**UEF//eRepository**

**DSpace** **https://erepo.uef.fi**

Artikkelit Terveystieteiden tiedekunta

2016

# Associations of the serum long-chain omega-3 polyunsaturated fatty acids and hair mercury with heart rate-corrected QT and JT intervals in men: the Kuopio Ischaemic Heart Disease Risk Factor Study

Tajik, Behnam

Springer International Publishing

article

info:eu-repo/semantics/acceptedVersion

© Springer-Verlag Berlin Heidelberg

All rights reserved

http://dx.doi.org/10.1007/s00394-016-1272-3

https://erepo.uef.fi/handle/123456789/7459

*Downloaded from University of Eastern Finland's eRepository*

For: European Journal of Nutrition

# Associations of the Serum Long-Chain Omega-3 Polyunsaturated Fatty Acids and Hair Mercury with Heart-Rate Corrected QT- and JT-intervals in Men: The Kuopio Ischaemic Heart Disease Risk Factor Study

Behnam Tajik, Sudhir Kurl, Tomi-Pekka Tuomainen, Jyrki K. Virtanen

All from the University of Eastern Finland, Kuopio Campus, Institute of Public Health and Clinical Nutrition, Kuopio, Finland

Address correspondence to: Jyrki K. Virtanen, Institute of Public Health and Clinical Nutrition, University of Eastern Finland, PO Box 1627, 70211 Kuopio, Finland. Tel: +358-40-3552957 Fax: +358-17-162936 E-mail: jyrki.virtanen@uef.fi.

**Keywords**: polyunsaturated fatty acids; heart electrophysiology; QT-interval; methylmercury; population study

**Abstract**

*Purpose* Long-chain omega-3 polyunsaturated fatty acids (PUFA) from fish have been associated with risk of cardiovascular diseases (CVD), especially sudden cardiac death (SCD). Mercury exposure, mainly due fish consumption, has been associated with higher risk. However, the impact of PUFAs or mercury on the ventricular cardiac arrhythmias, which often precede SCD, are not completely known. We investigated the associations of the serum long-chain omega-3 PUFAs and hair mercury with ventricular repolarization, measured by heart rate-corrected QT- and JT-intervals (QTc and JTc, respectively).

*Methods* A total of 1411 men from the prospective, population-based Kuopio Ischaemic Heart Disease Risk Factor Study, aged 42–60 years and free of CVD in 1984–1989, were studied.

*Results* Serum long-chain omega-3 PUFA concentrations were inversely associated with QTc and JTc (multivariate-adjusted *P*-trend across quartiles=0.02 and 0.002, respectively) and, during the mean 22.9-year follow-up, with lower SCD risk. However, further adjustments for QTc, JTc or hair mercury did not attenuate the associations with SCD. Hair mercury was not associated with QTc, JTc or SCD risk, but it slightly attenuated the associations of the serum long-chain omega-3 PUFA with QTc and JTc.

*Conclusions* Higher serum long-chain omega-3 PUFA concentrations, mainly a marker for fish consumption, were inversely associated with QTc and JTc in middle-aged and older men from Eastern Finland, but QTc or JTc did not attenuate the inverse associations of the long-chain omega-3 PUFA with SCD risk. This suggest that prevention of prolonged ventricular repolarization may not explain the inverse association of the long-chain omega-3 PUFA with SCD risk.

## Introduction

The long-chain omega-3 polyunsaturated fatty acids (PUFA) from fish may have beneficial impact on the risk of cardiac mortality, especially fatal myocardial infarction and sudden cardiac death (SCD), for example by reducing inflammation and lowering blood pressure [1]. However, the impact of the long-chain omega-3 PUFA on the ventricular cardiac arrhythmia, which often precede SCD, is unclear [1, 2].

The heart rate-corrected QT and JT intervals (QTc and JTc, respectively) on electrocardiogram (ECG) reflect the duration of ventricular repolarization [3]. However, since QT-interval includes both repolarization and part of depolarization phases during heart electrical cycle, JT-interval (QT duration–QRS duration) has been recommended as the more sensitive measure for assessing abnormality of ventricular repolarization [4]. It has been suggested that abnormal ventricular repolarization (prolonged QT- and JT-intervals) can predispose to a potentially fatal ventricular arrhythmias known as *torsades de pointes* [3, 5] and increase the risk of SCD [6, 7].

Few studies have investigated the impact of the long-chain omega-3 PUFA on the QTc and the findings are inconclusive. In dogs, infusion of long-chain omega-3 PUFA shortened the QTc and reduced risk of ischemia-induced fatal ventricular arrhythmias [8]. In population-based studies, higher fish intake has been related to a lower likelihood of prolonged QT-interval and lower risk of cardiac arrhythmias [9, 10]. In contrast, this was not observed in a small study, which investigated the association between the circulating levels of the long-chain omega-3 PUFA, an objective biomarker of exposure to these fatty acids, and QTc [11]. A small randomized controlled trial did not find an impact of fish oil supplementation on the QTc, either

[12]. To the best of our knowledge, no previous studies have evaluated the association between the long-chain omega-3 PUFA and JT-interval.

In addition to the long-chain omega-3 PUFA, fish may contain methylmercury, which has been associated with higher risk of coronary heart disease mortality and SCD in the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD), the study population for the current analysis [13, 14]. In these studies, higher mercury exposure also attenuated the inverse associations of the long-chain omega-3 PUFAs with the risk of cardiovascular outcomes. There is very little data from other studies regarding mercury exposure and risk of SCD, with the only other study showing no association [15].

We investigated the cross-sectional associations of the serum long-chain omega-3 PUFA with QTc- and JTc-intervals, as measurements of ventricular repolarization, among middle-aged and older men from the population-based KIHD study. We also evaluated whether high hair mercury concentration, a biomarker for long-term mercury exposure [16], is associated with QTc- and JTc- intervals and whether it could modify the associations with the long-chain omega-3 PUFA. In addition, in the secondary analysis we prospectively investigated whether adjusting for QTc or JTc-intervals would attenuate the associations of the serum long-chain omega-3 PUFA and hair mercury with the risk of incident SCD. This could suggest the impact on ventricular repolarization as one possible mechanism how these fatty acids and mercury could affect the risk of SCD in this study population [14, 17].

