Fish-derived long-chain omega-3 polyunsaturated fatty acids and methylmercury are associated with risk of cardiovascular disease. However, the mechanism underlying these associations are not completely known. In this doctoral thesis, higher concentrations of the serum long-chain omega-3 polyunsaturated fatty acids were found to have beneficial associations with cardiac functions. However, methylmercury diminished these benefits. These findings reveal potential new mechanisms whereby intake of fish, especially fish that have high content of the long-chain omega-3 polyunsaturated fatty acids but low methylmercury content, may improve cardiac health.
SERUM LONG-CHAIN OMEGA-3 POLYUNSATURATED FATTY ACIDS, METHYLMERCURY AND CARDIAC FUNCTIONS

A POPULATION-BASED COHORT STUDY
Behnam Tajik

SERUM LONG-CHAIN OMEGA-3 POLYUNSATURATED FATTY ACIDS, METHYLMERCURY AND CARDIAC FUNCTIONS

A POPULATION-BASED COHORT

To be presented by permission of the
Faculty of Health Sciences, University of Eastern Finland
for public examination in Canthia auditorium CA100, Kuopio,
on Friday, February 7th 2020, at 12 o’clock noon

Publications of the University of Eastern Finland
Dissertations in Health Sciences
No 552

Institute of Public Health and Clinical Nutrition, School of Medicine,
Faculty of Health Sciences
University of Eastern Finland
Kuopio 2020
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Distributor:
University of Eastern Finland
Kuopio Campus Library
P.O.Box 1627
FI-70211 Kuopio, Finland
www.uef.fi/kirjasto

Grano Oy 2020

ISBN: 978-952-61-3307-2 (PDF)
ISSNL: 1798-5706
ISSN: 1798-5706
ISSN: 1798-5714 (PDF)
Author’s address:  Institute of Public Health and Clinical Nutrition, University of Eastern Finland
               KUOPIO
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“All our knowledge begins with the senses, proceeds then to the understanding, and ends with reason. There is nothing higher than reason.”

Immanuel Kant
ABSTRACT

Convincing evidence has emerged from epidemiological studies indicating that the intake of fish and long-chain omega-3 polyunsaturated fatty acids (PUFAs) from fish is associated with a reduced risk of cardiovascular disease (CVD). Nonetheless, fish may also contain methylmercury, which is associated with the risk of CVD and it may attenuate the cardioprotective effects of long-chain omega-3 PUFAs. However, the mechanisms underlying these associations are not fully understood.

The aims of this thesis were to explore the associations of the serum long-chain omega-3 PUFAs and hair mercury concentrations, objective biomarkers of fish intake, with parameters of cardiac electrophysiology and performance, specifically QT- and JT-intervals (Study I), exercise cardiac power and its components (VO₂max and maximal systolic blood pressure during exercise) (Study II), resting heart rate, peak heart rate during exercise and heart rate recovery after exercise (Study III), and exercise-induced myocardial ischemia (Study IV), among older men from the Kuopio Ischaemic Heart Disease Risk Factor Study.

Serum long-chain omega-3 PUFA concentrations were inversely associated with QTc, JTh; however, a higher hair mercury concentration slightly attenuated the associations of the long-chain omega-3 PUFA with QTc and JTh (Study I). Moreover, higher serum long-chain omega-3 PUFA concentrations were associated with higher exercise cardiac power and VO₂max, but not with maximal systolic blood pressure during exercise. A higher hair mercury concentration modestly attenuated the associations of the long-chain omega-3 PUFA with VO₂max and exercise cardiac power (Study II). Higher serum long-chain omega-3 PUFA concentrations were associated with lower resting heart rates. No associations were observed with peak heart rate during exercise or heart rate recovery after exercise. A higher hair mercury concentration was associated with a lower peak heart rate and it also slightly attenuated the associations of the serum long-chain omega-3 PUFAs (Study III),
Finally, the occurrence of exercise-induced myocardial ischemia was lower among men with higher concentrations of the long-chain omega-3 PUFA. This association was mainly observed among those individuals with a history of coronary heart disease. A higher hair mercury concentration was associated with a higher occurrence of exercise-induced myocardial ischemia (*Study IV*).

In summary, higher serum long-chain omega-3 PUFA concentrations, mainly considered as a marker of fish intake in this study population, were beneficially associated with some parameters of cardiac electrophysiology and performance, which may point to some potential mechanisms for the cardioprotective effects of the long-chain omega-3 PUFAs. However, methylmercury may diminish this effect. Nonetheless, based on our findings, regular consumption of lean predatory fish that are high in mercury and low in long-chain omega-3 PUFA concentrations, is not recommended.

