest, I_{Ks} may have a greater effect in the presence of sympathetic stimulation,[26,27] which was not investigated in this study.

Prior clinical studies

Although there are other studies reporting different age- and sex-specific ECG measurements,[4,28] this is the first study reporting sex- and age-specific measurements for all the QT subintervals in healthy subjects.[4,14,28-31]

Our results regarding QTc measurements are in agreement with previous studies[4,28-31] and showed that men >18 years old have shorter QTc than women do and that this sex difference decreases with age. However, the age at which the QTc sex differences disappear is different in this study compared to that previously reported by Rautarhaju et al[4] Whereas they reported that the sex differences in QTc were no longer present in subjects >50 years of age, sex differences in QTc were still present at such age in our study's population. When dividing by decade, the QTc difference trended to disappear in subjects >60 years of age (online Appendix Supplementary Figure 2). The difference in the age at which sex differences in QTc disappear can be due to multiple factors such as the different heart rate correction method (individual-subject correction vs Fridericia), limited sample size of subjects aged >60 years or other differences in population characteristics.

When looking at the QTc subintervals, despite men having longer depolarization (QRS) and longer late repolarization (T_{peak}-T_{end}) phases compared to women, our results showed that the shorter QTc in men was completely explained by men having shorter early repolarization (J-T_{peak}c) than women do. Previous studies have also reported that men have longer QRS duration,[4,28,29] which is likely due to men having larger hearts that take longer to depolarize.[32] Previous studies of sex differences in T_{peak}-T_{end} are inconclusive,[14,30,31] and our results are in concordance with those reporting that men have longer late repolarization than women at resting heart rates. Our results showed shorter J-T_{peak}c in men than in women, and these findings are concordant with results from previous studies.[30,31] This study demonstrated a weak relationship between age and QRS, no age-related changes in T_{peak}-T_{end}, and that age-related J-T_{peak}c prolongation fully explains QTc prolongation with age in both men and women.

Mechanisms for sex and age differences in QT

In animal studies, testosterone has been shown to decrease I_{CaL} and enhance I_{Ks}.[7] Our simulations of testosterone-induced effects showed that testosterone's effects on both I_{CaL} and I_{Ks} contributed to sex differences in early repolarization. In ventricular cells, our results were in concordance with previous simulations of sex-specific and testosterone-induced effects studies.[27,33] Specifically, higher levels of testosterone in men compared to women resulted in men having shorter action potential duration, primarily caused by APD30 shortening. Simulations of body surface ECGs were in concordance with the clinical data and showed that men's shorter QT was fully explained by men having shorter early repolarization (J-T_{peak}c) compared to women. When assessing age-related changes in men, simulations of age-group–matched levels of testosterone in men showed early repolarization prolongation as testosterone levels decreased with age. This prolongation was entirely due to the effect of testosterone on I_{CaL}. These simulation results suggest that testosterone's effects on early repolarization play an important role in the sex difference in QTc observed in the clinical data.

Although it is known that sympathetic activity affects how I_{CaL} and I_{Ks} regulate the action potential duration, there is a lack of preclinical data on testosterone's effects on I_{CaL} and I_{Ks} under sympathetic stimulation.[26,27] All the ECGs in this study were acquired from healthy subjects in a controlled and quiet environment after a resting period in supine position, which minimizes the amount of sympathetic activation. However, the lack of sympathetic activation in the computational models might result in an underestimated effect of I_{CaL} and I_{Ks} in our simulations.[27] These potential underestimated effects may contribute to the differences in the magnitude of simulated changes when compared to the clinical data.

Testosterone-induced inhibition of I_{CaL} may provide a protective effect in men by preventing the occurrence of early afterdepolarizations, which can initiate torsade de pointes.[24,25] Furthermore, testosterone's effects on both I_{CaL} and I_{Ks} might contribute to an increased "repolarization reserve"[6,34,35] in men. When introducing "repolarization reserve," Roden[34] proposed that, in normal hearts, there are redundant mechanisms to accomplish normal repolarization. Thus, testosterone-induced I_{Ks} enhancement might provide the necessary redundancy to counteract the increasing torsade risk resulting from drug-induced hERG potassium current (I_{Kr}) block. This requires further investigation.