# Methods

## Study population

KIHD was designed to investigate risk factors for cardiovascular disease (CVD), atherosclerosis, and related outcomes in a prospective, population-based, randomly selected sample of men from eastern Finland [18]. A total of 2682 men (82.9 % of those eligible) who were 42, 48, 54 or 60 years old and living in the city of Kuopio or its surrounding areas were recruited to the baseline examinations in 1984-1989. The baseline characteristic of the entire study population have been described previously [18]. The KIHD protocol was approved by the Research Ethics Committee of the University of Eastern Finland and complies with Declaration of Helsinki. All the subjects signed a written informed consent.

From the analyses we excluded participants with a history of CVD (n=730), or those with missing data on the serum long-chain omega-3 PUFAs (n=103) or hair mercury (n=9). Since the wide QRS complex (QRS≥120 ms) has an effect on the QT-interval [4], we also excluded participants who had the QRS duration ≥120 ms (bundle branch block, n=429). The levels of exposures and other factors were generally similar between those with normal QRS complex and participants with prolonged QRS (≥120 ms) (*P*-values for differences >0.16). After the exclusions, 1411 men were included in the analysis.

## Measurements

Hair and venous blood samples were obtained between 8 A.M. and 10.00 A.M. at baseline examinations. Subjects were instructed to abstain from ingesting alcohol for three days and from smoking and eating for 12 hours prior to giving the sample. Comprehensive description of the determination of serum lipid and lipoproteins [19], assessment of medical history and

medications [19], smoking [19], alcohol consumption [19], resting blood pressure [19] and physical activity [20] have been reported previously. Hypertension diagnosis was defined as systolic/diastolic blood pressure >140/90 mmHg at study visit, clinical diagnosis of hypertension or use of hypertension medication. Serum C-reactive protein (CRP) was measured with an immunometric assay (Immulite High Sensitivity CRP Assay, DPC, Los Angeles, CA, USA). Dietary intakes were assessed by using 4-day food recording at the time of blood sampling [21]. Education and annual income were assessed by using self-administered questionnaires.

**Serum fatty acid and mercury measurements**

Serum fatty acids were determined in one gas chromatographic run without preseparation as described previously [22]. Serum fatty acids were extracted with chloroform-methanol. Chloroform phase was evaporated and treated with sodium methoxide, which methylated esterified fatty acids. Quantification was carried out with reference standards purchased from ν-Check Prep Inc. (MN). Each analyte had individual reference standard, and recovery of analytes was confirmed with an internal standard eicosan (arachidic acid $C_{20}H_{40}O_2$). Fatty acids were chromatographed in an NB-351 capillary column (HNU-Nordion, Helsinki, Finland) by a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard Company, Avondale, PA, since 1999 Agilent Technologies Inc.) with a flame ionization detector. Results were obtained in micromoles per liter. The coefficient of variation was 9.4% for eicosapentaenoic acid (EPA, 20:5n-3), 12.7% for docosapentaenoic acid (DPA, 22:5n-3) and 11.9% for docosahexaenoic acid (DHA, 22:5n-3). For the serum total long-chain omega-3 PUFAs, we used the sum of EPA, DPA and DHA.

Hair mercury was detected by flow injection analysis-cold vapor atomic absorption spectrometry and amalgamation [23]. Repeat hair samples and their mercury content were collected from 21 subject in 4 to 9 years (mean, 6 years) after baseline examination to survey the tracking of hair mercury values over time. Pearson correlation coefficient between the original and the repeat measurement was 0.91.

**Assessment of ECG**

All electrocardiographic intervals and amplitudes were measured automatically from standard 12-lead electrocardiographic recording [24]. Paper speed was 50 mm/s. The QT-interval was measured from the onset of the QRS complex to the end of the T wave; the last was characterized as the intersection of the isoelectric line and the tangent of the maximal slope on the downward limb of the T-Wave [25]. Because of the strong correlation between the QT-interval and heart rate, the heart rate-corrected QT- and JT-intervals were calculated by using the Bazett's formula [26]: $QTc = QT / \sqrt{RR}$ (RR = the interval between 3 consecutive R waves in the ECG) [27]; and JTc: = QTc-QRS.

**Ascertainment of follow-up events**

All SCD events that occurred by the end of 2013 were included. The sources of information were interviews, hospital documents, death certificates, autopsy reports, and medico-legal reports [28]. There were no losses to follow-up. The diagnostic classification of events was based on symptoms, ECG findings, cardiac enzyme elevations, autopsy findings (80%), and history of coronary heart disease together with the clinical and ECG findings of the paramedic staff. All the documents related to the death were cross checked in detail by two physicians. Deaths were

coded using to the ICD-9th Revision, codes 410 to 414 for non-SCD and 798.1 for SCD; or the ICD-10th Revision, codes I20 to I25 for non-SCD and I46 for SCD. A death was determined SCD when it occurred either within 1h after the onset of an abrupt change in symptoms or within 24 h after onset of symptoms when autopsy data did not reveal a non-cardiac cause of sudden death. The deaths due to aortic aneurysm rupture, cardiac rupture or tamponade, and pulmonary embolism were not included as SCD.

**Statistical Analysis**

The univariate associations between the serum total long-chain omega-3 PUFA (EPA+DPA+DHA) concentration and demographic, lifestyle and clinical characteristics at baseline were assessed by means and linear regression for continuous variables and $chi^2$-test for categorical variables. Correlations between the individual long-chain omega-3 PUFAs were evaluated by calculation of Spearman correlation. The mean values of QTc- and JTc-intervals in the quartiles of the long-chain omega-3 PUFA and hair mercury were analyzed using analysis of covariance (ANCOVA). Logistic regression models were used to estimate odds ratios (OR) for prolonged QTc and JTc in exposure quartiles, with the lowest category as the reference. We used the $95^{th}$ percentile of the distribution of QTc and JTc to define abnormal QTc (≥445.67 ms) and JTc (≥343.83 ms). In the secondary analyses, Cox proportional hazards regression model were used to evaluate the hazard ratio (HR) of incident SCD in categories of the long-chain omega-3 PUFA, QTc and JTc. Because of the small number of SCD events, tertiles of the long-chain omega-3 PUFA, QTc and JTc were used. The validity of the proportional hazards assumption was evaluated by using Schoenfeld residuals.