*Keywords: Cardiovascular disease; Diet; Fish; Long-chain omega-3 polyunsaturated fatty acids; Methylmercury; Cardiac electrophysiology and performance; Electrocardiogram parameters; Exercise test; QT- and JT-intervals; Exercise cardiac power; Maximum oxygen uptake; VO2max; Systolic blood pressure during exercise, Resting heart rate; Maximum heart rate during exercise; Heart rate recovery, Exercise-induced myocardial ischemia; Men; Middle-aged and older; Finland.*
Epidemiologisista tutkimuksista on runsaasti näyttöä siitä, että kalan ja kalan sisältämien pitkäketjuisten omega-3-rasvahappojen syönti on yhteydessä pienemään sydän- ja verisuonisairauksien riskiin. Toisaalta kaloissa voi myös olla metyylielohopeaa, joka saattaa lisätä sydän- ja verisuonisairauksien riskiä ja vähentää pitkäketjuisten omega-3-rasvahappojen hyötyjä. Näitä havaintoja selittäviä mekansimeja ei kuitenkaan vielä täysin tunneta.

Tämä tutkimuksen tavoitteena oli tutkia seerumin pitkäketjuisten omega-3-rasvahappojen ja hiusten elohopeapitoisuuden yhteyksiä sydämen toimintaan. Molempien merkittävä lähde on kala, ja sekä seerumin pitkäketjuisten omega-3-rasvahappojen pitoisuksia että hiusten elohopeapitoisuuksia voidaan käyttää kuvaamaan altistustasoa. Tutkimusten aiheina oli tutkia yhteyksiä QT- ja JT-intervalleihin (Tutkimus I), sydänrasitusvoimaan (exercise cardiac power) ja sen komponentteihin (Tutkimus II), sydämen leposykkeeseen sekä rasituksen aiakseen maksimisykkeeseen ja rasituksen jälkeiseen sykkeen palautumiseen (Tutkimus III), ja rasituksen aiheuttaman sydänlihaksen iskemian ilman iskemian ilman ilman ilman ilmaantumisen riskiin (Tutkimus IV). Tutkimusaineistona oli keski-ikäisiä ja vanhempia miehiä Sepelvaltimotauidun vaaratekijät tutkimuksesta.

Tutkimuksessa havaittiin, että suurempi seerumin pitkäketjuisten omega-3-rasvahappojen pitoisuus oli yhteydessä lyhyempään QTc- ja JTc-aikaan. Hiusten elohopeapitoisuudella ei havaittu yhteyttä QTc- tai JTc-aikoihin, mutta suurempi hiusten elohopeapitoisuus heikensi seerumin pitkäketjuisten omega-3-rasvahappojen ja QTc- ja JTc-aikojen välistä käänteistä yhteyttä (Tutkimus I). Suurempi seerumin pitkäketjuisten omega-3-rasvahappojen pitoisuus oli myös yhteydessä suurempaan sydänrasitusvoimaan ja maksimaaliseen hapenottokykyyn, mutta ei maksimaaliseen rasituksen aiakseen systoliseen verenpaineseen. Suuri hiusten elohopeapitoisuus hiukan heikensi yhteyksiä (Tutkimus II). Suurempi seerumin pitkäketjuisten omega-3-rasvahappojen pitoisuus oli yhteydessä...
matalampaan leposykeeseen, mutta yhteyttä ei havaittu rasituksen aikaiseen maksimisykkeeseen tai sykkeen palautumiseen rasituksen jälkeen. Hiusten suuri elohopeapitoisuus yhdistyi matalampaan rasituksen aikaiseen maksimisykkeeseen ja se myös hiukan heikensi omega-3-rasvahopoilla havaittuja yhteyksiä (Tutkimus III). Tutkimus IV:ssä rasituksen aikaisen sydänlihaksen iskemian riski oli pienempi miehillä, joilla oli suurempi pitkäketjuisten omega-3-rasvahappojen pitoisuus seerumissa. Yhteys havaittiin varsinkin sepelvaltimotautia sairastavilla miehillä. Suurempi hiusten elohopeapitoisuus yhdistyi suurempaan rasituksen aikaisen sydänlihaksen iskemian riskiin.


Avainsanat: Sydän- ja Verisuonisairaudet; Kala; Pitkäketjuiset Omega-3-rasvahapot; Metyylielohopea; Sydämen sähköinen toiminta; Elektrokardiogrammi; Rasitustesti; QT- ja JT- aika; Sydänrasitusvoimaa; Maksimaalinen hapenottokyky; Rasituksen aikainen maksimaalinen verenpaine; Leposyke; Rasituksen jälkeinen sydämen sykkeen palautuminen; Keski-ikäiset ja vanhemmat miehet.
ACKNOWLEDGEMENTS

The present doctoral thesis study was carried out in the Institute of Public Health and Clinical Nutrition, Kuopio campus, University of Eastern Finland.

Foremost, I would like to express my sincere gratitude to my principal supervisor, Associate Professor Jyrki K. Virtanen, Ph.D., for the continuous support of my PhD study and research. Thank you for your endless patience, motivation, enthusiasm, and immense knowledge. I could not imagine having a better advisor and mentor for my PhD study.

Special thanks should also be given to Professor Tomi-Pekka Tuomainen, M.D., Ph.D. Thank you for introducing me to the field of epidemiology and giving me the opportunity to grow in this field of research, for believing in me and giving me the necessary pep-talks whenever I started doubting myself. Your wealth of knowledge in the field of epidemiology is inspiring. I would like to offer my most sincere appreciation to Adjunct Professor Sudhir Kurl, M.D., Ph.D., for keeping me motivated throughout the writing this thesis. Thank you for your encouragement, insightful comments and suggestions all through my doctoral thesis.

