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BASIC SCIENCE

Docosahexaenoic acid normalizes QT interval in long QT type 2 transgenic rabbit models in a genotype-specific fashion

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Aim Long QT syndrome (LQTS) is a cardiac channelopathy predisposing to ventricular arrhythmias and sudden cardiac death. Since current therapies often fail to prevent arrhythmic events in certain LQTS subtypes, new therapeutic strategies are needed. Docosahexaenoic acid (DHA) is a polyunsaturated fatty acid, which enhances the repolarizing I_{Ks} current.

Methods and results We investigated the effects of DHA in wild type (WT) and transgenic long QT Type 1 (LQT1; loss of I_{Ks}), LQT2 (loss of I_{Kr}), LQT5 (reduction of I_{Ks}), and LQT2–5 (loss of I_{Kr} and reduction of I_{Ks}) rabbits. *In vivo* ECGs were recorded at baseline and after 10 μM/kg DHA to assess changes in heart-rate corrected QT (QTc) and short-term variability of QT (STVQT). *Ex vivo* monophasic action potentials were recorded in Langendorff-perfused rabbit hearts, and action potential duration (APD_{75}) and triangulation were assessed. Docosahexaenoic acid significantly shortened QTc *in vivo* only in WT and LQT2 rabbits, in which both α- and β-subunits of I_{Ks}-conducting channels are functionally intact. In LQT2, this led to a normalization of QTc and of its short-term variability. Docosahexaenoic acid had no effect on QTc in LQT1, LQT5, and LQT2–5. Similarly, *ex vivo*, DHA shortened APD_{75} in WT and normalized it in LQT2, and additionally decreased AP triangulation in LQT2.

Conclusions Docosahexaenoic acid exerts a genotype-specific beneficial shortening/normalizing effect on QTc and APD_{75} and reduces pro-arrhythmia markers STVQT and AP triangulation through activation of I_{Ks} in LQT2 rabbits but has no effects if either α- or β-subunits to I_{Ks} are functionally impaired. Docosahexaenoic acid could represent a new genotype-specific therapy in LQT2.

Keywords Docosahexaenoic acid • Repolarization • Action potential duration • Long QT syndrome • QT normalization • Ion currents • Rabbit models • Genotype-specific therapy

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What's new?

- I_{Ks}-activator docosahexaenoic acid shortens QTc/APD_{75} and reduces short-term variability of QT in transgenic long QT Type 2 (LQT2) and in wild-type rabbits.
- This docosahexaenoic acid (DHA) effect leads to a complete normalization of QT, STVQT, and APD_{75} in LQT2.
- In contrast, DHA does not exert any relevant effects on ventricular repolarization in transgenic LQT1, LQT5, and LQT2–5 rabbits with impaired I_{Ks} function.
- These genotype-specific effects of DHA on repolarization suggest that both α- and β-subunits to I_{Ks} (KCNQ1 and KCNE1) need to be functionally intact for I_{Ks}-activation by DHA.

Introduction

Long QT syndrome (LQTS) is a genetic channelopathy with impaired cardiac repolarization that can lead to Torsades de Pointes (TdP) ventricular tachycardias, arrhythmic syncopes, or sudden cardiac death (SCD).[1] In >80% of LQTS patients one of the main ventricular repolarizing potassium currents, I_{Ks} *and* I_{Kr}, is reduced or absent due to loss-of-function mutations in genes encoding for the corresponding potassium channel subunits such as α-subunit to I_{Ks}-conducting potassium channel (KCNQ1) [long QT Type 1 (LQT1)], α-subunit to I_{Kr}-conducting potassium channel (KCNH2) [long QT Type 2 (LQT2)], or β-subunit to I_{Ks}-conducting potassium channel (KCNE1) [long QT Type 5 (LQT5)].[2] Beta-blockers, such as nadolol or propranolol, represent the first-line therapy in LQT1 and 2, but offer a rather unspecific protection from arrhythmic events by reducing the triggering sympathetic stimuli. Reports suggest a 5-year risk of cardiac events up to 32% in symptomatic LQTS patients despite an appropriate beta-blocker therapy.[3] Therefore, there is still an urgent need for new therapeutic strategies, which reduce the incidence of arrhythmias in LQTS.

Docosahexaenoic acid (DHA) is an omega-3 fatty acid of marine origin, which contributes to various biological activities such as (i) the modulation of gene expression, (ii) the regulation of the physical properties of membranes, and (iii) the production of eicosanoids. Importantly, DHA was described to increase I_{Ks} currents and may thereby affect cardiac repolarization.[4] Interactions between DHA and the channel complex constituted by KCNQ1 and KCNE1 in guinea pig cardiomyocytes have been studied by Moreno *et al.*,[5] suggesting that DHA exerts an activating effect on I_{Ks} by altering the reciprocal electrostatic interactions between KCNQ1 and KCNE1. In this regard, Liin *et al.*[4] suggested that KCNE1 may influence the sensibility of KCNQ1 to DHA or other fatty acids due to a protonation of KCNQ1. Apart from cellular electrophysiology experiments, recent *ex vivo* whole heart and *in vivo* experiments in guinea pig models demonstrated that DHA may indeed shorten cardiac repolarization/QT interval in healthy and drug-induced LQTS guinea pig models.[6]

In this study, we take these experiments a step further, investigating potentially beneficial shortening effects on QT and action potential duration (APD) in genetic rabbit models for LQTS, also investigating potential genotype-specific effects. The rabbit is a species that plays an important role in LQTS-related arrhythmia research: the function and gating kinetics of the underlying cardiac repolarizing ion-channels/currents, the shape of action potential (AP), and cardiac responses to pharmacological interventions show very close resemblance to human cardiac physiology.[7] Transgenic rabbit models for various subtypes of LQTS have been developed and mimic the human disease phenotypes.[8,9]

The LQT1 and LQT2 rabbit models lack I_{Ks} or I_{Kr} currents, respectively due to loss-of-function mutations in *KCNQ1* or *KCNH2*[8]; LQT5 rabbits have reduced I_{Ks} current due to a mutation in the beta-subunit *KCNE1*[9]; and LQT2–5 rabbits lack I_{Kr} and have reduced activity of I_{Ks}.[10] These LQTS rabbit models thus provide the opportunity to look into the effects of DHA on ventricular cardiac repolarization in a genotype-specific fashion.

Methods

A more detailed method section is found in Supplementary material online.

Ethical aspects

All animal experiments were performed in compliance with EU legislation (directive 2010/63/EU) and the German (TierSchG and TierSchVersV) animal welfare laws, after approval by the local Institutional Animal Care and Use Committees in Germany (Regierungspraesidium Freiburg; approval number G14/111). The experimental use of *Xenopus laevis* was reviewed and approved by the regional board of ethics in Linköping, Sweden (Case no 1941). Animal housing and handling were in accordance with good animal practice as defined by the Federation of European Laboratory Animal Science Association. Animal studies were reported in compliance with the ARRIVE guidelines.

Rabbit models

The study was conducted on wild type (New Zealand White, WT, $n = 11$) and different transgenic LQTS rabbit models overexpressing human mutant KCNQ1-Y315S (LQT1, lack of I_{Ks}, $n = 9$), KCNH2-G628S (LQT2, lack of I_{Kr}, $n = 11$), KCNE1-G52R (LQT5, impaired I_{Ks}, $n = 12$) and KCNH2-G628S + KCNE1-G52R (LQT2–5, lack of I_{Kr} and impaired I_{Ks}, $n = 12$) in the heart.[8,9] Both male and female animals were equally included in the study.

Telemetric ECG monitoring

To study the potential beneficial genotype-specific repolarization shortening effect of DHA in awake, free-moving, non-sedated rabbits *in vivo,* 6 WT, 8 LQT1, 6 LQT2, 8 LQT2–5, and 8 LQT5 rabbits were subjected to subcutaneous telemetric ECG transmitter implantations as described.[10]. 24-H telemetric ECG recordings (representing standard ECG limb leads I–III) were carried out at baseline (drug-free) and following 10 µM/kg BW IM administration of DHA on the subsequent day. Conventional ECG parameters (RR, PR, QT, and QTc intervals) were measured. At baseline, pairs of RR and QT intervals were assessed every 30 min as averaged values over 5 s to collect in total 48 QT-RR pairs for each animal. Similarly, ECGs were analysed within the first 90 min following the administration of DHA (starting at 5 min post-injection at which time-point DHA effects were already observed) every 4–5 min to obtain at least 20 datasets of RR, QT, PR, and QRS values. For each rabbit, an individual heart rate correction formula was used to calculate QTc. The individual correction formula was created by plotting the baseline QT and RR pairs (48 pairs for

each rabbits) on a Cartesian co-ordinate system and fitting a linear regression curve. The steepness (a) of the acquired individual QT-RR regression curve [QT (y) = $a\times$RR (x) + b] was used for the individual heart rate correction formula [QTc = QT$-a\times$(RR$-$250)].[10]

In vivo 12-lead ECG recording in anaesthetized rabbits

To study DHA effects *in vivo,* 12-lead ECGs were recorded at baseline and (within 20 min) after IV administration of 10 µM/kg BW DHA in WT (n = 6), LQT1 (n = 8), LQT2 (n = 5), LQT 2–5 (n = 4), and LQT5 (n = 7) rabbits anaesthetized with ketamine (12.5 mg/kg BW) and xylazine (3.75 mg/kg BW) IM. All parameters were stable within 10–15 min after DHA bolus. We analysed DHA effects in the stable phase at 20 min post-bolus. DHA effects on conventional ECG parameters and on pro-arrhythmia markers such as QT dispersion and short-term QT variability[11] were assessed. The heart rate correction of the QT was performed as described in Telemetric ECG monitoring section.