171 The confounders in the analyses were selected based on established risk factors for CHD,
172 previously published associations with CHD in the KIHD study, or on associations with
173 exposures or outcomes in the present analysis. Two models were conducted to adjust for
174 potential cofounders in the cross-sectional analyses. The model 1 was adjusted for age (years)
175 and examination year. The model 2 included the variables in the model 1 plus body mass index
176 (kg/m2), type 2 diabetes (yes/no), smoking status (never smoker, previous smoker, current
177 smoker <20 cigarettes/day and current smoker ≥20 cigarettes/day), leisure-time physical activity
178 (kcal/day), education (years), income (euro/year), treated hypertension (including the use of beta-
179 blockers, yes/no), alcohol intake (g/week) and energy intake (kcal/day). The multivariate-
180 adjusted model (Model 2) was used to evaluate the HR of incident SCD. In the analyses of the
181 PUFAs and hair mercury with the risk of SCD, the model was further adjusted for QTc or JTc.
182 Statistical significance of the interactions on a multiplicative scale was assessed by stratified
183 analysis with hair mercury divided by the median and likelihood ratio tests with a cross-product
184 term.
185 Cohort mean was used to replace missing values in covariates (<0.5%). Tests of linear trend
186 across categories were conducted by assigning the median values for each category of exposure
187 variable and treating those as a single continuous variable. All *P*-values were two-sided
188 ($\alpha$=0.05). Data were analyzed using the SPSS software version 21 for windows (Armonk, NY:
189 IBM Corp.).
190

# Results

## Baseline characteristics

Baseline characteristics of the participants are presented in Table 1. Men with higher serum EPA+DPA+DHA concentration were more likely to be older and have a higher education, annual income, body mass index, leisure time physical activity, serum 25-hydroxyvitamin D, serum HDL cholesterol and hair mercury concentrations, and alcohol intake. They also had lower serum total and LDL cholesterol and CRP concentrations and lower total energy intake and were less likely to use beta-blockers. The mean±SD serum concentrations, as a percentage of all serum fatty acids, was 4.70±1.61% for EPA+DPA+DHA, 2.64±0.74% for DHA, 1.69±0.92% for EPA and 0.55±0.10% for DPA. The correlations between the individual long-chain omega-3 PUFA were 0.70 for EPA and DHA, 0.56 for EPA and DPA, and 0.41 for DHA and DPA.

## Association of the serum long-chain omega-3 PUFA with QTc and JTc

After adjustment for age and examination year (Model 1), higher serum EPA+DPA+DHA concentration was inversely associated with the QTc and JTc (the mean difference between extreme quartiles was 3.2 ms (95% CI -0.1 – 6.4 ms, *P*-trend across quartiles=0.03) for QTc and 4.4 ms (95% CI 1.1 – 7.7 ms, *P*-trend across quartiles=0.006) for JTc, Table 2). Further multivariate adjustments had little impact on the associations (Model 2). Additional adjustment for hair mercury content slightly attenuated the associations. For example, the mean difference between extreme quartiles of EPA+DPA+DHA was 2.5 ms (95% CI -0.8 – 5.9 ms, *P*-trend=0.08) for QTc and 3.7 ms (95% CI 0.3 – 7.2 ms, *P*-trend=0.02) for JTc (Model 2, other data not shown).

Prolonged QTc and JTc were found in 104 (7.4%) and 101 (7.2%) of the 1411 men, respectively. After multivariate-adjustments, the odds for prolonged QTc was 46% lower (95% CI -2 – 72%, *P*-trend across quartiles=0.04) and the odds for prolonged JTc was 43% lower (95% CI -6 – 69%, *P*-trend across quartile=0.08) in the highest vs. the lowest serum EPA+DPA+DHA quartile (Model 2, Table 3). When the fatty acids were investigated individually, generally similar inverse associations with the QTc and JTc were observed with EPA, DPA and DHA (Tables 2&3). When evaluated continuously, each 0.5 percentage unit increase in EPA+DPA+DHA, EPA, DPA and DHA was associated with 9.4% (95% CI 1.0 – 16.1%), 15.2% (95% CI 1.8 – 26.7%), 68.7% (95% CI 8.8 – 89.3%), and 13.4% (95% CI 0.3 – 26.0%) lower odds for prolonged QTc and 9.4% (95% CI 2.1 – 16.2%), 16.3% (95% CI 3.3 – 27.5%), 64.6% (95% CI -1.7 – 87.7%), and 16.4% (95% CI 1.9 – 28.7%) lower odds for prolonged JTc, respectively. Further adjustment for hair mercury had no appreciable impact on the associations. For example, in the highest vs. lowest EPA+DPA+DHA quartile the OR for prolonged QTc was 0.52 (95% CI 0.27 – 1.01, *P*-trend=0.04) and for prolonged JTc 0.57 (95% CI 0.29 – 1.08, *P*-trend=0.08) (other data not shown).

**Association of hair mercury with QTc and JTc**

The mean±SD hair mercury concentration was 1.9±2.0 μg/g. Hair mercury concentration was not statistically significantly associated with the QTc and JTc (Tables 2&3). Further adjustment for serum long-chain omega-3 PUFA did not materially alter the results [extreme quartile difference 0.7 ms for QTc (95% CI -2.8 – 4.2 ms, *P*-trend=0.50) and 1.3 ms for JTc (95% CI -2.3 – 4.9 ms, *P*-trend=0.34). We did not find evidence that hair mercury concentration would modify the

associations between the serum long-chain omega-3 PUFA, QTc and JTc, either (*P* for interactions >0.26).