I would like to extend my special thanks to Professor Jukka T. Salonen, M.D., Ph. D. and Kai P. Savonen M.D. Ph. D., for their insightful comments and valuable contribution in my research.

This PhD project would never have been completed without the support of all the professional staff and colleagues in the Institute of Public Health and Clinical Nutrition, especially Professor Jussi Kauhanen, M.D., Ph.D., and Professor Pekka Mäntyselkä M.D., Ph.D., for granting me the permission to conduct my research work in the Kuopio Ischaemic Heart Disease Risk Factor Study cohort.

I express my appreciation to all the foundations and organizations that financially supported this Ph.D. work; Olvi Foundation, Juho Vainio Foundation, Antti and Tyyne Soininen Foundation, Paulo Foundation, Saara Kuusisto and Salme Penna Foundation, Kuopio University Foundation and University of Eastern Finland Doctoral Program.

I send my warmest gratitude to all my friends in Iran and Finland for their encouragement and support. There are no suitable words to express my deep sense of gratitude towards my wonderful parents, Naser Tajik and Azar Cheraghi. Thank you for your endless love, support and kindness. You were always beside me during both the happy and hard moments to push and motivate me.
Finally, I would like particularly to thank my role model, my older brother, Behzad, who has been my mentor all through my life. Words fail to express my appreciation; you are my hero.

Kuopio, January 2020

Behnam Tajik
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# ABBREVIATIONS

<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADA</td>
<td>American Dietetic Association</td>
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<tr>
<td>AHA</td>
<td>American Heart Association</td>
</tr>
<tr>
<td>ALA</td>
<td>Alpha-Linolenic Acid</td>
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<tr>
<td>BP</td>
<td>Blood pressure</td>
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<tr>
<td>CHD</td>
<td>Coronary Heart Disease</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaenoic Acid</td>
</tr>
<tr>
<td>DPA</td>
<td>Docosapentaenoic Acid</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>ECP</td>
<td>Exercise cardiac power</td>
</tr>
<tr>
<td>EPA</td>
<td>Eicosapentaenoic Acid</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density Lipoprotein</td>
</tr>
<tr>
<td>HR</td>
<td>Heart Rate</td>
</tr>
<tr>
<td>KIHD</td>
<td>Kuopio Ischaemic Heart Disease Risk Factor Study</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density Lipoprotein</td>
</tr>
<tr>
<td>PTWI</td>
<td>Permissible tolerable weekly intake</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated Fatty Acids</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Controlled Trial</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic Blood Pressure</td>
</tr>
<tr>
<td>SCD</td>
<td>Sudden Cardiac Death</td>
</tr>
<tr>
<td>VO2max</td>
<td>Maximal Oxygen Uptake</td>
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Cardiovascular disease (CVD) is the leading worldwide cause of mortality and it has long been recognized as a major public health threat, particularly among older adults (Benjamin, Virani et al. 2018). Even though there has been a decline in CVD mortality in Finland during the recent decades, CVD has remained the principal cause of death (Wilkins, Wilson et al. 2017). It is well established that a substantial proportion of this chronic disease could be prevented by adoption of a healthy diet since various dietary factors have been linked to the risk of CVD (Micha, Renata, Peñalvo et al. 2017). Therefore, understanding the impact of dietary factors on the etiopathogenesis of CVD and clarifying the underlying mechanisms plays a crucial role in tackling this issue and improving public health.

Fish have been one of the most extensively examined food items in the prevention of CVD. Fish, especially oily fish, are the major source of long-chain omega-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) (Micha, Renata et al. 2017). The impact of these fatty acids on cardiovascular health has been intensively studied after the first cross-cultural epidemiologic studies conducted in the Greenland Inuit population, Alaskan natives and Japanese, which revealed an inverse association between the intake of marine-derived omega-3 PUFAs and the incidence of CVD (Kromann, Green 1980, Middaugh 1990, Hirai, Hamazaki et al. 1980). However, the mechanisms underlying the cardioprotective of these fatty acids are not fully known.

Observational and experimental studies have confirmed the presence of an inverse association between intake of seafood-derived long-chain omega-3 PUFAs and the CVD risk, especially fatal coronary heart disease (CHD) (Rimm, Appel et al. 2018). The favourable impact of the long-chain omega-3 PUFAs on clinical risk factors of CVD, notably on the concentrations of triglycerides, lipoproteins and inflammatory markers as well as on vascular function, the tendency for platelet aggregation, insulin resistance, and blood pressure is well known (Mozaffarian, Wu 2011). However, several questions remain - how are the associations of the long-chain omega-3 PUFAs linked with cardiac electrophysiology and performance, which are indicators of CVD risk.