Monophasic action potential recording

To investigate the effect of DHA *ex vivo*, monophasic action potentials (MAP) were recorded in Langendorff-perfused WT (n = 7), LQT1 (n = 7), LQT2 (n = 6), LQT 2–5 (n = 9), and LQT5 (n = 9) rabbit hearts as described before.[10] The duration of the MAPs at 75% of repolarization as well as the MAP triangulation (APD_{90}–APD_{30})—a marker of arrhythmogenicity—were measured at baseline and after 10 min perfusion with 20 µM DHA.

Two-electrode voltage-clamp experiments on *Xenopus* oocytes

Oocytes were isolated from the African claw frog *X. laevis* through in-house frog surgery, following RNA injection and two-electrode voltage-clamp experiments as described in detail.[12,13] In brief, 50 ng of complementary RNA of human KCNQ1 (NM_000218), human KCNE1 (NM_000219), rabbit KCNQ1 (NM_008252197.2), and rabbit KCNE1 (NM_001109822) were injected into defolliculated *Xenopus* oocytes (at a 3:1 KCNQ1/KCNE1 ratio for co-expression). *Xenopus* oocytes were recorded in the two-electrode voltage-clamp configuration using a Dagan CA-1B high-performance oocyte clamp amplifier (Dagan, MN, USA). Control or DHA-supplemented control solutions (2–20 µM) were perfused extracellularly to the oocyte chamber using a Minipuls 3 peristaltic pump (Gilson, WI, USA) with a perfusion rate of 1 mL/min. Solution was perfused until a stable effect on current amplitude was observed (about 5–10 min of perfusion). Electrophysiological recordings were obtained using Clampex 10.7 software (Molecular Devices, CA, USA). Measurements were performed with a holding voltage of $-$80 mV followed by test voltages ranging $-$90 to +80 mV for 5 s each in 10 mV increments, followed by a tail voltage to $-$20 mV. The DHA effect on the maximum conductance (V_{50}) and maximal conductance (G_{max}) and the relative current at 0 mV was determined as previously described.[13]

In silico modelling

Computational simulations of single cells and tissue strands were performed using the electrophysiological rabbit cardiomyocyte model by Shannon *et al.*[eRef1] Model parameters g_{Ks} *and* g_{Kr} (maximum conductances of I_{Ks} and I_{Kr}) were adjusted to obtain APD_{90} values consistent with APD_{90} measured in Langendorff-perfused hearts of WT and LQT2 rabbits[14] instead of isolated WT cardiomyocytes as in the original model. As I_{Kr} is absent in LQT2 cardiomyocytes harbouring the KCNH2-G628S loss-of-function mutation,[8] in LQT2 models, g_{Kr} was set to 0 mS/cm^2 and g_{Ks} was fitted to be consistent with APD_{90} in LQT2 hearts. DHA effects on (human and rabbit) KCNQ1/KCNE1 I_{Ks} were included into the LQT2 model by increasing g_{Ks} to 200%, in accordance with the patch clamp data.

To quantify DHA effects on the cellular repolarization reserve, we conducted single-cell simulations, in which we additionally varied g_{Ks} *and* $g_{Ca,\,L}$ (the maximum conductance of L-type calcium channel) in the range of 0–100% and 100–500%, respectively. These changes are designed to increase likelihood of pro-arrhythmic behaviour, such as early afterdepolarizations (EADs), *n*: 1 blocks, or permanent depolarization.[eRef2] For each model variant and at different pacing frequencies, we quantified, which fraction of the parameter space produced pro-arrhythmic APs or blocks.

Statistics

All data that support the findings of this study are available from the corresponding author upon reasonable request. Data are expressed as mean ± standard error of the mean (SEM). Statistical and Power analyses were performed by Prism 8.0 (Graphpad, San Diego, CA, USA), Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA), and Prism StatMate. Graphs were created by Prism 8.0. After verification of normal distribution, comparisons between different genotype groups were performed using one-way ANOVA. Comparisons between values recorded before and after DHA administration within the same groups were carried out using two-tailed paired Student's *T*-tests. The acceptable maximal α error was set at 5%.

Results

Docosahexaenoic acid activates human and rabbit KCNQ1/KCNE1 channels expressed in *Xenopus* oocyte

Docosahexaenoic acid-induced activation of human KCNQ1 alone is observed as a shifted voltage dependence of channel opening (ΔV_{50}) towards more negative voltages (*Figure 1A*) and an increased G_{max} (*Figure 1B*).[13,14] While DHA did not shift V_{50} of human KCNQ1/KCNE1, (*Figure 1D*), DHA increased G_{max} of human KCNQ1/KCNE1 (*Figure 1E*) to a larger extent. The total activating effect of DHA, caused by the combined effect of shifting the V_{50} and increasing the G_{max}, at a voltage close to the systolic plateau voltage during a cardiac AP can be estimated by quantifying the relative increase in K^+ current (I_{DHA}/I_{Ctrl}) at 0 mV.[eRef3] On average, for human KCNQ1 alone the relative increase of I_{DHA}/I_{Ctrl} was 1.3 ± 0.09 (n = 6) for 20 µM of DHA (*Figure 1C*), while it was larger for human KCNQ1/KCNE1 with I_{DHA}/I_{Ctrl} of 2.0 ± 0.6 (n = 4) for 20 µM of DHA (*Figure 1F*, data from Ref.[13]). Similar experiments on rabbit KCNQ1/KCNE1 expressed in *Xenopus* oocytes showed that DHA shifts V_{50} towards more negative voltages (*Figure 1G*), increases G_{max} (*Figure 1H*), and increases the relative K^+ current I_{DHA}/I_{Ctrl} at 0 mV (*Figure 1I*) by 2.1 ± 0.2 (n = 7) for 20 µM of DHA. Altogether, the data summarized in *Figure 1* show that DHA activates human KCNQ1, human KCNQ1/KCNE1 and rabbit KCNQ1/KCNE1, with the largest activating effect at a physiologically relevant voltage for the cardiac plateau phase for the two KCNQ1/KCNE1 channels and with less effect on human KCNQ1 channels.