While this study focused on the potential relationship between testosterone and shortened early repolarization time (J-T_{peak}), a separate recent study found a relationship between testosterone levels and ST-J elevation in leads other than V_1 to V_3 ("early repolarization pattern").[36] The relationship between testosterone, early repolarization duration, and early repolarization pattern deserves further study.

Limitations

This was a cross-sectional study, which included same-day baseline ECG recordings per subject; thus, within-subject changes over time could not be assessed. However, it represents a clear snapshot of the sex and age normal limits of the studied ECG intervals. In addition, children were not included in this study and the number of subjects ≥60 years old is limited. There is high variability (ie, >10% within each age group) in the average testosterone levels in men reported in the literature.[17,37,38] Thus, although overall trends might be similar, quantitative results may vary depending on the average values selected for characterization of each age group of men. Finally, other age-specific, sex-specific (eg, women having smaller ventricles compared to men)[32],

and genomic-based differences (eg, differences in the expression of genes encoding key cardiac ion channels)[27] may also contribute to the observed differences in the clinical data.

Conclusions

In healthy adult subjects at rest, shorter QTc in men than women is due to shorter early repolarization (J-T_{peak}c); and these differences diminish with increasing age because of a greater increase in early repolarization in men. Simulations suggested that the primary reason for lengthening QTc as men age (and testosterone levels decrease) is due to testosterone's effects on I_{CaL}. With the larger difference in testosterone levels between women and men, testosterone's effects on I_{Ks} also contribute to differences in QTc, although the effect of I_{CaL} is still larger. Further research should investigate how these findings translate to torsade de pointes risk, including under sympathetic stimulation that can increase the role of I_{Ks}.

Disclosures

The authors have no conflicts of interest to report.