In addition to the long-chain omega-3 PUFAs, fish, especially certain species of large predatory fish and marine mammals, may contain methylmercury, an environmental contaminant and the most poisonous form of mercury (Roman, Walsh et al. 2011). A growing body of evidence has demonstrated the potential adverse effect of methylmercury on cardiovascular health and it has been speculated that it may also diminish the cardioprotective effect of long-chain omega-3 PUFAs (Oomen,

Although the association of mercury with some CVD risk factors, such as blood pressure (Houston 2011, Valera, Dewailly et al. 2011), heart rate variability (Valera, Dewailly et al. 2013), lipid peroxidation and production of free radicals (Kobal, Horvat et al. 2004) are well-established, very little is known about the association of methylmercury with parameters of cardiac electrophysiology and performance, which may represent new mechanisms to explain the adverse effect of methylmercury on the cardiovascular health.

Thus, the aim of this doctoral thesis was to investigate the associations of the serum long-chain omega-3 PUFAs and hair mercury concentrations, i.e. objective biomarkers for exposures, in relation to cardiac electrophysiology and performance in a Finnish population-based cohort.
2  REVIEW OF THE LITERATURE

2.1  CARDIOVASCULAR DISEASE

2.1.1  Public health relevancy

CVD are the leading cause of morbidity and mortality all around the world. In 2017, about 17.9 million deaths (31.5% of all global deaths) were attributed to CVD, particularly from heart attacks and strokes (Benjamin, Virani et al. 2018). In the United State, it has been estimated that 43.9% of the population will develop some form of CVD by 2030 (Benjamin, Virani et al. 2018). Moreover, the direct and indirect economic costs attributable to CVD in the United States in 2013 were estimated to be over $316 billion and this value is expected to almost triple to $918 billion in 2030 (Benjamin, Virani et al. 2018). According to the latest report by the American Heart Association (AHA) Goals and Metrics Committee of the Strategic Planning Task Force, the goal should be to achieve a 20% reduction in CVD mortality by 2020 in United States population (Lloyd-Jones, Hong et al. 2010, Benjamin, Virani et al. 2018).

Across the European region, CVD accounts for more than 50% of all deaths (Timmis, Townsend et al. 2017). Ischaemic heart disease and stroke are the main causes responsible of CVD mortality, the former accounting for every second CVD death, the latter for about 14% (Timmis, Townsend et al. 2017). In Finland, despite a sharp decline in death rates attributable to CVD between 1990 and 2015 due to changes in the lifestyle, diet, smoking habits, serum cholesterol and blood pressure levels by the increase in awareness of the general population stimulated by comprehensive national treatment projects (e.g. North Karelia Project) (Borodulin, Vartiainen et al. 2014, Jousilahti, Laatikainen et al. 2016), CVD is still the main cause of death followed by cancer and nervous system disorders (particularly dementia) (Benjamin, Virani et al. 2018). In 2014, CVD was responsible for 38% of all deaths in the Finnish population (Wilkins, Wilson et al. 2017). Therefore, the provision of adequate and cost-effective care for CVD prevention has attracted growing attention from national and local governments and international organizations, as well as from the general public.

2.1.2  CVD risk factors

An enormous number of factors are linked with the risk of CVD. The best known and traditional risk factors for CVD are aging, male gender, smoking behavior, high blood pressure, high triglycerides, total cholesterol and low-density lipoprotein (LDL) cholesterol concentrations, low serum high-density lipoprotein (HDL) cholesterol concentration, obesity, physical inactivity, low socioeconomic status, and
diabetes (Khot, Khot et al. 2003, Clark, DesMeules et al. 2009, Mozaffarian, Wu 2011). Moreover, some other risk factors, which are known as novel risk factors of CVD, such as inflammatory markers, oxidative stress, endothelial dysfunction, thrombosis, myocardial inefficiency and left ventricular hypertrophy, should also be taken into account when assessing the risk (Mozaffarian, Wu 2011) (Figure 1).

The risk of CVD may not always be entirely explained by the existence of traditional and novel risk factors. Some patients suffer from CVD events, such as myocardial infarction, angina or heart failure without having any prior CVD risk factors (Jones, Pothier et al. 2004). Therefore, other supplemental methods are needed for early detection of the CVD events among asymptomatic patients, of which resting and exercise electrocardiogram (ECG) have been claimed to be the most useful (D’agostino, Grundy et al. 2001).

### 2.2 ECG and CVD

The ECG is a widely used, accurate and noninvasive screening tool in clinical practice, which provides reliable information regarding the electrical and muscular functions of atrial and ventricular myocytes. It is usually the first step for the detection of atherosclerosis, acute myocardial infarction and heart attack (Rezazadeh, Seno 2013). These activities which are measured consist of three main phases, depolarization, repolarization and the recovery period. On the surface ECG, right and left atrial depolarizations are expressed by the P-wave. Atrial repolarization is not detectable in ECG due to its low amplitude (Jayaraman, Gandhi et al. 2015). Ventricular depolarization is made up of the QRS complex, whereas the repolarization phase of the ventricle consists of the J-point, ST-segments, and T- and U-waves (Yan, Lankipalli et al. 2003) (Figure 2).
Several abnormalities in resting and exercise ECG have been consistently reported to be associated with a higher risk of CVD events and cardiac mortality, especially in middle aged and older asymptomatic patients (Chou, Arora et al. 2011, Alpert 2018, US Preventive Services Task Force 2018). Moreover, direct associations have been observed between an ECG abnormality and the traditional CVD risk factors such as age and male gender (Denes, Garside et al. 2013, Healy, Lloyd-Jones 2016), smoking behavior (Moller, Byberg et al. 2006, Gepner, Piper et al. 2013), hypertension (Kawamura, Yamamoto et al. 1996), diabetes (Kawamura, Yamamoto et al. 1996), obesity (Soteriades, Targino et al. 2011, Moller, Byberg et al. 2006) and lipid profile (Lathadevi, Anusha 2012). Therefore, ECG parameters are accurate indicators for the early detection and prevention of CVD.