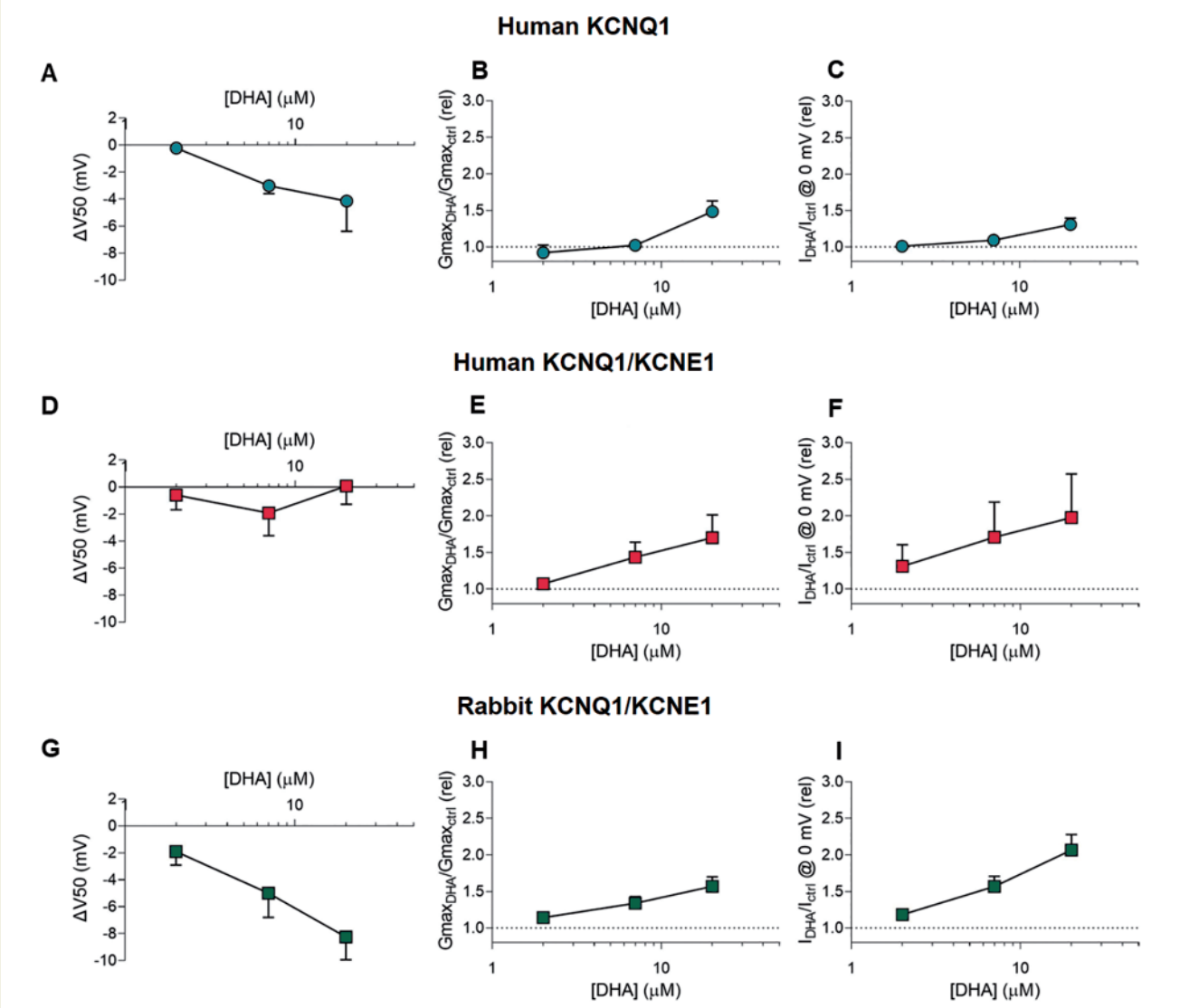

Figure 1 Effect of DHA on human and rabbit KCNQ1/KCNE1 channel expressed in *Xenopus* oocytes. Effect of 2–20 μM of DHA on V_{50} (*A*), G_{max} (*B*), and the current at 0 mV (*C*) for human KCNQ1. All data are presented as mean ± SEM. *n* = 3–6 depending on concentration. (*D–F*) Same as in (*A–C*) but for human KCNQ1/KCNE1. All data are presented as mean ± SEM. *n* = 4. (*G–I*) Same as in (*D–F*) but for rabbit KCNQ1/KCNE1. All data are presented as mean ± SEM. *n* = 7. (Data presented in panels *A, D, E*, and *F* have been previously published in Refs.[12,13].) DHA, docosahexaenoic acid;KCNE1, β-subunit to I_{Ks}-conducting potassium channel; KCNQ1, α-subunit to I_{Ks}-conducting potassium channel; SEM, standard error of the mean.

Baseline electrocardiographic characteristics *in vivo*

The average heart rate was similar in animals from the different genotypes (RR-intervals, WT: 253 ± 19 ms; LQT1: 264 ± 6.2 ms; LQT2: 259 ± 17 ms; LQT2–5: 272 ± 39 ms; and LQT5: 272 ± 39 ms).

The average heart-rate corrected QTc interval duration, however, differed between WT and transgenic LQTS rabbit models (*Figure 2A*). In line with previous data,[9,10] LQT1, LQT2, and LQT2–5 rabbits showed a significantly longer QTc than WT rabbits (all *P*-values < 0.01), while LQT5 rabbits did not show any QT changes compared with WT. As the QT prolongation was particularly pronounced at slower heart rates, the QT/RR ratio was steeper in LQT2 and LQT2–5 rabbits than in WT controls (*P*-values < 0.001) (Supplementary material online, *Figure S1A*).

Baseline *ex vivo* recordings on Langendorff-perfused isolated rabbit hearts

At a stimulation rate of 2 Hz, mean left ventricular APD_{75} recorded in WT rabbit hearts was 135.0 ± 7.02 ms. In transgenic LQTS rabbits, mean APD_{75} was longer only in LQT2 rabbit hearts (LQT2, 162.3 ± 7.44 ms, ΔAPD_{75} compared with WT, +27.2 ± 7.01 ms, *P*-value = 0.022). No difference was found in mean APD_{75} of LQT1 (mean APD_{75} =

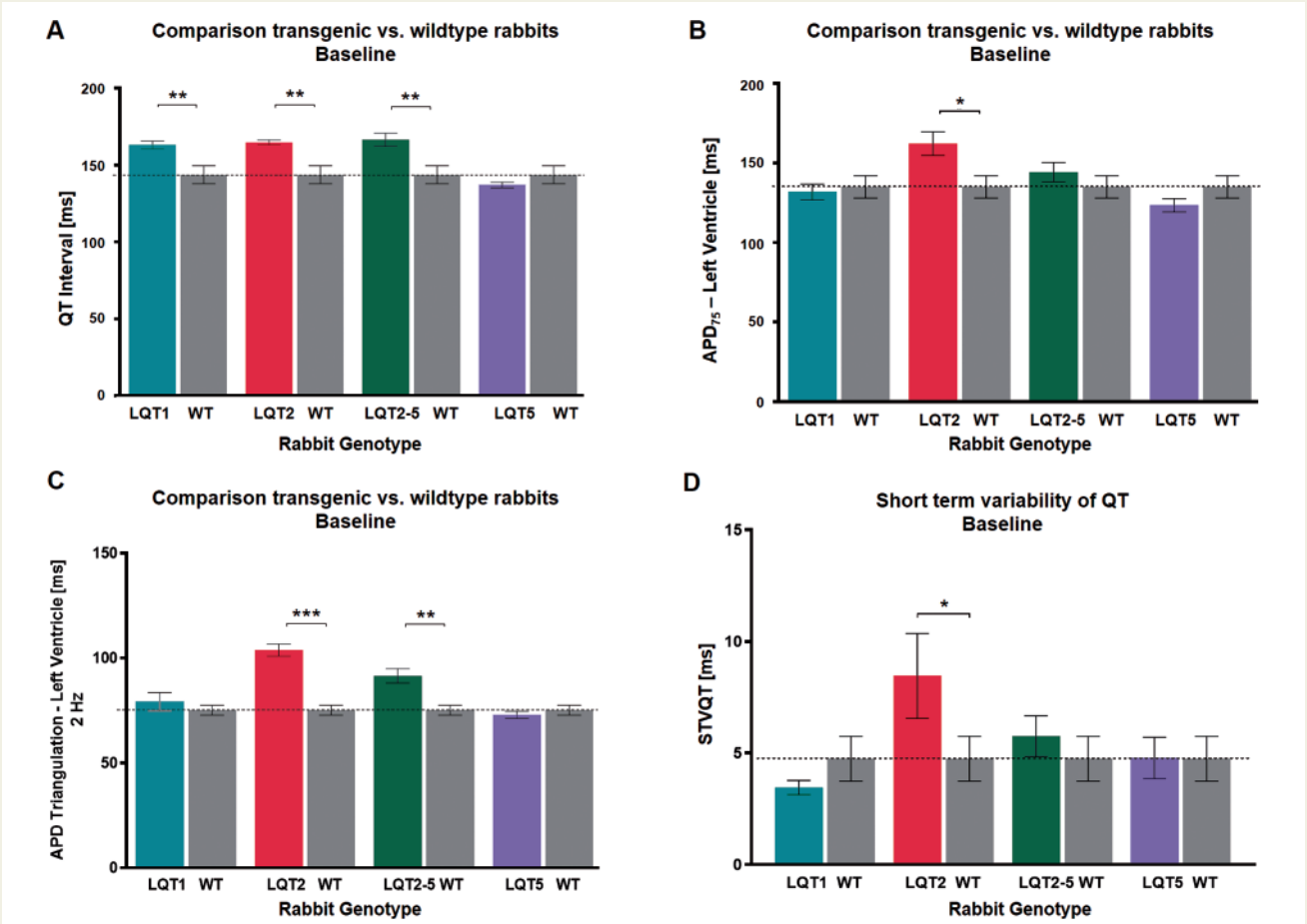

Figure 2 Baseline electrical differences between different LQTS genotypes *in vivo and ex vivo*. (*A*) Heart-rate corrected QTc interval. (*B*) Action potential duration at 75% of repolarization. (*C*) Action potential triangulation in transgenic LQT1. (*D*) Short-term variability of QT. (*A–D*) LQT1: green, *in vivo n* = 8, *ex vivo n* = 7; LQT2: red, *in vivo n* = 6, *ex vivo n* = 6; LQT2–5: violet, *in vivo n* = 8, *ex vivo n* = 9; and LQT5: blue, *in vivo n*= 8, *ex vivo n* = 9; WT littermates: grey, *in vivo n* = 6, *ex vivo n* = 7. Differences are indicated as * for *P*-value < 0.050, ** for *P*-value < 0.010, and *** for *P*-value < 0.001. All data are presented as mean ± SEM. The WT (grey) bars are shown next to each LQTS genotype bar to better indicate the difference to WT for each individual genotype. LQTS, long QT syndrome; LQT1, long QT Type 1; LQT2, long QT Type 2; LQT5, long QT Type 5; LQT2–5, combined form of long QT Types 2 and 5; SEM, standard error of the mean; WT, wild type.

131.9 ± 4.92 ms), LQT2–5 (mean APD_{75} = 144.3 ± 6.07 ms), or LQT5 rabbit hearts (mean APD_{75} = 120.7 ± 4.59 ms) compared with WT rabbit hearts (*Figure 2B*), similarly as in previous studies.[10,eRef4] AP triangulation—a marker for the prolongation of phase 3 repolarization—was more pronounced in LQT2 and LQT2–5 than in WT rabbit hearts (WT: 75.2 ± 2.34 ms; LQT2: 103.8 ± 2.83 ms, *P*-value < 0.001 vs. WT; and LQT2–5: 91.5 ± 3.39 ms, *P*-value = 0.002 vs. WT; *Figure 2C*). No significant differences in AP triangulation were apparent between LQT1, LQT5, and WT rabbits.

Effects of docosahexaenoic acid on telemetric ECG in conscious rabbits

In LQT1 rabbits, DHA administration shortened mean RR intervals from 264.0 ± 6.24 to 235.5 ± 5.00 ms (*P*-value = 0.006), thus inducing a slight increase in average heart rate. In all other genotypes only marginal, non-significant changes in RR interval were observed.