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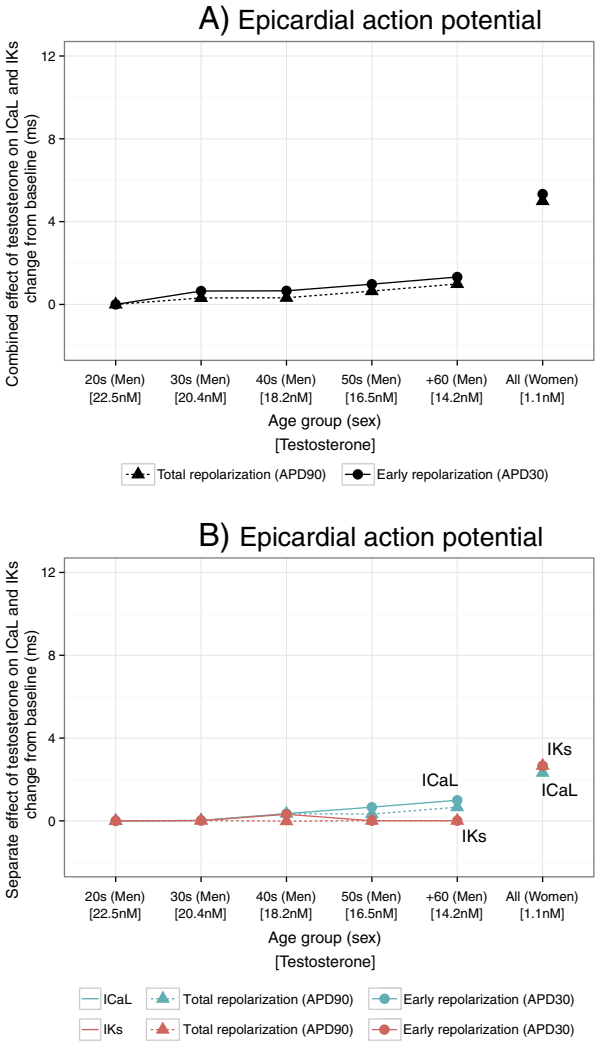
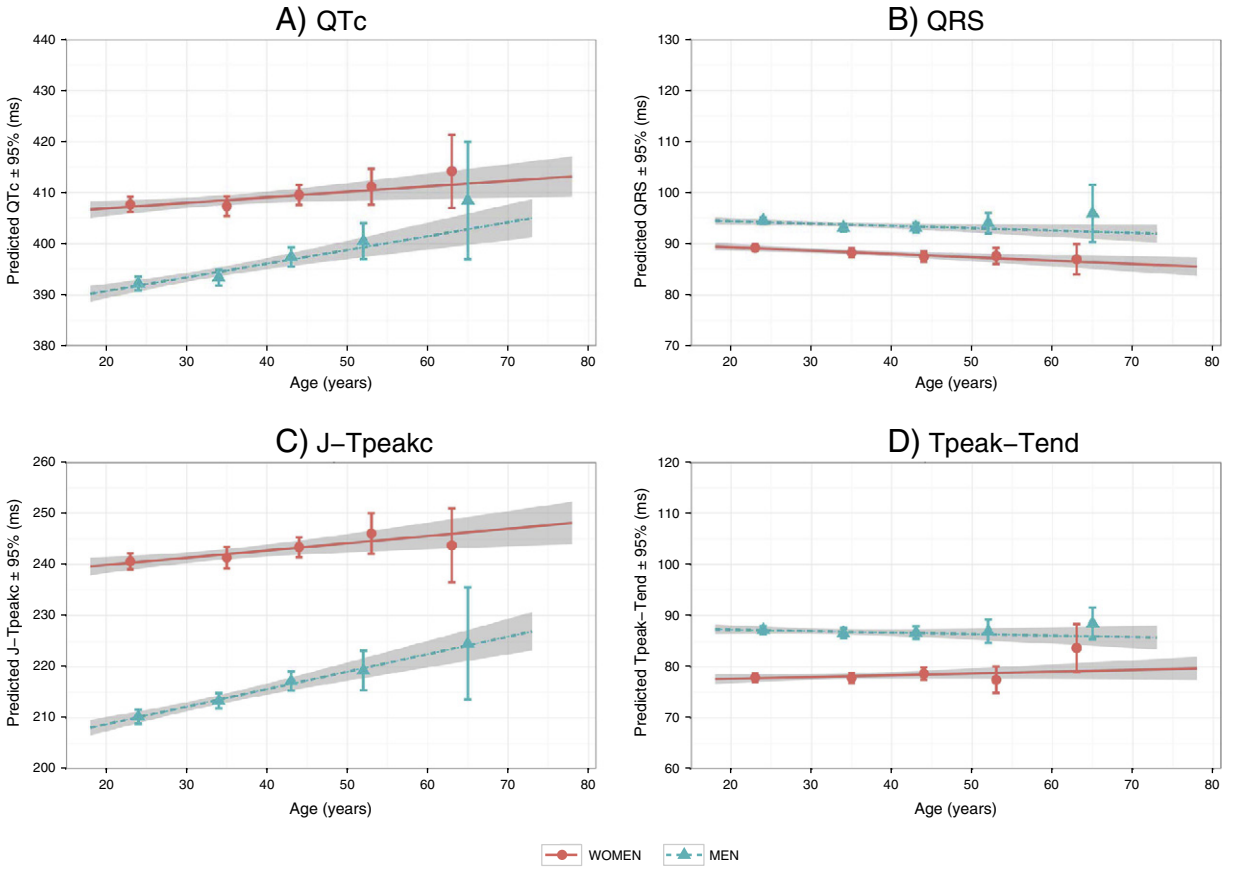
Appendix. Supplementary materials

Supplementary Figure 1



A, Combined effects of testosterone on total (triangles) and early repolarization (circles) on epicardial action potential in different age groups in men and in all women. **B**, Separate effects of testosterone on I_{CaL} (blue) and I_{Ks} (red) and their contribution to total (triangles) and early (circles) repolarization on epicardial action potential. Changes in milliseconds from reference group (men in their 20s). Horizontal axis labels show each group's mean testosterone level from literature.[39,40]

Supplementary Figure 2



Linear model predictions for men (blue dashed lines) and women (red solid lines) and 95% CIs (gray area) of changes in QTc (**A**), QRS (**B**), J-T_{peak}c (**C**), and T_{peak}-T_{end} (**D**) with age by sex vs observed measurements by age group. Men (triangles) and women (circles) were grouped by decade except for the oldest group, which contains subjects ≥60 years old, and plotted with the x-axis location of the median age per group and mean ± 95% CIs on the y-axis. Vertical axes are scaled to the same range to facilitate visual comparisons of differences between sexes, age groups, and intervals. Sample size and mean values of each age group are reported in Table.

Supplementary References

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