2.3 PARAMETERS USED IN THIS STUDY IN RELATION TO CARDIAC FUNCTIONS

2.3.1 QT- and JT- intervals

The QT is an interval in ECG trace, starting from the first deflection of the QRS complex to the end of T-wave and it is an accurate measure of the duration of the ventricular action potential. It represents a crucial stage in electrical cardiac activity since it includes Q-wave (depolarization of the interventricular septum), R-wave (depolarization of the main mass of ventricles), S-wave (last phase of ventricular repolarization ), ST-segment (plateau of myocardial action potential) and T-wave (ventricular repolarization phase immediately before ventricular relaxation or ventricular diastole) (Rautaharju, Surawicz et al. 2009, Monitillo, Leone et al. 2016). The QT-interval includes both ventricular repolarization and part of the
depolarization phases during heart electrical cycle, therefore the JT-interval (QTc–QRS duration) has been recommended as a more sensitive measure for assessing abnormalities of ventricular repolarization (Rautaharju, Surawicz et al. 2009).

Since the QT- and JT-intervals are highly correlated with heart rate, heart rate-corrected QT- and JT-intervals (QTc and JTc) have been used in the different clinical settings to achieve a more accurate risk stratification of arrhythmic events (Ahnve 1985). Common formulas of calculating QTc and JTc are Bazett’s (QTcB = QT / √RR) and Fridericia’s Formulas (QTcF = QT / 3√RR) (Stramba-Badiale, Karnad et al. 2018, Vandenberk, Vandael et al. 2016). (RR interval is the time between the one QRS complex to the onset of the next QRS complex (Giles, Draper et al. 2016)).

The normal ranges of QTc and JTc in healthy populations are between 350-460 ms and 270-350 ms, respectively, with 10-20% variation (Viskin 2009, Lehmann, Hardy et al. 1999). Any value more than this range is considered as a prolonged QTc and JTc (Johnson, Ackerman 2009). Genetic factors, electrolyte imbalance, and unwanted effects of some medications (e.g. some antidepressant, antipsychotic, antihistamines, antiarrhythmic and anti-nausea medications) are the main causes of prolonged QTc and JTc values (Montanez, Ruskin et al. 2004). Moreover, it has been reported that prolonged QTc and JTc are highly correlated with some traditional CVD risk factors such as aging, obesity and unbalanced diet (Benoit, Mendelsohn et al. 2005, Akylbekova, Crow et al. 2009).

It has been suggested that abnormal ventricular repolarization (prolonged QT- and JT-intervals) is a pro-arrhythmic risk factor, which may increase the risk of a CVD event (Zhang, Y., Post et al. 2011), especially in middle aged and older men (Beinart, Zhang et al. 2014). This association might be partially explained by the role of prolonged QT- and JT-intervals in the ventricular arrhythmias (torsades de pointes) and ventricular hypertrophy, which may lead to serious cardiac events, especially sudden cardiac death (SCD) (Zabel, Hohnloser et al. 1997, Davey 2000, Elming, Sonne et al. 2003).

2.3.2 Exercise cardiac power

A low exercise capacity during an exercise test has been established as an independent predictor of risk for the total mortality and cardiovascular events (Kokkinos, P., Myers et al. 2008, Korpelainen, Lämsä et al. 2016). Cardiorespiratory fitness, typically represented by maximal oxygen uptake (VO2max) during exercise tests, refers to the ability of the respiratory and cardiovascular system to deliver oxygen to the muscles during exercise, and it is recognized as the golden standard of exercise capacity, especially habitual exercise (Kokkinos, Myers et al. 2018). VO2max is higher among men and it is greatly affected by aging, training severity and anthropometric measurements (Paap, Takken 2014).

Convincing evidence has emerged from the population-based studies indicating that a higher VO2max value is inversely associated with the risk of cardiovascular

Although VO2max during exercise is an accurate indicator of the efficiency with which the cardiovascular and respiratory systems can transport and use oxygen during physical stress (cardiac output and cardiac preload) (Laukkanen, Kurl et al. 2002), its usefulness is limited by the fact that it does not provide information about the cardiac peripheral resistance (cardiac afterload), which is mainly indicated by systolic blood pressure (SBP) during exercise.