Docosahexaenoic acid influenced cardiac repolarization in a genotype-specific fashion: In WT and—more pronouncedly—in LQT2 rabbits, DHA shortened the absolute QT and heart-rate corrected QTc intervals (ΔQTc: WT, −12.0 ± 1.88 ms, *P*-value = 0.001; LQT2, −20.7 ± 1.71 ms, *P*-value < 0.001), while no changes were observed in LQT1, LQT5, and LQT2–5 rabbits, whose I_{Ks}-function is impaired (*Figure 3A and B*). Important to note, this QTc-shortening effect in WT and LQT2 rabbits was seen in each individual rabbit (*Figure 3D*).

In LQT2 rabbits, the QT shortening effect of DHA could be seen at all heart rates and flattened the QT/RR ratio curve (*P*-value = 0.01) (Supplementary material online, *Figure S1B*).

To investigate whether DHA may normalize the prolonged QTc to physiological values, we compared QTc of transgenic LQTS rabbits following DHA administration to QTc of WT rabbits at baseline (*Figure 3C*). DHA administration normalized QTc in LQT2 rabbits completely (*P*-value for difference between WT and LQT2 + DHA = 0.950). QTc intervals in LQT1 and LQT2–5 rabbits, in contrast,

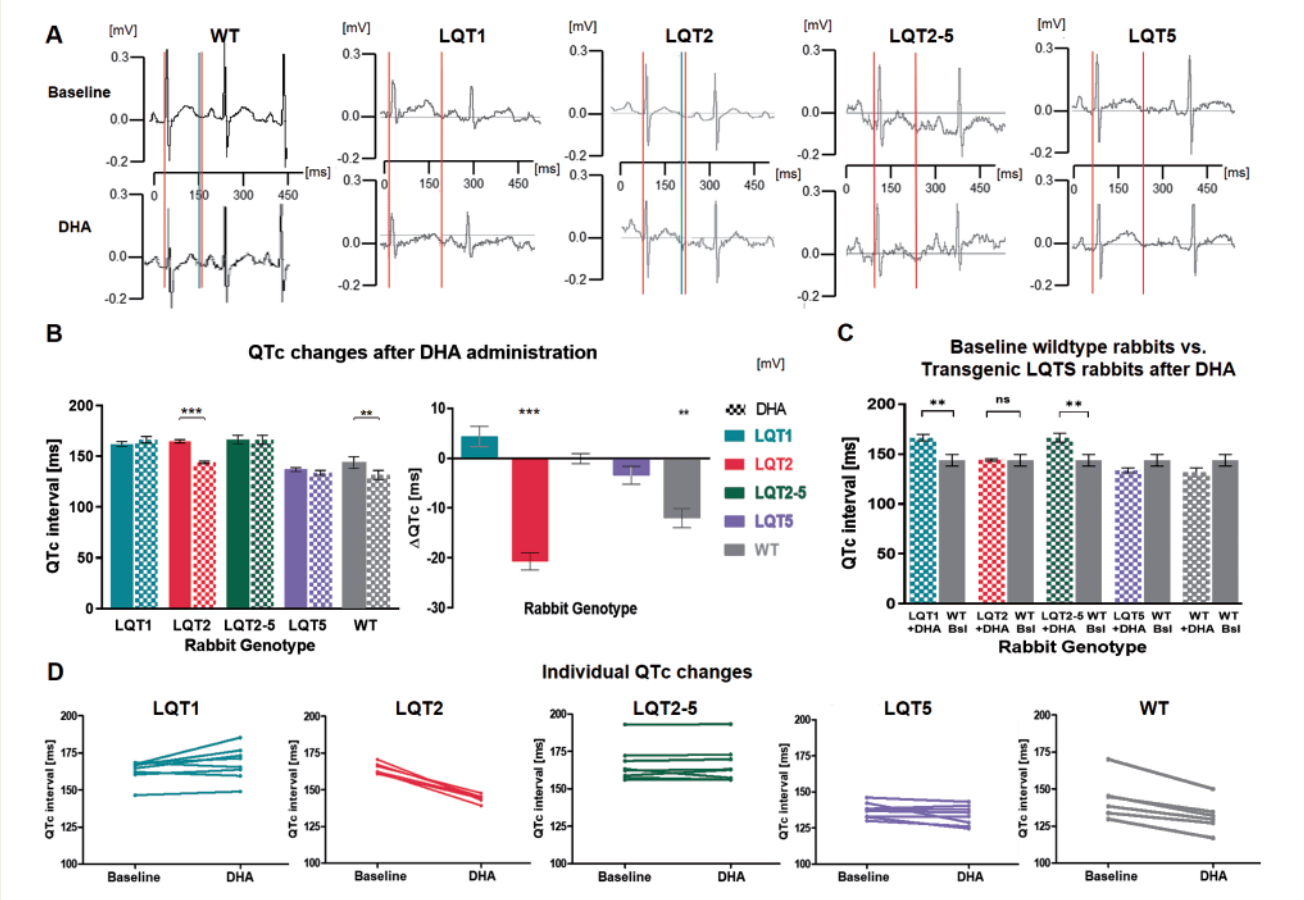

Figure 3 Effects of DHA on QT interval *in vivo* in telemetric ECGs. (*A*) Representative examples of telemetric ECGs recorded in WT, LQT1, LQT2, LQT2–5, and LQT5 rabbits before (above) and after (below) administration of DHA. Q and the end of the T-wave are indicated with vertical lines. (*B*) Left panel: QTc comparison before (fully coloured columns) and after (dotted columns) administration of DHA in WT, LQT1, LQT2, LQT2–5, and LQT5 rabbits. Right panel: changes in QTc in the different genotypes are indicated as ΔQTc. (*C*) Comparison of QTc in LQTS rabbits treated with DHA vs. baseline QTc in WT animals (grey), indicates a normalization in LQT2 (lack of differences between LQT2+DHA and WT at baseline). Grey bars are repeated to better show the difference to WT for each individual genotype. (*D*) Representation of DHA-induced QTc changes in the individual rabbits (left: QTc values before and right: after administration of DHA). (*B–D*) Sample numbers: LQT1 (green) $n = 8$, LQT2 (red) $n = 6$, LQT2–5 (violet) $n = 8$, LQT5 (blue) $n = 8$, and WT rabbits (grey) $n = 6$. Differences are indicated as * for P-value < 0.050, ** for P-value < 0.010, and *** for P-value < 0.001. All data are presented as mean ± SEM. DHA, docosahexaenoic acid;LQTS, long QT syndrome; LQT1, long QT Type 1; LQT2, long QT Type 2; LQT5, long QT Type 5; LQT2–5, combined form of long QT Types 2 and 5; SEM, standard error of the mean; WT, wild type.

remained prolonged after DHA administration (LQT1, P-value = 0.003; LQT2–5, P-value = 0.008). In LQT5 rabbits without any overt QT prolongation, QTc remained unchanged after DHA.

Effects of docosahexaenoic acid on 12-lead ECG in anaesthetized rabbits

Docosahexaenoic acid administration accelerated heart rate (shortened RR interval) only in LQT1 ($RR_{baseline}$: 347.42 ± 17.04 ms, RR_{DHA}: 371.75 ± 17.37 ms, P-value < 0.001) and not in the other genotype groups. DHA had no effects on PR or QRS in any of the genotypes.

After intravenous administration of DHA in anaesthetized rabbits, a statistically significant shortening of QTc was observed in WT (ΔQTc: −7.31 ± 1.52 ms, P-value = 0.005) and in LQT2 (ΔQTc: −11.35 ± 1.77 ms, P-value = 0.003), while no changes of QTc were observed in LQT1, LQT2–5, and LQT5 rabbits (Supplementary material online, *Figure S2A*).

At baseline, temporal QT variability [characterized by the short-term variability of QT interval (STVQT)] was significantly higher in LQT2 rabbits compared with LQT1 or WT rabbits (baseline $STVQT_{LQT2}$: 8.46 ± 1.90 ms, baseline $STVQT_{LQT1}$ 3.45 ± 0.32 ms, baseline $STVQT_{WT}$ 4.75 ± 1.00 ms; One-way ANOVA P-value = 0.036) (*Figure 2D*). Due to a significant reduction of STVQT upon DHA-infusion in LQT2 rabbits ($\Delta STVQT_{LQT2}$: −2.33 ± 0.58 ms, P-value = 0.016), after 20 min administration of DHA STVQT was similar in all genotypes (one-way ANOVA P-value = 0.566), indicating a normalization of STVQT in LQT2 (*Figure 4A–C* and Supplementary material online, *Figure S3*).