**Risk of sudden cardiac death**

During the mean follow-up of 22.9 years, 85 SCD events occurred (6.0% of the men). Serum EPA+DPA+DHA was associated with a lower risk of SCD [multivariate-adjusted extreme tertile HR=0.50 (95% CI 0.29 to 0.86; *P*-trend=0.02)] (Online Resource 1). Similar association was observed with DHA, but the associations with EPA and DPA were weaker and not statistically significant. However, further adjustment for QTc or JTc had no impact on the associations (Online Resource 1). Hair mercury was not associated with the risk of SCD (Online Resource 1)

After adjustment for age and examination year, both the QTc and JTc were associated with a higher risk of SCD [HR=2.18 (95% CI 1.26 to 3.78; *P*-trend=0.026) in the highest vs. the lowest tertile of QTc] and [HR=1.89 (95% CI 1.11 to 3.21; *P*-trend=0.017) in the highest vs. the lowest tertile of JTc] (Online Resource 2). Further adjustments for potential confounders attenuated the associations and they were no longer statistically significant.

## Discussion

In this study among 1411 middle-aged and older men free of CHD from Eastern Finland, we found that the serum long-chain omega-3 PUFA were inversely associated with QTc and JTc. However, adjusting for QTc or JTc did not attenuate the inverse associations between the long-chain n-3 PUFA and risk of SCD. Hair mercury concentration was not associated with the QTc, JTc or risk of SCD; however, it slightly attenuated the associations of the long-chain omega-3 PUFA with QTc and JTc.

To our knowledge, only one small study has evaluated the association of the circulating long-chain omega-3 PUFA with the QTc in human subjects [11]. In that study higher concentration of the long-chain omega-3 PUFA was not associated with the QTc duration among 53 healthy men and women with the mean age of 31 years. In contrast, Billman et al. [8] found that infusion of 1.0 g to 10 g free long-chain omega-3 PUFA to 13 dogs resulted in shortening of the QTc. Among 5096 men and women aged ≥65 years from the Cardiovascular Health Study, higher intake of fish and long-chain omega-3 PUFA was associated with a significantly lower likelihood of prolonged QTc [9]. Similarly, Chrysohoou et al. [10] found that, among 3042 men and women aged 18-89 years, those who consumed more than 300 g fish/week had, on average, 14% lower QTc. In contrast, no effect on the QTc was found in a small randomized controlled trial by Geelen et al. [12] among 42 healthy middle-aged men and women after 12-week supplementation with 3.5 g/d of fish oil. However, the small size of the trial makes it difficult to draw conclusions on the effectiveness of fish oil supplementation in lowering of the QTc. To the best of our knowledge, this is the first study evaluated the association between the long-chain omega-3 PUFA and JT-interval.

A possible mechanism underlying the inverse association between the serum long-chain omega-3 PUFA and QTc and JTc may be explained by the impact of the long-chain omega-3 PUFA on the ventricular repolarization process. Ventricular repolarization is a complicated process, which is attributed by membrane ion channel activity, cellular ion concentration, and autonomic tone [29]. Beneficial impact of the long-chain omega-3 PUFA on the ventricular repolarization may result from the role of these fatty acids in the function of ion channels in heart cell membranes, such as reduction in the activity of membrane sodium channels and modulation of the activity of membrane L-type calcium channel, which are essential for heart rhythm [30].

The serum long-chain omega-3 PUFAs could also be a marker for some other compounds in fish, such as selenium or vitamin D. However, a recent study did not find an association between vitamin D and QT interval duration [31], and in our study there was no difference in serum selenium concentrations in the quartiles of the serum long-chain omega-3 PUFA, suggesting that these compounds would not explain the associations with the fatty acids.

In the current study we found that higher serum long-chain omega-3 PUFA concentration was inversely associated with the risk of SCD. This finding is consistent with the previous finding in KIHD [13]. We also observed suggestive associations between the duration of QTc and JTc and the risk of SCD, which supports the previous findings that indicated that abnormally prolonged QTc may be a risk factor of SCD [6, 7]. However, adjustments for QTc or JTc did not attenuate the associations between the serum long-chain omega-3 PUFA concentrations and risk of SCD. This suggests that the inverse associations of the long-chain omega-3 PUFA with the risk of SCD are not explained by their inverse associations with the QTc and JTc. One possible explanation is that the impact of the long-chain omega-3 PUFA on QTc and JTc among these generally healthy men is not strong enough for it to attenuate the associations with the risk of SCD.

We have previously found that higher hair mercury concentration, reflecting long-term exposure to mercury, was associated with a higher risk of CVD, including coronary heart disease mortality and SCD, in the KIHD cohort [14, 23]. In those studies, the inverse associations of the long-chain omega-3 PUFA with the CVD outcomes were also stronger after adjusting for hair mercury In the current study we did not find such associations, but instead adjustment for mercury slightly attenuated the associations with the long-chain omega-3 PUFA. This might be explained by the inverse, although not statistically significant, association between hair mercury

and QTc and JTc. Although there is some evidence that mercury exposure can affect heart rate variability [16], our findings do not support an adverse impact on the QTc and JTc.

The strengths of our study include the use of serum long-chain omega-3 PUFA and hair mercury instead of dietary intakes, both established biomarkers for intake [16, 32]. Because serum fatty acids and hair mercury are objective biomarkers for exposure, their use reduced the bias by misclassification, which would reduce the associations towards the null. Other strengths include the extensive examination of potential confounders and a rather large study population with data on ECG parameters. Correcting the QT and JT intervals for heart rate and excluding participants with prolonged QRS complex likely reduced the inter-person variability, which improved the sensitivity to find associations between the fatty acids and hair mercury and ECG parameters. A potential limitation was that the participants were middle-aged and older men from Eastern Finland, so the findings may not be generalizable to other populations or to women. Although the analytical variabilities in the serum fatty acid measurements (CV %) in our study were similar to or lower than what has been reported in other studies [32], such variability could attenuate the true associations between the fatty acids and outcomes. In the analyses with incident SCD, the long-follow up may have attenuated the associations, which were based only on single exposure assessment at baseline. Despite the long follow-up, we had a limited number of incident SCD events, which limited the power to find statistically significant associations with SCD risk. Also, because of the observational study design, conclusions about causality cannot be drawn.

In conclusion, higher circulating concentrations of the long-chain omega-3 PUFA, mainly a marker of fish consumption in this study population, were inversely associated with QTc and JTc in middle-aged and older men from eastern Finland, whereas mercury exposure had no

association. However, because adjustment for QTc or JTc did not attenuate the associations of the long-chain omega-3 PUFAs with the risk of SCD, our results suggest that the inverse association of the long-chain omega-3 PUFA with the SCD risk is not explained by the prevention of prolonged ventricular repolarization in this study population. Further studies in diverse study populations are needed to elucidate the impact of the long-chain omega-3 PUFA on ventricular repolarization and to investigate other potential mechanisms, which could explain the inverse association of these fatty acids with the risk of SCD.