It has been well-known that higher resting SBP is directly associated with the risk of mortality (Brunström, Carlberg 2018, Bundy, Li et al. 2017, Stevens, Wood et al. 2016). Moreover, high exercise-induced SBP is a risk factor of stroke (Kurl, S., Laukkanen et al. 2005), hypertension (Kannel, Wolf et al. 1981, Singh, Larson et al. 1999), CVD events (McHam, Marwick et al. 1999, Mundal, Kjeldsen et al. 1996) and CVD mortality (Chaitman 2018).

Exercise cardiac power (ECP) is defined as the ratio of directly measured maximal VO2max with the peak SBP during an exercise test (Kurl, Laukkanen et al. 2005). ECP is a more accurate marker of cardiac output, since it not only evaluates the cardiac function derived from preload (VO2max), but also provides information about cardiovascular resistance and cardiac afterload (Kurl, S., Laukkanen et al. 2005).

ECP is known to be an independent predictor of cardiovascular events. Previously in the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) cohort, a lower ECP value was associated with an increased risk of CHD and CVD mortality (Kurl, Laukkanen et al. 2005, Kurl, Jae et al. 2015, Kurl, Jae et al. 2015, Kurl, Mäkikallio et al. 2016).

### 2.3.3 Heart rate

Heart rate (HR) is a noninvasive measure which reflects the myocardial performance and coronary blood flow (Caetano, Alves 2015). HR is known as a well-established predictor of cardiovascular events, independently of traditional cardiovascular risk factors (Sabbah, Ilsar et al. 2011). Numerous epidemiological studies have detected a direct significant association between abnormal resting HR and cardiovascular and overall mortality in healthy populations (Zhang, Shen et al. 2016, Aune, Sen et al. 2017) as well as in patients with existing hypertension, CHD and chronic heart failure (Böhm, Reil et al. 2015). It has been also shown that a higher resting HR (more than 100 beats/min) is associated with an increased risk of myocardial ischemia, ventricular arrhythmias, stroke and the progression of atherosclerosis, which may

The peak exercise-induced HR and HR recovery after exercise are other indicators of cardiac autonomic function (Cole, Blackstone et al. 1999, Jouven, Empana et al. 2005). It has been reported that both peak exercise-induced HR and HR recovery after exercise decrease with aging and are slightly higher in men (Paap, Takken 2014). There is epidemiological and clinical evidence demonstrating that low peak HR during an exercise test is independently associated with cardiac mortality (Jouven, Empana et al. 2005, Savonen, Lakka et al. 2006, Sandvik, Erikssen et al. 1995, Tan, Allen et al. 2017). HR recovery after exercise cessation is also known as an independent predictor of cardiovascular mortality in healthy populations (Cole, Blackstone et al. 1999, Qiu, Cai et al. 2017, Mora, Redberg et al. 2003, Savonen, KP, Kiviniemi et al. 2011) as well as in patients with clinically evident CVD (Vivekananthan, Blackstone et al. 2003, Watanabe, Thamilarasan et al. 2001). HR recovery is mainly evaluated either one or two minutes after the cessation of the physical stress. Generally similar associations with the risk of cardiac mortality have been reported when HR recovery was assessed after one minute or two minutes after exercise cessation (Qiu, Cai et al. 2017).

2.3.4 Exercise-induced myocardial ischemia

Myocardial ischemia occurs when there is an imbalance between myocardial oxygen consumption and oxygen delivery to the myocardium (Turer, Hill 2010). Myocardial oxygen consumption is determined by the left ventricular wall tension, blood pressure and myocardial substrates while coronary blood flow is mainly evaluated by the coronary vascular resistance (Turer, Hill 2010). Physical activity is another crucial factor which is directly related to the myocardial oxygen consumption and oxygen delivery to the myocardium. The intensity and duration of physical activity may influence the sub-endocardial blood flow (Matsuzaki, Patritti et al. 1984).

Myocardial ischemia results from the reduction of myocardial blood flow and contractile function (Detry 1996, Crossman 2004). Atherosclerosis, blood clotting and coronary arterial spasm may lead to myocardial oxygen imbalance, and consequently myocardial ischemia (Gimbrone, García-Cardeña 2016). Symptoms of myocardial ischemia are angina (angina pectoris), chest discomfort, ischaemic left ventricular dysfunction and cardiac arrhythmias; however, some experience asymptomatic myocardial ischemia (Crossman 2004).

The ST-segment depression, slowed conduction on the QRS complex and T-wave inversion in the resting and exercise-induced ECG surface denote myocardial ischemia in the symptomatic and asymptomatic patients (Surawicz 1998, Wagner, Sevilla et al. 1988, Spekhorst, SippensGroenewegen et al. 1990, Kleber, Janse et al. 1986, Bacharova, Szathmary et al. 2013). For example, ST-segment depression is known as the most accurate indicator (Bacharova, Szathmary et al. 2013, Ross 1976).
It has been demonstrated that myocardial ischemia is an independent predictor of cardiovascular events and cardiac mortality (Wetmore, Broce et al. 2012, Elhendy, Chapman et al. 2005, Rahimi, Duncan et al. 2015). Moreover, exercise-induced myocardial ischemia, as indicated in an ECG stress test, predicts the risk of atherosclerosis and the prognosis of future cardiac events (Lalonde, Poirier et al. 2015, Hagnäs, Kurl et al. 2015, Hagnäs, Lakka et al. 2017).