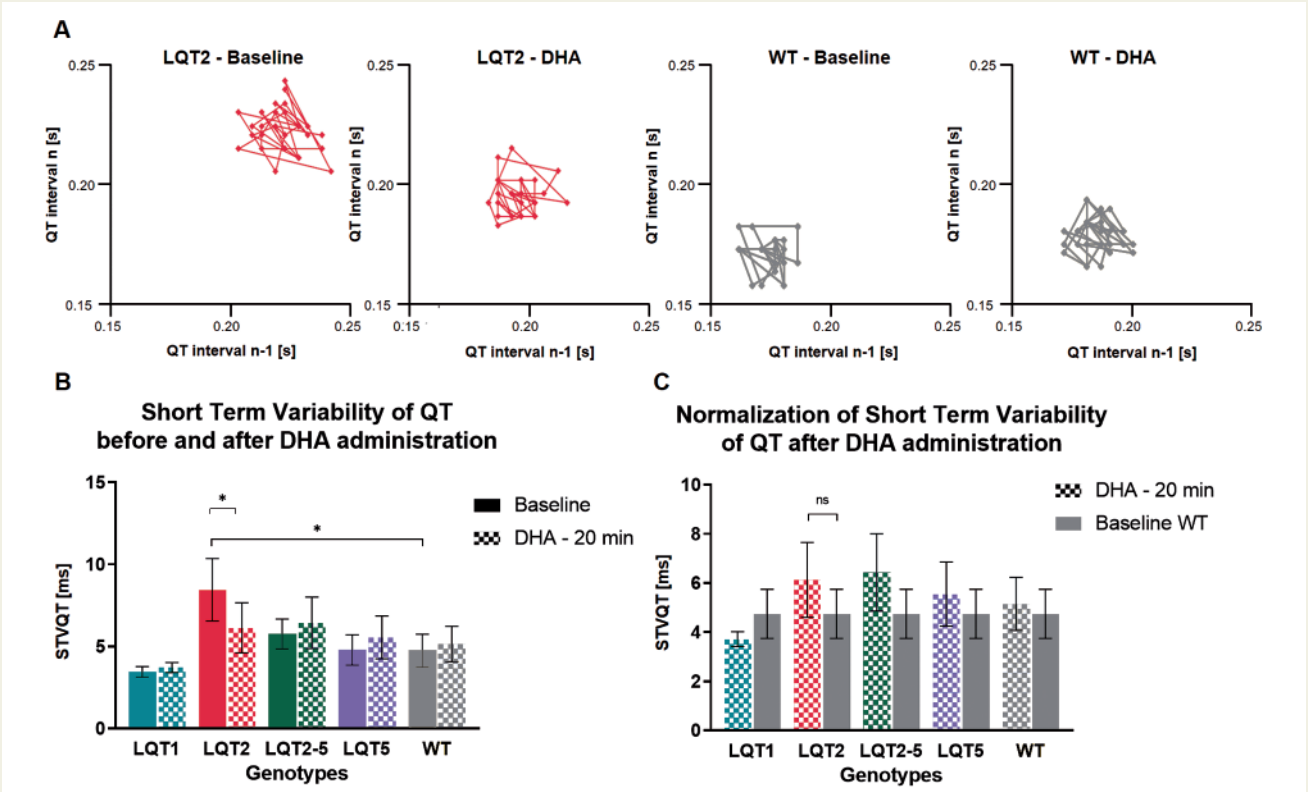

Figure 4 Effects of DHA on STVQT *in vivo*. (*A*) Representative examples of short term variability of QT measured in WT and LQT2 rabbits before (left) and after (right) administration of DHA. (*B*) Histograms showing STVQT before (fully coloured columns) and after (dotted columns) administration of DHA in WT, LQT1, LQT2, LQT2–5, and LQT5 rabbits. Differences are indicated with * for *P*-value < 0.050. All data are presented as mean ± SEM. (*C*) Histograms showing a comparison between STVQT in transgenic LQTS rabbits treated with DHA vs. baseline STVQT in WT animals (grey); a normalization in LQT2 can be observed (lack of significant differences between LQT2+DHA and WT at baseline). Differences are indicated as * for *P*-value <0.050, and as ** for *P*-value <0.010. All data are presented as mean ± SEM. Grey bars are repeated to better show the difference to WT for each individual genotype. DHA, docosahexaenoic acid; LQTS, long QT syndrome; LQT1, long QT Type 1; LQT2, long QT Type 2; LQT5, long QT Type 5; LQT2–5, combined form of long QT Types 2 and 5; STVQT, short-term variability of QT interval; SEM, standard error of the mean; WT, wild type.

Spatial QT dispersion neither showed relevant genotype-related differences at baseline nor was it significantly influenced by DHA in any of the genotype groups (Supplementary material online, *Figure S2B*).

Effects of docosahexaenoic acid in perfused whole hearts *ex vivo*

Docosahexaenoic acid shortened mean APD_{75} in WT (ΔAPD_{75}: −12.3 ± 2.22 ms, *P*-value < 0.001) and LQT2 rabbits (ΔAPD_{75}: −18.1 ± 3.54 ms, *P*-value = 0.004) (*Figure 5A–B*). The DHA-shortening in LQT2 was so pronounced that in LQT2 hearts perfused with DHA no longer any prolongation of APD_{75} was observed in comparison to WT hearts at baseline conditions ($APD_{75\text{-}LQT2\text{-}DHA}$: 144.12 ± 5.50 ms, $APD_{75\text{-}WT}$: 135.03 ± 7.02 ms, *T*-test *P*-value = 0.342), indicating a DHA-induced normalization of APD_{75} in LQT2 (*Figure 5C*). Importantly, the APD-shortening effect of DHA in WT and LQT2 rabbits was seen in each individual rabbit (*Figure 5D*). No relevant changes of the APD_{75} were recorded in LQT1, LQT2–5, or LQT5 rabbit (*Figure 5A–B*).

We further analysed whether DHA's APD_{75}-shortening effects in LQT2 and WT rabbits differed in the various LV regions. At 2 Hz pacing rate, APD_{75} shortening effects were observed in both LQT2 and WT rabbits in the LV mid-lateral wall and in LV lateral base regions. However, only in WT but not in LQT2 rabbits APD_{75}-shortening was also significant at the LV apex and LV medial base regions (*Figure 6A*). Notably, spatial APD_{75} dispersion was not altered by DHA neither in WT nor in LQT2—similarly as observed with QT dispersion.

Action potential triangulation was significantly reduced after DHA administration only in LQT2 rabbits (from baseline 103.8 ± 2.83 to 96.9 ± 2.41 ms; *P*-value < 0.001; *Figure 6B*), though not in WT, LQT1, LQT2–5, or LQT5 rabbits. Despite its reduction, in DHA-treated LQT2 rabbits AP triangulation remained more elevated than in WT rabbits at baseline (*Figure 6C*).

Effects of docosahexaenoic acid on action potential duration and arrhythmogenesis *in silico*

The modelled APs (*Figure 7A*) demonstrate a longer APD in LQT2 than in WT and a pronounced DHA-induced shortening of APD in LQT2, supporting our experimental observations. Single-cell