**Acknowledgments** The study was supported by the University of Eastern Finland.

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

1. Mozaffarian D, Wu JH (2011) Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. J Am Coll Cardiol 58:2047-2067

2. Billman GE (2013) The effects of omega-3 polyunsaturated fatty acids on cardiac rhythm: a critical reassessment. Pharmacol Ther 140:53-80

3. Rautaharju PM, Surawicz B, Gettes LS (2009) AHA/ACCF/HRS recommendations for the standardization and interpretation of the electrocardiogram: part IV: the ST segment, T and U waves, and the QT interval a scientific statement from the American Heart Association Electrocardiography and Arrhythmias Committee, Council on Clinical Cardiology; the American College of Cardiology Foundation; and the Heart Rhythm Society Endorsed by the International Society for Computerized Electrocardiology. J Am Coll Cardiol 53:982-991

4. Crow RS, Hannan PJ, Folsom AR (2003) Prognostic significance of corrected QT and corrected JT interval for incident coronary heart disease in a general population sample stratified by presence or absence of wide QRS complex: the ARIC Study with 13 years of follow-up. Circulation 108:1985-1989

5. Rautaharju PM, Zhang Z, Prineas R, Heiss G (2004) Assessment of prolonged QT and JT intervals in ventricular conduction defects. Am J Cardiol 93:1017-1021

6. Morita H, Wu J, Zipes DP (2008) The QT syndromes: long and short. The Lancet 372:750-763

7. De Bruyne M, Hoes A, Kors J, Hofman A, Van Bemmel J, Grobbee D (1999) Prolonged QT interval predicts cardiac and all-cause mortality in the elderly. Eur Heart J 20:278-284

8. Billman GE, Kang JX, Leaf A (1997) Prevention of ischemia-induced cardiac sudden death by n− 3 polyunsaturated fatty acids in dogs. Lipids 32:1161-1168

9. Mozaffarian D, Prineas RJ, Stein PK, Siscovick DS (2006) Dietary fish and n-3 fatty acid intake and cardiac electrocardiographic parameters in humans. J Am Coll Cardiol 48:478-484

10. Chrysohoou C, Panagiotakos DB, Pitsavos C, Skoumas J, Krinos X, Chloptsios Y, Nikolaou V, Stefanadis C (2007) Long-term fish consumption is associated with protection against arrhythmia in healthy persons in a Mediterranean region--the ATTICA study. Am J Clin Nutr 85:1385-1391

11. Brouwer IA, Zock PL, van Amelsvoort LG, Katan MB, Schouten EG (2002) Association between n-3 fatty acid status in blood and electrocardiographic predictors of arrhythmia risk in healthy volunteers. Am J Cardiol 89:629-631

12. Geelen A, Brouwer IA, Zock PL, Kors JA, Swenne CA, Katan MB, Schouten EG (2002) (N-3) fatty acids do not affect electrocardiographic characteristics of healthy men and women. J Nutr 132:3051-3054

13. Virtanen JK, Laukkanen JA, Mursu J, Voutilainen S, Tuomainen T (2012) Serum long-chain n-3 polyunsaturated fatty acids, mercury, and risk of sudden cardiac death in men: a prospective population-based study. PloS One 7:e41046

14. Virtanen JK, Voutilainen S, Rissanen TH, Mursu J, Tuomainen TP, Korhonen MJ, Valkonen VP, Seppanen K, Laukkanen JA, Salonen JT (2005) Mercury, fish oils, and risk of acute coronary events and cardiovascular disease, coronary heart disease, and all-cause mortality in men in eastern Finland. Arterioscler Thromb Vasc Biol 25:228-233

15. Wennberg M, Bergdahl IA, Hallmans G, Norberg M, Lundh T, Skerfving S, Stromberg U, Vessby B, Jansson JH (2011) Fish consumption and myocardial infarction: a second prospective biomarker study from northern Sweden. Am J Clin Nutr 93:27-36

16. Roman HA, Walsh TL, Coull BA, Dewailly E, Guallar E, Hattis D, Marien K, Schwartz J, Stern AH, Virtanen JK, Rice G (2011) Evaluation of the cardiovascular effects of methylmercury exposures: current evidence supports development of a dose-response function for regulatory benefits analysis. Environ Health Perspect 119:607-614

17. Houston MC (2011) Role of mercury toxicity in hypertension, cardiovascular disease, and stroke. The Journal of Clinical Hypertension 13:621-627

18. Salonen JT (1988) Is there a continuing need for longitudinal epidemiologic research? The Kuopio Ischaemic Heart Disease Risk Factor Study. Ann Clin Res 20:46-50

19. Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Seppanen R, Salonen R (1992) High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. Circulation 86:803-811

20. Lakka TA, Venalainen JM, Rauramaa R, Salonen R, Tuomilehto J, Salonen JT (1994) Relation of leisure-time physical activity and cardiorespiratory fitness to the risk of acute myocardial infarction in men. N Engl J Med 330:1549-1554

21. Voutilainen S, Rissanen TH, Virtanen J, Lakka TA, Salonen JT, Kuopio Ischemic Heart Disease Risk Factor Study (2001) Low dietary folate intake is associated with an excess incidence of acute coronary events: The Kuopio Ischemic Heart Disease Risk Factor Study. Circulation 103:2674-2680

22. Laaksonen D, Lakka T, Lakka H, Nyyssönen K, Rissanen T, Niskanen L, Salonen J (2002) Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men. Diabetic Med 19:456-464

23. Salonen JT, Seppanen K, Nyyssonen K, Korpela H, Kauhanen J, Kantola M, Tuomilehto J, Esterbauer H, Tatzber F, Salonen R (1995) Intake of mercury from fish, lipid peroxidation, and