2.4 OMEGA-3 PUFA AND METHYLMERCURY

2.4.1 Biochemistry, dietary intake and sources

PUFAs are fat molecules that contain more than one double bond (an unsaturated carbon bond) in their structures. There are two major classes of PUFAs; omega-6 and omega-3. The name of the omega-3 PUFAs comes from the location of the double bond which is on the third carbon atom from the methyl end of fatty acid chain (De Caterina 2011). Omega-3 PUFAs are a key component of phospholipids, which play a crucial role in various biological processes, notably cell membranes formation and energy storage (Cao, Schwichtenberg et al. 2006). The major omega-3 families include alpha-linolenic acid (ALA, C18:3n-3), eicosapentaenoic acid (EPA, C20:5n-3), docosapentaenoic acid (DPA, C22:5n-3), and docosahexaenoic acid (DHA, C22:6n-3).
ALA is considered as an essential fatty acid, since it cannot be synthetized in the human body, due to the lack of $\Delta_{12}$ and $\Delta_{15}$ desaturases (Jones, Papamandjaris 2001). ALA is present in plants, seeds, nuts, some vegetable oils (e.g. flaxseed, chia, soybean, walnut and canola oils), and in green leafy vegetables (in very limited amounts) (Lavie, Milani et al. 2009). The major dietary ALA sources in Finland are vegetable spreads and oils, grains and meat products (Valsta, Salminen et al. 1996). The mean intake of omega-3 PUFA in Finland is 3.6 g/day (3.9 g/day in men and 3.3 g/day in women), of which 2.8 g/day is ALA (men 3.1 g/day and women 2.6 g/day) (FinDiet 2017).

ALA can be converted in liver to stearidonic acid, and then EPA, DPA and DHA (ALA to EPA conversion rate ranges from 0.2% to 8%, ALA to DPA 0.1%-6% and <1% of ALA to DHA). Genetic variation is the key factor which may influence the
enzymes regulating fatty acid synthesis and metabolism, and affects the rate of formation of omega-3 PUFAs in the body (Domenichiello, Kitson et al. 2015, Goyens, Spilker et al. 2006). This conversion is done during the desaturation reaction catalysed process via delta-5 and delta-6-desaturase enzymes (Whelan 2008, Pawlosky, Hibbeln et al. 2001) (Figure 4).
Figure 4. The metabolism of omega-3 PUFAs; modified from (Pawlosky, Hibbelsn et al. 2001)
EPA and DHA are obtained primarily from marine sources. Their content in marine food depends on the type of fish as well as the type of foods that the fish consumes (Miller, Nichols et al. 2008). Fatty fish, especially cold-water fish such as salmon, mackerel, tuna, herring, and sardines are good dietary sources of long-chain omega-3 PUFAs (Shahidi, Ambigaipalan 2018) (Table 1). Moreover, it has been reported that farmed fish contain more EPA and DHA, as compared to wild-trapped fish (Cladis, Kleiner et al. 2014). The supplementary sources of EPA and DHA are fish oil, krill oil, cod liver oil, and algal oil (Shahidi, Ambigaipalan 2018).

The intakes of fish vary substantially around the world (Micha, R., Khatibzadeh et al. 2015). The average intake of fish in Finland is 36 g/d in men and 27 g/d in women (FinDiet 2017), which is close to the average intake in Western Europe (Micha, Khatibzadeh et al. 2015). Before the 1980s, most of the fish was wild-caught domestic fish; however, since then, there has been an increase in demand, and the consumption of farmed and exported fish (e.g. Norwegian salmon) has drastically increased (Setälä, Honkanen et al. 1998).
EPA and DHA can be synthesized in the human liver, although in limited amounts, during ALA conversion (Figure 4). Some factors may influence the rate of this bioconversion such as age, gender and genetic variability (Burdge, Graham, Wootton 2002). It has been reported that young healthy women have the highest rate of ALA conversion to EPA and DHA of 21% and 8%, respectively (Burdge, Graham, Wootton 2002), possibly due to the synergistic effects of oestrogen (Burdge, Graham