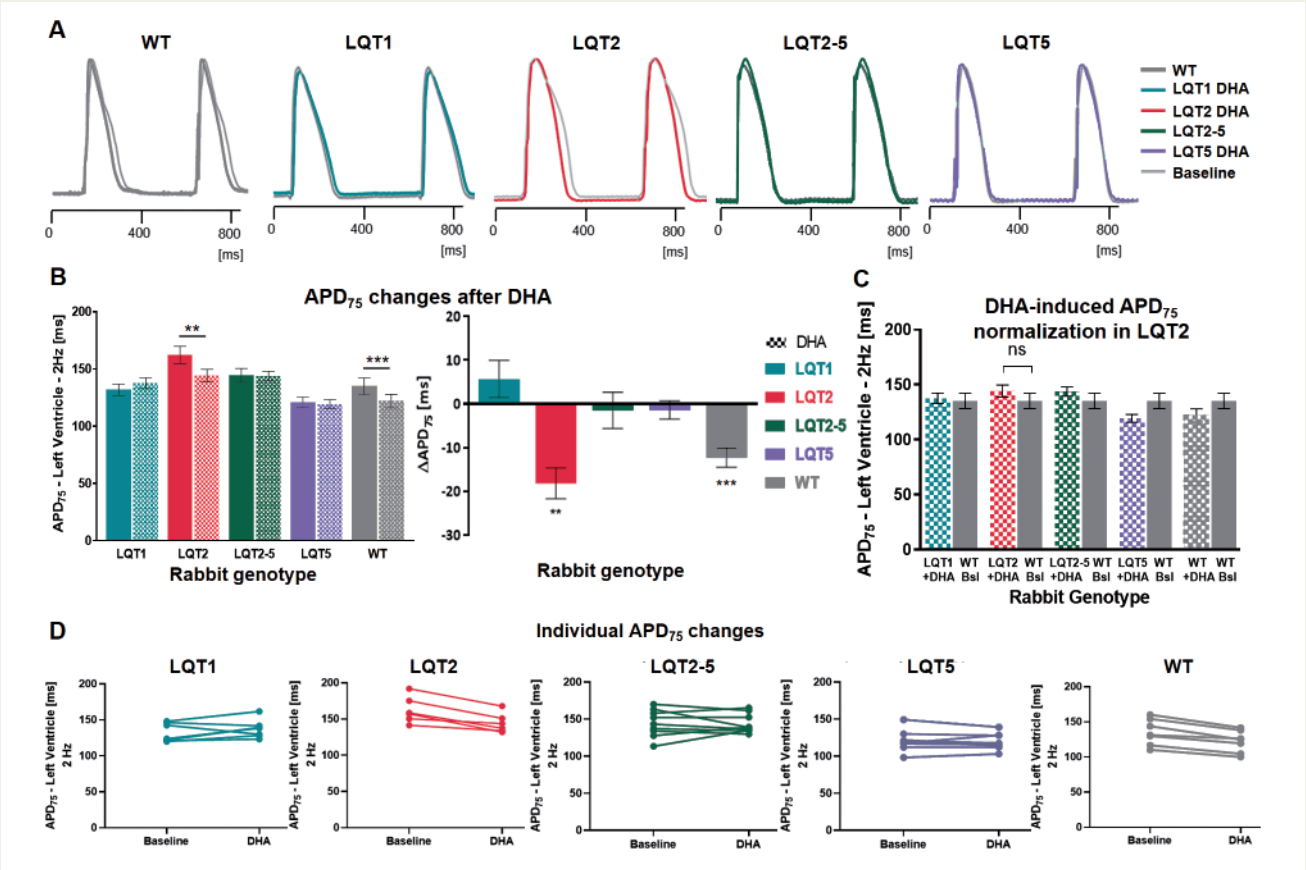

Figure 5 Effects of DHA on action potential duration *ex vivo*. (*A*) Representative examples of recorded MAPs before (in grey) and after administration of DHA in perfused WT (black), LQT1 (green), LQT2 (red), LQT2–5 (violet), and LQT5 (blue) rabbit hearts. The APD shortening appears during phase 3, which corresponds to the phase in which I_{Ks} is conducted. (*B*) Left panel: APD_{75} in WT, LQT1, LQT2, LQT2–5, and LQT5 rabbits before and after DHA administration. Right panel: changes in APD_{75} in the different genotypes are indicated as ΔAPD_{75}. (*C*) Comparison of APD_{75} in LQTS rabbits treated with DHA vs. baseline QTc in WT animals (grey) indicates a normalization in LQT2 (lack of differences between LQT2+DHA and WT at baseline). Grey bars are repeated to better show the difference to WT for each individual genotype. (*D*) Representation of DHA-induced APD_{75} changes in the individual rabbits (left: APD_{75} values before; right: after administration of DHA). (*B–D*) Sample numbers: LQT1 (green) $n = 7$, LQT2 (red) $n = 6$, LQT2–5 (violet) $n = 9$, LQT5 (blue) $n = 9$, and WT rabbits (grey) $n = 7$. Differences are indicated as * for P-value < 0.050, ** for P-value < 0.010, and *** for P-value < 0.001. All data are presented as mean ± SEM. DHA, docosahexaenoic acid; LQTS, long QT syndrome; LQT1, long QT Type 1; LQT2, long QT Type 2; LQT5, long QT Type 5; LQT2–5, combined form of long QT Types 2 and 5; MAP, monophasic action potential; SEM, standard error of the mean; WT, wild type.

simulations with varied conductivities of g_{Ks} *and* $g_{Ca,L}$ aiming at quantifying (potential positive) DHA effects on the cellular repolarization reserve were performed. The analysis of the parameter space of g_{Ks} *and* $g_{Ca,L}$ revealed that the LQT2 model produce EAD, blocks, and permanent depolarization for many more combinations of parameters than the WT model (*Figure 7B* for pacing at 1.0–2.5 Hz, Supplementary material online, *Figure S4* for pacing at 1.0–4.5 Hz), and importantly, that DHA exerted an anti-arrhythmic effect by reducing these arrhythmogenic AP compared with LQT2, reaching a level very close to WT cells. *Figure 7C* shows the prevalence for arrhythmogenic behaviour over all tested pacing frequencies, expressed as the ratio of parameter combinations that do not result in EAD, blocks, or permanent depolarization, compared with WT at 1 Hz. Again, LQT2 + DHA cells had consistently lower arrhythmic behaviour than LQT2 cells over all frequencies, nearly reaching the level of WT cells, particularly at faster stimulation frequencies.

Discussion

In this study, we investigated the potential beneficial APD/QT-shortening effects of the I_{Ks}-activator DHA, a natural polyunsaturated fatty acid (PUFA),[5] *in vivo and ex vivo* on the whole heart level in four different transgenic rabbit models of LQTS.

In WT and more pronouncedly in transgenic LQT2 rabbit models, DHA significantly shortened QTc and reduced the beat-to-beat variability of repolarization, quantified as STVQT *in vivo*, and shortened APD_{75} and reduced AP triangulation *ex vivo*. Notably, in LQT2 rabbits the effect of DHA led to a normalization of QTc, STVQT, and APD_{75} to the level observed in healthy WT rabbits, suggesting that DHA may exert a beneficial therapeutic effect in LQT2.

Several experimental studies have investigated the effects of different PUFAs on cardiac electrophysiology. We have chosen DHA, which increased the magnitude of I_{Ks} current in KCNQ1/KCNE1-

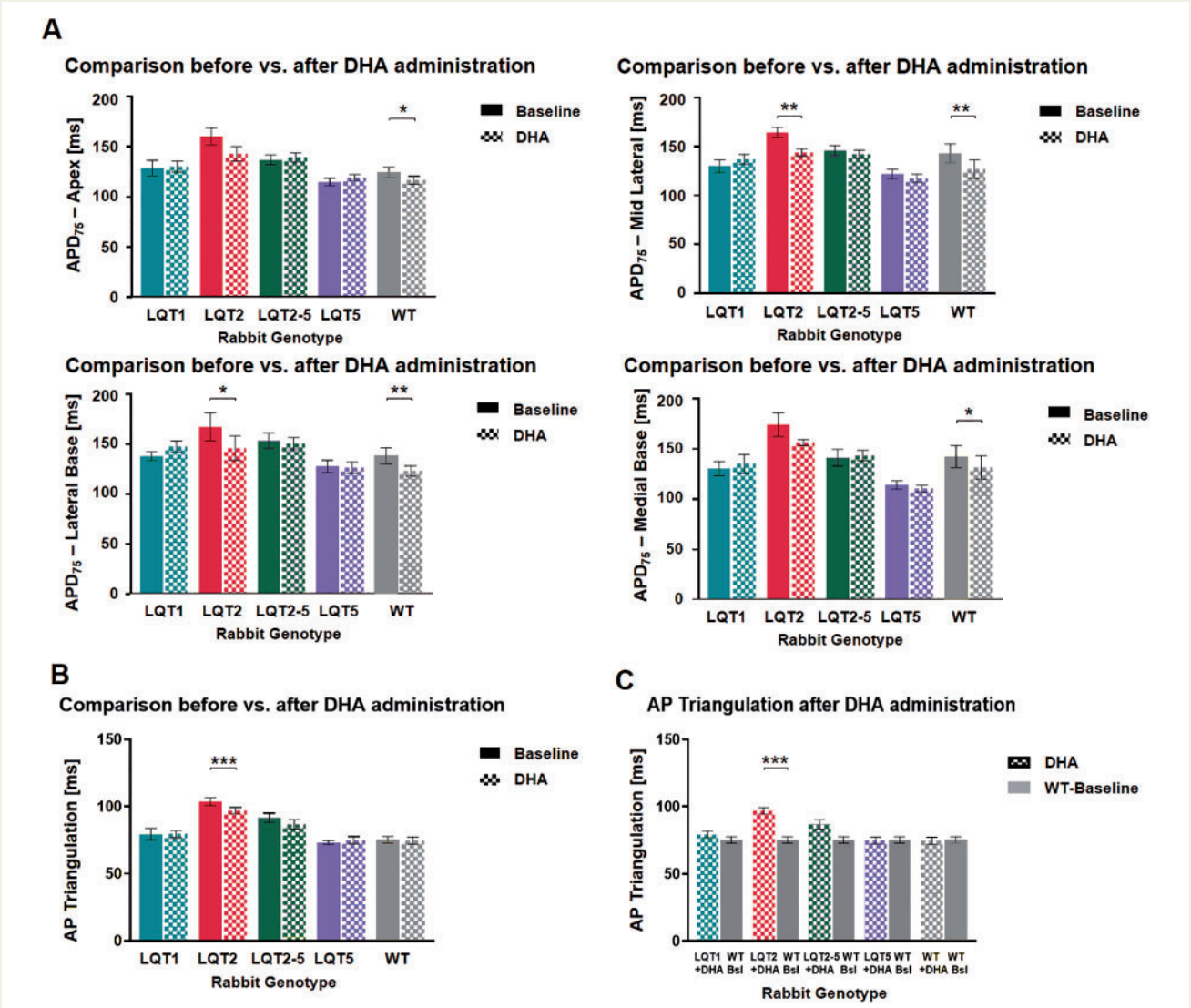

Figure 6 Effects of DHA on regional APD and AP triangulation *ex vivo*. (*A*). Effects of DHA on APD_{75} in apex, mid lateral wall, lateral basis, and medial basis in WT, LQT1, LQT2, LQT2–5, and LQT5 rabbits. (*B*) AP triangulation before (fully coloured columns) and after (dotted columns) administration of DHA in WT, LQT1, LQT2, LQT2–5, and LQT5 rabbits. (*C*) Comparison between APD_{75} in LQTS rabbit hearts after administration of DHA and AP triangulation in WT rabbit hearts before administration of DHA. In contrast to QTc and APD_{75}, a normalization of AP triangulation cannot be observed in LQT2 after DHA administration; sample numbers: LQT1 (green) $n = 7$, LQT2 (red) $n = 6$, LQT2–5 (violet) $n = 9$, LQT5 (blue) $n = 9$, and WT rabbits (grey) $n = 7$. Differences are indicated as * for P-value < 0.050, ** for P-value < 0.010, and *** for P-value < 0.001. All data are presented as mean ± SEM. AP, action potential; APD, action potential duration; DHA, docosahexaenoic acid; LQTS, long QT syndrome; LQT1, long QT Type 1; LQT2, long QT Type 2; LQT5, long QT Type 5; LQT2–5, combined form of long QT Types 2 and 5; MAP, monophasic action potential; SEM, standard error of the mean; WT, wild type.

transfected COS7 cells and in guinea pig cardiomyocytes more than, for example, eicosapentaenoic acid, another omega-3 fatty acid of marine origin. These findings have been further supported by the groups of Liin, Bentzen, and Larsson,[4,6,12] who have observed that natural and modified PUFAs activate the I_{Ks}-conducting channel through a lipoelectric interaction between the negatively charged PUFA head group and positively charged aminoacidic residues in the alpha-subunit KCNQ1.