406 the risk of myocardial infarction and coronary, cardiovascular, and any death in eastern Finnish
407 men. Circulation 91:645-655
408 24. Furberg CD, Manolio TA, Psaty BM, Bild DE, Borhani NO, Newman A, Tabatznik B,
409 Rautaharju PM, Cardiovascular Health Study Collaborative Research Group (1992) Major
410 electrocardiographic abnormalities in persons aged 65 years and older (the Cardiovascular
411 Health Study). Am J Cardiol 69:1329-1335
412 25. Statters DJ, Malik M, Ward DE, CAMM A (1994) QT dispersion: problems of
413 methodology and clinical significance. J Cardiovasc Electrophysiol 5:672-685
414 26. Ahnve S (1985) Correction of the QT interval for heart rate: review of different formulas
415 and the use of Bazett's formula in myocardial infarction. Am Heart J 109:568-574
416 27. Butrous GS, Schwartz PJ (1989) Clinical aspects of ventricular repolarization. Farrand
417 Press, London, UK, 1989
418 28. Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas AM, Pajak A (1994)
419 Myocardial infarction and coronary deaths in the World Health Organization MONICA Project.
420 Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries
421 in four continents. Circulation 90:583-612
422 29. Guideline IHT (2005) The non-clinical evaluation of the potential for delayed ventricular
423 repolarization (Qt Interval Prolongation) by human pharmaceuticals. S7B
424 (http://www.ich.org/products/guidelines/safety/article/safety-guidelines.html). Accessed 6
425 December 2015
426 30. Endo J, Arita M (2016) Cardioprotective mechanism of omega-3 polyunsaturated fatty
427 acids. J Cardiol 67:22-7

31. Zhang Y, Post WS, Dalal D, Bansal S, Blasco-Colmenares E, Jan De Beur S, Alonso A, Soliman EZ, Whitsel EA, Brugada R (2011) Serum 25-hydroxyvitamin D, calcium, phosphorus, and electrocardiographic QT interval duration: findings from NHANES III and ARIC. J Clin Endocrinol Metab 96:1873-1882

32. Hodson L, Skeaff CM, Fielding BA (2008) Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Prog Lipid Res 47:348-380

**Table 1. Baseline characteristics according to quartiles of serum total long-chain omega-3 polyunsaturated fatty acids**

| Variables | Serum total long-chain omega-3 polyunsaturated fatty acids quartile | | | | *P* for trend |
|---|---|---|---|---|---|
| | Q1 (n=352) | Q2 (n=353) | Q3 (n=353) | Q4 (n=353) | |
| Age (years) | 51.8 (5.5)[a] | 51.6 (5.4) | 52.0 (5.4) | 52.5 (5.2) | 0.04 |
| Education (years) | 8.9 (3.3) | 8.9 (3.5) | 9.0 (3.6) | 9.6 (4.0) | 0.01 |
| Income (euro) | 12,923 (6,821) | 14,088 (9,256) | 14,809 (10.830) | 15,351 (9,848) | 0.001 |
| Body mass index (kg/m2) | 26.6 (3.5) | 26.5 (3.4) | 26.9 (3.6) | 27.0 (3.4) | 0.05 |
| Current smoker (%) | 32.7 | 29.7 | 31.7 | 28.3 | 0.29 |
| Leisure-time physical activity (kcal/day) | 127 (161) | 129 (157) | 132 (144) | 157 (212) | 0.01 |
| C-reactive protein (mg/L) | 2.83 (6.99) | 1.82 (2.65) | 2.16 (3.25) | 1.74 (2.53) | 0.01 |
| Serum triglycerides (mmol/L) | 1.52 (0.94) | 1.29 (0.83) | 1.17 (0.53) | 1.04 (0.49) | <0.001 |
| Serum HDL cholesterol (mmol/L) | 1.20 (0.27) | 1.28 (0.27) | 1.33 (0.28) | 1.35 (0.32) | <0.001 |
| Serum LDL cholesterol (mmol/L) | 3.82 (0.95) | 3.98 (0.95) | 4.15 (1.02) | 4.12 (0.99) | <0.001 |
| Blood glucose (mmol/L) | 4.80 (1.28) | 4.62 (0.84) | 4.72 (0.85) | 4.73 (0.94) | 0.86 |
| Systolic blood pressure (mm Hg) | 135 (17) | 133 (15) | 134 (15) | 134 (17) | 0.42 |
| Diastolic blood pressure (mm Hg) | 90 (11) | 89 (10) | 90 (10) | 89 (10) | 0.45 |
| Energy intake (kcal/d) | 2468 (686) | 2446 (582) | 2390 (631) | 2297 (583) | <0.001 |
| Alcohol intake (g/d) | 53 (85) | 64 (104) | 88 (142) | 84 (115) | <0.001 |

| | | | | | |
|---|---|---|---|---|---|
| Diabetes (%) | 5.1 | 2.5 | 5.4 | 4.8 | 0.67 |
| Hypertension (%) | 58.2 | 52.7 | 57.2 | 53.5 | 0.41 |
| Lipid-lowering medication during follow-up (%) | 45.7 | 46.2 | 43.9 | 48.2 | 0.56 |
| Hypertension medication during follow-up (%) | 79.0 | 74.5 | 74.8 | 78.5 | 0.87 |
| Serum 25-hydroxyvitamin D (nmol/L) | 37.6 (18.9) | 39.6 (18.8) | 43.5 (17.5) | 49.3 (17.2) | <0.001 |
| Serum selenium (µg/L) | 104.2 (22.9) | 106.7 (22.9) | 103.9 (22.8) | 105.5 (19.9) | 0.84 |
| Serum EPA (% of all serum fatty acids) | 0.96 (0.25) | 1.27 (0.23) | 1.71 (0.30) | 2.80 (1.11) | <0.001 |
| Serum DPA (% of all serum fatty acids) | 0.48 (0.08) | 0.53 (0.07) | 0.56 (0.78) | 0.65 (0.10) | <0.001 |
| Serum DHA (% of all serum fatty acids) | 1.72 (0.29) | 2.20 (0.26) | 2.55 (0.29) | 3.38 (0.68) | <0.001 |
| Hair mercury (µg/g) | 1.15 (1.29) | 1.47 (1.60) | 2.15 (2.03) | 2.64 (2.40) | <0.001 |

[a] Results are means (SD) for continuous variables and percentages for categorical data.

EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; HDL, high density lipoprotein; LDL, low density lipoprotein; Q, quartile.

435

**Table 2. Mean QTc and JTc intervals in quartiles of serum long-chain omega-3 polyunsaturated fatty acids and hair mercury**

| | Exposure quartile | | | | | |
|---|---|---|---|---|---|---|
| | 1 (n =352) | 2 (n=353) | 3 (n =353) | 4 (n =353) | *P* for trend | Mean difference (95% confidence interval) (ms) |
| *EPA+DPA+DHA (%)* | <3.64 | 3.64 – 4.35 | 4.36 – 5.37 | >5.37 | | |
| Mean QTc interval (ms), model 1 | 417 (415 – 419)[a] | 417 (415 – 419) | 416 (413 – 418) | 414 (412 – 416) | 0.03 | 3.2 (-0.1 – 6.4) |
| Mean QTc interval (ms), model 2 | 417 (415 – 419) | 417 (415 – 420) | 415 (413 – 417) | 414 (412 – 416) | 0.02 | 3.2 (0.003 – 6.4) |
| Mean JTc interval (ms), model 1 | 316 (313 – 318) | 315 (313 –317) | 314 (312 – 317) | 311 (309 – 314) | 0.006 | 4.4 (1.1 – 7.7) |
| Mean JTc interval (ms), model 2 | 316 (313 – 318) | 316 (313 – 318) | 314 (312 – 316) | 311 (309 – 313) | 0.002 | 4.6 (1.3 – 7.8) |
| *EPA (%)* | <1.12 | 1.12 – 1.48 | 1.49 – 2.01 | >2.01 | | |
| Mean QTc interval (ms), model 1 | 418 (416 – 420) | 417 (415 – 419) | 414 (411 – 416) | 415 (413 – 417) | 0.04 | 3.1 (-0.2 – 6.3) |
| Mean QTc interval (ms), model 2 | 418 (416 – 420) | 417 (415 – 420) | 414 (412 – 416) | 415 (412 – 417) | 0.02 | 3.5 (0.3 – 6.7) |
| Mean JTc interval (ms), model 1 | 316 (314 – 319) | 315 (313 – 317) | 313 (310 – 315) | 312 (310 – 315) | 0.01 | 4.1 (0.8 – 7.4) |
| Mean JTc interval (ms), model 2 | 316 (314 – 319) | 315 (313 – 318) | 312 (310 – 315) | 312 (310 – 314) | 0.003 | 4.6 (1.3 – 7.9) |
| *DPA (%)* | <0.48 | 0.48 – 0.54 | 0.55 – 0.61 | >0.61 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Mean QTc interval (ms), model 1 | 420 (417 – 422) | 415 (413 – 417) | 414 (412 – 417) | 415 (413 – 417) | 0.01 | 4.5 (1.3 – 7.7) |
| Mean QTc interval (ms), model 2 | 418 (416 – 421) | 415 (413 – 417) | 415 (412 – 417) | 416 (414 – 418) | 0.15 | 2.6 (-0.6 – 5.8) |
| Mean JTc interval (ms), model 1 | 319 (317 – 321) | 313 (311 – 316) | 312 (310 – 314) | 312 (310 – 314) | <0.001 | 6.7 (3.5 – 10.0) |
| Mean JTc interval (ms), model 2 | 318 (315 – 320) | 313 (311 – 316) | 312 (310 – 314) | 313 (311 – 315) | 0.006 | 4.8 (1.5 – 8.1) |
| | | | | | | |
| *DHA (%)* | <1.96 | 1.96 – 2.37 | 2.38 – 2.83 | >2.83 | | |
| Mean QTc interval (ms), model 1 | 417 (414 – 419) | 417 (414 – 419) | 416 (414 – 419) | 414 (412 – 416) | 0.09 | 2.6 (-0.6 – 5.8) |
| Mean QTc interval (ms), model 2 | 417 (415 – 419) | 417 (415 – 419) | 416 (414 – 418) | 414 (412 – 416) | 0.05 | 3.0 (-0.2 – 6.3) |
| Mean JTc interval (ms), model 1 | 315 (313 – 317) | 315 (313 – 317) | 315 (312 – 317) | 311 (309 – 314) | 0.02 | 3.6 (0.3 – 6.9) |
| Mean JTc interval (ms), model 2 | 315 (313 – 318) | 315 (313 – 318) | 314 (312 – 317) | 311 (309 –313) | 0.008 | 4.2 (0.9 – 7.5) |
| | | | | | | |
| *Hair mercury (μg/g)* | <0.61 | 0.61 – 1.22 | 1.23 – 2.41 | >2.41 | | |
| Mean QTc interval (ms), model 1 | 416 (414 – 418) | 417 (414 – 419) | 415 (413 – 418) | 416 (413 – 418) | 0.71 | 0.3 (-3.0 – 3.7) |
| Mean QTc interval (ms), model 2 | 416 (414 – 419) | 417 (415 – 419) | 416 (414 – 418) | 415 (412 – 417) | 0.18 | 1.9 (-1.4 – 5.2) |
| Mean JTc interval (ms), model 1 | 315 (312 – 317) | 315 (313 – 317) | 313 (311 – 315) | 313 (311 – 316) | 0.37 | 1.2 (-2.2 – 4.7) |
| Mean JTc interval (ms), model 2 | 315 (313 – 317) | 315 (313 – 317) | 313 (311 – 316) | 312 (310 – 315) | 0.09 | 2.8 (-0.6 – 6.1) |

[a] Values are means (95% confidence interval).

Model 1 adjusted for age and examination year.

Model 2 adjusted for model 1 plus body mass index, diabetes, smoking, leisure-time physical activity, education, income, treated hypertension, and intakes of energy and alcohol.