Along this line, they also described a significant shortening of the prolonged QT and prolonged APD in a guinea pig model of drug-induced LQTS.[9] Our results confirm this observation of QT/APD-shortening in wild-type rabbits—and importantly—in transgenic LQTS rabbit models. Moreover, as we used several different transgenic rabbit models for different LQTS subtypes, in which different alpha- and beta-subunits of repolarizing ion channels are impaired (namely, KCNQ1 in LQT1, KCNH2 in LQT2, KCNE1 in LQT5, and KCNQ1 and KCNE1 in LQT2–5), important information regarding the mechanistic action of DHA on I_{Ks} could be obtained.

Moreno *et al.*[5] suggested that the DHA-induced acceleration of ventricular repolarization was based on a modification of the

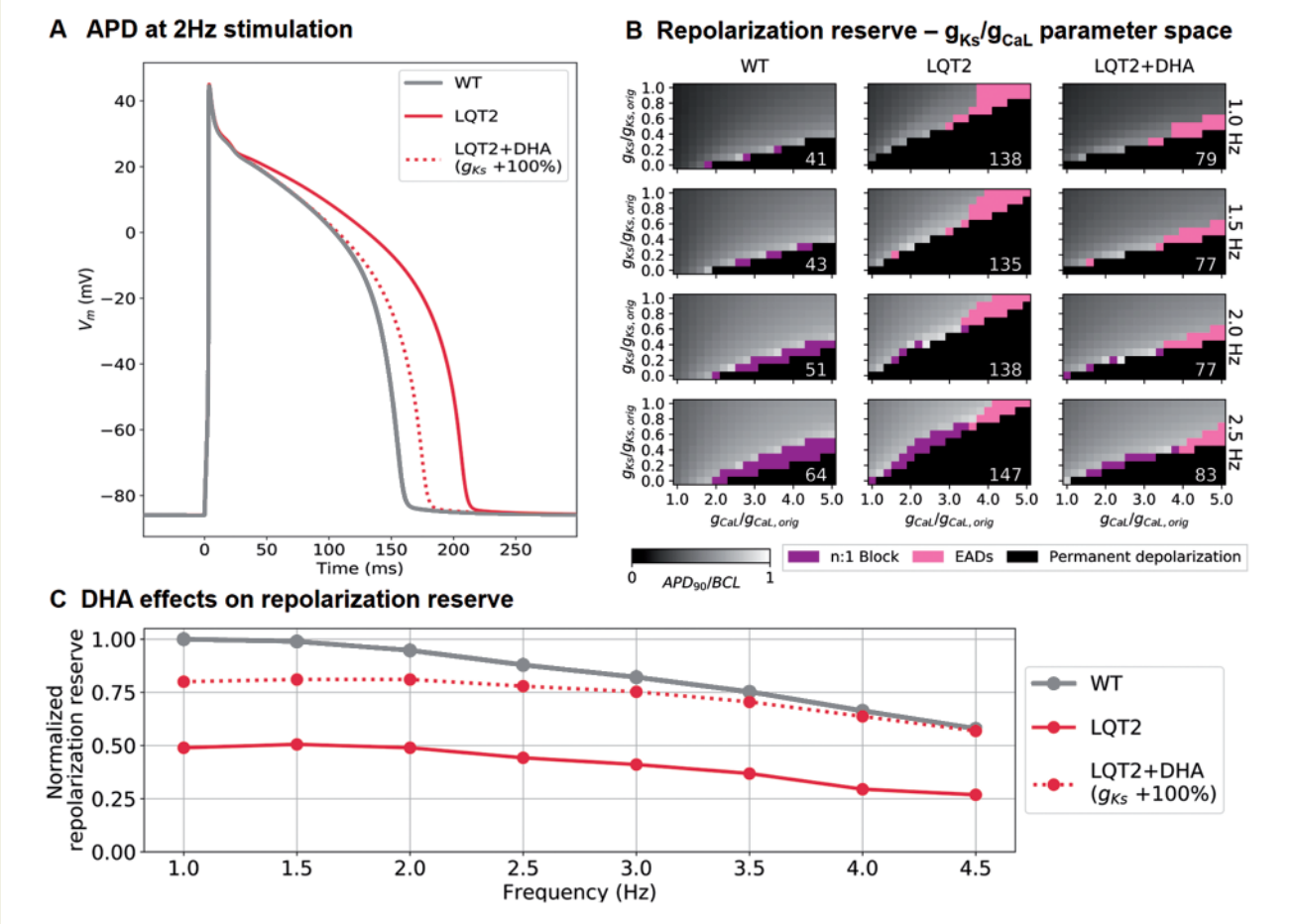

Figure 7 *In silico* modelling of DHA effects. (*A*) Simulated APs at 2 Hz based on Shannon model for WT, LQT2 (g_{Kr} = 0%), and LQT2+DHA (g_{Ks} +100%) cardiomyocytes. (*B*) Results of single-cell *in silico* simulations for combinations of parameters g_{Ks} and $g_{Ca,L}$ at 1.0–2.5 Hz. Every square represents one simulation. Shaded squares represent parameters where pacing was successful (shade encodes APD_{90}). Pink and purple squares represent simulations where 2:1 block and EAD occur, respectively. Black squares represent simulations where the cell was unable to repolarize. Numbers in bottom right corners of each plot indicate simulations resulting in block, EAD or permanent depolarization. (*C*) Normalized repolarization reserve calculated from the number of single-cell pacing simulations in the parameter space $0 < g_{Ks}/g_{Ks,orig} < 1$ (step 0.1) and $1 < g_{CaL}/g_{CaL,orig} < 5$ (step 0.2) that did not result in EAD, block or permanent depolarization. Reference for normalization is the number of simulations for WT at 1 Hz that did not show these pro-arrhythmic features. AP, action potential; DHA, docosahexaenoic acid; EAD, early after depolarization; LQT2, long QT Type 2; WT, wild type.

interaction between the alpha- and beta-subunits KCNQ1 and KCNE1 that leads to an enhancement of I_{Ks} current. Accordingly, I_{Ks}-activating effects of DHA could not be observed, when KCNQ1 channels were expressed alone,[5] suggesting that the presence of functional KCNE1 subunits was essential for the observed electrophysiological DHA-effects. We now demonstrated in *Xenopus* oocyte experiments that DHA activates human KCNQ1, human KCNQ1/KCNE1 and rabbit KCNQ1/KCNE1 I_{Ks} to different extents, with the largest activating effect in the two KCNQ1/KCNE1 channels and with less effect on human KCNQ1 channels (when the beta-subunit KCNE1 is missing), indicating an importance of both subunits for a pronounced DHA-induced I_{Ks} activation. The larger relative increase in K^+ current at 0 mV for human KCNQ1/KCNE1 compared with KCNQ1 can be largely explained by the different voltage dependence of the two channels. Half maximal conductance is reached at about +20 mV for human KCNQ1/KCNE1 and at about −30 mV for KCNQ1,[eRef5] which means that at 0 mV KCNQ1 has already approached its maximal conductance, giving less possibility for DHA to induce combined activating effects. In line with these hypotheses, no QT/APD-shortening effects were observed neither in LQT5 and LQT2–5 rabbits with impaired KCNE1-subunits nor in LQT1 rabbits with impaired KCNQ1—while pronounced QT/APD-shortening effects were observed in LQT2 and WT with intact KCNE1 and KCNQ1 function. This strongly suggests that DHA may exert a genotype-specific beneficial effect only in LQTS subtypes with intact I_{Ks} function.

Recently, possible PUFA interaction sites have been identified at the voltage-sensor and the pore region of the KCNQ1 channel, using coarse-grained and all-atom molecular dynamics simulations.[15] Although DHA is a protonable PUFA, which allows it to cross the

cell membrane and to exert effects through intracellular or extracellular interactions, the functional PUFA sites in KCNQ1 seem to be located in the outer membrane leaflet.[15] Along those lines, it is important to note, that the KCNQ1 mutation expressed in our transgenic LQT1 rabbit models is a dominant negative pore mutation with complete loss of functional I_{Ks}, in which no DHA-induced APD shortening can be expected. This may of course be different in other KCNQ1 variants in other regions of the gene and raises the possibility that some clinically relevant mutations may influence the DHA effect (e.g. when located in PUFA-interacting regions); and in some LQT1 variants one might still see some DHA-induced rescue of the phenotype. Hence, it is very important to stress that DHA may—in addition to the genotype-specific beneficial DHA effect in LQTS genotypes with normal KCNQ1 and KCNE1 function—also have mutation-specific beneficial effects in some LQT1 and/or LQT5 mutations.