436

**Table 3. Odds ratios for prolonged QTc and JTc intervals in quartiles of serum long-chain omega-3 polyunsaturated fatty acids and hair mercury**

| | Exposure quartile | | | | *P* for trend |
|---|---|---|---|---|---|
| | 1 (n =352) | 2 (n=353) | 3 (n =353) | 4 (n =353) | |
| *EPA+DPA+DHA (%)* | <3.64 | 3.64 – 4.35 | 4.36 – 5.37 | >5.37 | |
| N of cases (%) | 27 (7.7) | 28 (7.9) | 31 (8.8) | 18 (5.1) | |
| OR for prolonged QTc, Model 1 | 1(reference group) | 1.04 (0.60 – 1.81)[a] | 1.10 (0.64 – 1.89) | 0.70 (0.32 – 1.13) | 0.11 |
| OR for prolonged QTc, Model 2 | 1(reference group) | 1.07 (0.61 – 1.88) | 0.99 (0.57 – 1.73) | 0.54 (0.28 – 1.02) | 0.04 |
| N of cases (%) | 29 (8.2) | 25 (7.1) | 28 (7.9) | 19 (5.4) | |
| OR for prolonged JTc, Model 1 | 1(reference group) | 0.86 (0.49 – 1.50) | 0.97 (0.56 – 1.67) | 0.62 (0.34 – 1.13) | 0.15 |
| OR for prolonged JTc, Model 2 | 1(reference group) | 0.91 (0.52 – 1.61) | 0.90 (0.51 – 1.58) | 0.57 (0.31 – 1.06) | 0.08 |
| *EPA (%)* | <1.12 | 1.12 – 1.48 | 1.49 – 2.01 | >2.01 | |
| N of cases (%) | 33 (9.4) | 26 (7.4) | 21 (5.9) | 24 (6.8) | |
| OR for prolonged QTc, Model 1 | 1(reference group) | 0.71 (0.41 – 1.23) | 0.54 (0.30 – 0.96) | 0.59 (0.33 – 1.03) | 0.08 |
| OR for prolonged QTc, Model 2 | 1(reference group) | 0.76 (0.44 – 1.31) | 0.52 (0.29 – 0.95) | 0.52 (0.29 – 0.93) | 0.03 |

| | | | | | |
|---|---|---|---|---|---|
| N of cases (%) | 34 (9.7) | 24 (6.8) | 24 (6.8) | 19 (5.4) | |
| OR for prolonged JTc, Model 1 | 1(reference group) | 0.68 (0.39 – 1.18) | 0.67 (0.39 – 1.17) | 0.52 (0.29 – 0.94) | 0.05 |
| OR for prolonged JTc, Model 2 | 1(reference group) | 0.71 (0.40 – 1.23) | 0.66 (0.37 – 1.16) | 0.46 (0.25 – 0.84) | 0.02 |
| *DPA (%)* | <0.48 | 0.48 – 0.54 | 0.55 – 0.61 | >0.61 | |
| N of cases (%) | 38 (10.8) | 27 (7.6) | 18 (5.1) | 21 (5.9) | |
| OR for prolonged QTc, Model 1 | 1(reference group) | 0.72 (0.43 – 1.20) | 0.47 (0.26 – 0.85) | 0.55 (0.31 – 0.96) | 0.02 |
| OR for prolonged QTc, Model 2 | 1(reference group) | 0.80 (0.47 – 1.37) | 0.55 (0.30 – 0.99) | 0.64 (0.36 – 1.15) | 0.07 |
| N of cases (%) | 37 (10.5) | 26 (7.4) | 21 (5.9) | 17 (4.8) | |
| OR for prolonged JTc, Model 1 | 1(reference group) | 0.69 (0.40 – 1.16) | 0.56 (0.32 – 0.98) | 0.44 (0.24 – 0.81) | 0.01 |
| OR for prolonged JTc, Model 2 | 1(reference group) | 0.77 (0.45 – 1.32) | 0.66 (0.37 – 1.17) | 0.52 (0.28 – 0.96) | 0.03 |
| *DHA (%)* | <1.96 | 1.96 – 2.37 | 2.38 – 2.83 | >2.83 | |
| N of cases (%) | 24 (6.8) | 32 (9.1) | 33 (9.3) | 15 (4.2) | |
| OR for prolonged QTc, Model 1 | 1(reference group) | 1.34 (0.77 – 2.32) | 1.38 (0.79 – 2.38) | 0.61 (0.31 – 1.19) | 0.14 |
| OR for prolonged QTc, Model 2 | 1(reference group) | 1.23 (0.70 – 2.17) | 1.16 (0.66 – 2.04) | 0.51 (0.25 – 1.01) | 0.04 |
| N of cases (%) | 27 (7.7) | 28 (7.9) | 26 (7.4) | 20 (5.7) | |
| OR for prolonged JTc, Model 1 | 1(reference group) | 1.02 (0.59 – 1.77) | 0.94 (0.54 – 1.65) | 0.70 (0.38 – 1.27) | 0.11 |

| | | | | | |
|---|---|---|---|---|---|
| OR for prolonged JTc, Model 2 | 1(reference group) | 0.95 (0.54 – 1.67) | 0.82 (0.46 – 1.47) | 0.61 (0.32 – 1.14) | 0.10 |
| *Hair mercury (μg/g)* | <0.61 | 0.61 – 1.22 | 1.23 – 2.41 | >2.41 | |
| N of cases (%) | 24 (6.8) | 25 (7.1) | 22 (6.2) | 9.4 (33) | |
| OR for prolonged QTc, Model 1 | 1(reference group) | 0.99 (0.55 – 1.78) | 0.78 (0.43 – 1.44) | 1.13 (0.64 – 2.00) | 0.56 |
| OR for prolonged QTc, Model 2 | 1(reference group) | 0.95 (0.53 – 1.72) | 0.72 (0.39 – 1.35) | 0.93 (0.52 – 1.68) | 0.92 |
| N of cases (%) | 23 (6.5) | 27 (7.7) | 22 (6.2) | 29 (8.2) | |
| OR for prolonged JTc, Model 1 | 1(reference group) | 1.19 (0.66 – 2.12) | 0.91 (0.49 – 1.68) | 1.20 (0.66 – 2.16) | 0.65 |
| OR for prolonged JTc, Model 2 | 1(reference group) | 1.15 (0.64 – 2.08) | 0.90 (0.48 – 1.69) | 1.04 (0.57 – 1.91) | 0.97 |

[a] Values are odds ratios (95% confidence interval).

Model 1 adjusted for age and examination year.

Model 2 adjusted for model 1 plus body mass index, diabetes, smoking, leisure-time physical activity, education, income, treated hypertension, and intakes of energy and alcohol.

437