The fact that not only global QTc and APD were shortened/normalized by DHA in LQT2 rabbits but also classical *in vivo and ex vivo* pro-arrhythmia markers such as STVQT and AP triangulation, suggests that DHA might also exert anti-arrhythmic effects. STVQT, which characterizes temporal instability in cardiac repolarization, has been validated as a novel marker regarding arrhythmic risk also in human LQTS[16]—and it was also most pronounced in transgenic LQT2 rabbits with the highest risk for spontaneous TdP arrhythmias.[8,10] Importantly, DHA normalized STVQT to the level observed in WT rabbits, strongly suggesting that not only overall cardiac repolarization was stabilized but also the pro-arrhythmic temporal instability of repolarization was reduced. Similarly, the arrhythmogenicity marker AP triangulation, which is particularly increased in LQT2 rabbits with a marked pro-arrhythmic phenotype,[8,10] was also reduced by DHA in LQT2. The failure to normalize AP triangulation completely may be due to the fact that the extent of AP triangulation is strongly dependent on I_{K1} *and* I_{Kr}, and somewhat less on I_{Ks}.[17]

To further investigate potential anti-arrhythmic effects of DHA in LQT2, we have conducted *in silico* modelling experiments incorporating the observed DHA-induced increase in I_{Ks}. In these we identified a reduction in EAD formation and in 2:1 blocks as well as an improved repolarization reserve in LQT2 cells with shortened APD due to an increase in I_{Ks} by +100% that corresponds to the experimentally observed DHA-effect on I_{Ks}. Despite such encouraging evidence, the potential anti-arrhythmic effects certainly need to be validated in larger, long-term studies directly investigating DHA-effects on spontaneous (and provoked) TdP ventricular arrhythmias and SCD in LQTS.

In both WT and in LQT2 rabbits, an APD-shortening was observed in all investigated left ventricular regions, despite not reaching statistical significance in the apex and in the medial base in LQT2. This may be the reason why DHA had no effect on APD dispersion and—particularly—did not increase APD dispersion. This is important as it has previously been shown that the activation of one specific potassium channel may exert pro-arrhythmic effects by increasing the dispersion of repolarization or by causing excessive regional APD-shortening effects, because of underlying regional heterogeneity in the expression of cardiac potassium channels.[18] Why the pharmacological activation of I_{Kr} increased APD dispersion,[18] while the activation of I_{Ks} by DHA did not, is currently unclear. One might speculate that the underlying regional differences in I_{Ks} and I_{Kr} that are unmasked by the activation of the reciprocal current might differ in their extent. The fact that DHA also affects other channel proteins, such as voltage-gated Na^+ and Ca^{2+} channels that are heterogeneously expressed throughout the ventricles may counteract some of the regional effects of I_{Ks} activation. DHA effects on other (unknown) protein that alter excitability in pacemaker cells which counteract the decrease in excitability by DHA's activating effect on I_{Ks}, might also underlie the observation that DHA impacts on heart rate in LQT1 but not in WT and LQTS rabbits with intact I_{Ks}.

As rabbits show pronounced similarities to humans in terms of cardiac electrophysiology, and—particularly—as the transgenic LQTS rabbits mimic all major aspects of the human LQTS disease phenotypes, it stands to reason that the observed beneficial DHA effects in LQT2 rabbits could have a translational impact on future genotype-specific treatment approaches in LQTS. Thus far, no studies have investigated DHA effects in human LQTS patients. Moreover, the available literature offers only limited data regarding the effects of PUFA and more specifically of DHA on cardiac electrophysiology—as the focus of these studies was more on prevention of cardiovascular diseases—where PUFA supplementation seems to be beneficial.[19,eRef6] Recently, though, Yagi *et al.*[20] observed that low levels of DHA were associated with cardiogenic syncope in patients affected by Brugada syndrome, suggesting that DHA may play an important role in preventing ventricular fibrillation in this cohort of patients. Our observations in transgenic LQTS rabbit models may open the door for a translational, clinical evaluation of DHA as novel genotype-specific therapy in LQTS. To this aim, additional experiments directly demonstrating anti-arrhythmic effects in animal models and—importantly—first confirmatory studies in human patients are still warranted.

Conclusion

We demonstrated that DHA exerts a genotype-specific beneficial shortening/normalizing effect on QTc, STVQT, APD_{75}, and AP triangulation through activation of I_{Ks} in LQT2 rabbits but has no effects if either KCNQ1 (α-subunit to I_{Ks}) or KCNE1 (β-subunits to I_{Ks}) are functionally impaired (as in LQT1, LQT5, and LQT2–5). Thus, DHA could represent a new genotype-specific therapeutic option in LQT2 syndrome (or other LQTS subtypes with intact α- and β-subunits to I_{Ks}).

Supplementary material

Supplementary material is available at *Europace* online.

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Conflict of interest: none declared.

Data availability

All data are available on request at the Institute for Physiology of the University of Bern.

References

1. Priori SG, Blomstrom-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, Camm J *et al.* 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. *Europace* 2015;**17**:1601–87.
2. Schwartz PJ, Crotti L, Insolia R. Long-QT syndrome: from genetics to management. *Circ Arrhythm Electrophysiol* 2012;**5**:868–77.
3. Cho Y. Management of patients with long QT syndrome. *Korean Circ J* 2016;**46**: 747–52.
4. Liin SI, Yazdi S, Ramentol R, Barro-Soria R, Larsson HP. Mechanisms underlying the dual effect of polyunsaturated fatty acid analogs on Kv7.1. *Cell Rep* 2018;**24**: 2908–18.
5. Moreno C, de la Cruz A, Oliveras A, Kharche SR, Guizy M, Comes N *et al.* Marine n-3 PUFAs modulate IKs gating, channel expression, and location in membrane microdomains. *Cardiovasc Res* 2015;**105**:223–32.
6. Skarsfeldt MA, Liin SI, Larsson HP, Bentzen BH. Polyunsaturated fatty acid-derived I(Ks) channel activators shorten the QT interval ex-vivo and in-vivo. *Acta Physiol* 2020;**229**:e13471.
7. Nerbonne JM. Molecular basis of functional voltage-gated K+ channel diversity in the mammalian myocardium. *J Physiol* 2000;**525 Pt 2**:285–98.
8. Brunner M, Peng X, Liu GX, Ren XQ, Ziv O, Choi BR *et al.* Mechanisms of cardiac arrhythmias and sudden death in transgenic rabbits with long QT syndrome. *J Clin Invest* 2008;**118**:2246–59.
9. Major P, Baczkó I, Hiripi L, Odening KE, Juhász V, Kohajda Z *et al.* A novel transgenic rabbit model with reduced repolarization reserve: long QT syndrome caused by a dominant-negative mutation of the KCNE1 gene. *Br J Pharmacol* 2016;**173**:2046–61.
10. Hornyik T, Castiglione A, Franke G, Perez-Feliz S, Major P, Hiripi L *et al.* Transgenic LQT2, LQT5, and LQT2-5 rabbit models with decreased repolarisation reserve for prediction of drug-induced ventricular arrhythmias. *Br J Pharmacol* 2020;**177**:3744–59.
11. Orosz A, Baczkó I, Nagy V, Gavallér H, Csanády M, Forster T *et al.* Short-term beat-to-beat variability of the QT interval is increased and correlates with parameters of left ventricular hypertrophy in patients with hypertrophic cardiomyopathy. *Can J Physiol Pharmacol* 2015;**93**:765–72.
12. Liin SI, Silverå Ejneby M, Barro-Soria R, Skarsfeldt MA, Larsson JE, Starck Härlin F *et al.* Polyunsaturated fatty acid analogs act antiarrhythmically on the cardiac IKs channel. *Proc Natl Acad Sci U S A* 2015;**112**:5714–9.
13. Bohannon BM, Wu X, Wu X, Perez ME, Liin SI, Larsson HP. Polyunsaturated fatty acids produce a range of activators for heterogeneous IKs channel dysfunction. *J Gen Physiol* 2020;**152**:e201912396.
14. Bodi I, Sorge J, Castiglione A, Glatz SM, Wuelfers EM, Franke G *et al.* Postpartum hormones oxytocin and prolactin cause pro-arrhythmic prolongation of cardiac repolarization in long QT syndrome type 2. *Europace* 2019;**21**:1126–38.
15. Yazdi S, Nikesjö J, Miranda W, Corradi V, Tieleman DP, Noskov SY *et al.* Identification of PUFA interaction sites on the cardiac potassium channel KCNQ1. *J Gen Physiol* 2021;**153**:e202012850.
16. Hinterseer M, Beckmann B-M, Thomsen MB, Pfeufer A, Dalla Pozza R, Loeff M *et al.* Relation of increased short-term variability of QT interval to congenital long-QT syndrome. *Am J Cardiol* 2009;**103**:1244–8.
17. Romero L, Pueyo E, Fink M, Rodríguez B. Impact of ionic current variability on human ventricular cellular electrophysiology. *Am J Physiol Heart Circ Physiol* 2009; **297**:H1436–45.
18. Bentzen BH, Bahrke S, Wu K, Larsen AP, Odening KE, Franke G *et al.* Pharmacological activation of Kv11.1 in transgenic long QT-1 rabbits. *J Cardiovasc Pharmacol* 2011;**57**:223–30.
19. Parish S, Mafham M, Offer A, Barton J, Wallendszus K, Stevens W *et al.*; ASCEND Study Collaborative Group. Effects of omega-3 fatty acid supplements on arrhythmias. *Circulation* 2020;**141**:331–3.
20. Yagi S, Soeki T, Aihara K-I, Fukuda D, Ise T, Kadota M *et al.* Low serum levels of eicosapentaenoic acid and docosahexaenoic acid are risk factors for cardiogenic syncope in patients with Brugada syndrome. *Int Heart J* 2017;**58**:720–23.

The eReferences 1–6 can be found in the online eReference list in the supplementary material.