<table>
<thead>
<tr>
<th>Dietary Sources</th>
<th>EPA</th>
<th>DPA</th>
<th>DHA</th>
<th>EPA+DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seafoods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anchovy</td>
<td>763</td>
<td>41</td>
<td>1292</td>
<td>2055</td>
</tr>
<tr>
<td>Herring, Atlantic</td>
<td>909</td>
<td>71</td>
<td>1105</td>
<td>2014</td>
</tr>
<tr>
<td>Salmon, farmed</td>
<td>862</td>
<td>393</td>
<td>1104</td>
<td>1966</td>
</tr>
<tr>
<td>Salmon, wild</td>
<td>411</td>
<td>368</td>
<td>1429</td>
<td>1840</td>
</tr>
<tr>
<td>Mackerel, Atlantic</td>
<td>504</td>
<td>106</td>
<td>699</td>
<td>1203</td>
</tr>
<tr>
<td>Bluefish</td>
<td>323</td>
<td>79</td>
<td>665</td>
<td>988</td>
</tr>
<tr>
<td>Sardines, Atlantic</td>
<td>473</td>
<td>0</td>
<td>509</td>
<td>982</td>
</tr>
<tr>
<td>Golden bass</td>
<td>172</td>
<td>143</td>
<td>733</td>
<td>905</td>
</tr>
<tr>
<td>Swordfish</td>
<td>127</td>
<td>168</td>
<td>772</td>
<td>899</td>
</tr>
<tr>
<td>Shark</td>
<td>258</td>
<td>89</td>
<td>431</td>
<td>689</td>
</tr>
<tr>
<td>Pollock, Atlantic</td>
<td>91</td>
<td>28</td>
<td>451</td>
<td>542</td>
</tr>
<tr>
<td>Oysters, wild</td>
<td>274</td>
<td>16</td>
<td>210</td>
<td>484</td>
</tr>
<tr>
<td>Tuna, white</td>
<td>233</td>
<td>18</td>
<td>629</td>
<td>862</td>
</tr>
<tr>
<td>Tuna, light</td>
<td>91</td>
<td>17</td>
<td>237</td>
<td>328</td>
</tr>
<tr>
<td>Lobster</td>
<td>117</td>
<td>6</td>
<td>78</td>
<td>195</td>
</tr>
<tr>
<td>Other Dietary sources</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td>0</td>
<td>7</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>Chicken breast</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Beef</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Pork</td>
<td>0</td>
<td>10</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 1. Food sources of long-chain omega-3 PUFAs

EPA, Eicosapentaenoic acid; DPA, Docosapentaenoic acid; DHA, Docosahexaenoic acid; PUFAs, Polyunsaturated fatty acids.

Modified from (Mozaffarian, Wu 2012)
Therefore, the source of EPA and DHA largely stems from an exogenous origin via adequate dietary intake (Lavie, Milani et al. 2009). Moreover, DHA can be retro-converted to EPA and DPA in limited amounts, based on the DHA intake (Mozaffarian, Wu 2012).

Unlike the situation for EPA and DHA, less is known regarding the source and health effects of DPA. DPA mainly originates from the endogenous elongation of EPA and its levels are clearly correlated with the EPA concentration. However, the retro-conversion of DHA to DPA is very limited (Kaur, Cameron-Smith et al. 2011).

In addition to being sources of long-chain omega-3 PUFAs, protein, vitamin D, iodine and selenium, it is known that fish, especially large and old predatory fish such as shark, swordfish, tilefish, king mackerel, pike and bigeye tuna, also contain methylmercury, an organic form of mercury (Schuhmacher, Batiste et al. 1994) (Table 2). Mercury can be also found as a natural and elemental form in the air (Hansen, Danscher 1997), water (Beldowski, Pempkowiak 2003) dental amalgams (Lyttle, Bowden 1993), and some plants (Horvat, Nolde et al. 2003).
Mercury exists in three forms i.e. elemental (metallic), inorganic (liquid metallic mercury, mercury vapor, mercurous, mercuric salts), and organic mercury (methylmercury, ethylmercury, phenylmercury) (Clarkson, Magos 2006, Bernhoft 2012).

Mercury is released into the environment from different sources, for example mineral deposits and coal-fired power stations. After mercury settles in lakes, streams, and oceans, the elemental form of mercury is converted into the organic form of mercury, methylmercury, by aquatic anaerobic sulfate-reducing microorganisms. Fish and other marine animals accumulate methylmercury.

In the human body, methylmercury is absorbed by the gastrointestinal system and enters the bloodstream. It remains in the human body for a long time due to its slow conversion to inorganic mercury, and due to the fact that organisms do not possess a mechanism to metabolize or excrete mercury (Genchi, Sinicropi et al. 2017). The

<table>
<thead>
<tr>
<th>Species</th>
<th>Mercury concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tilefish</td>
<td>1.45</td>
</tr>
<tr>
<td>Shark</td>
<td>0.99</td>
</tr>
<tr>
<td>Swordfish</td>
<td>0.98</td>
</tr>
<tr>
<td>Mackerel, King</td>
<td>0.73</td>
</tr>
<tr>
<td>Pike</td>
<td>0.38-0.40</td>
</tr>
<tr>
<td>Tuna</td>
<td>0.38</td>
</tr>
<tr>
<td>Bluefish</td>
<td>0.36</td>
</tr>
<tr>
<td>Burbot</td>
<td>0.22-0.26</td>
</tr>
<tr>
<td>Scorpionfish</td>
<td>0.23</td>
</tr>
<tr>
<td>Perch, freshwater</td>
<td>0.14</td>
</tr>
<tr>
<td>Cod</td>
<td>0.10-0.11</td>
</tr>
<tr>
<td>Whitefish</td>
<td>0.03-0.08</td>
</tr>
<tr>
<td>Trout, freshwater</td>
<td>0.07</td>
</tr>
<tr>
<td>Catfish</td>
<td>0.05</td>
</tr>
<tr>
<td>Herring</td>
<td>0.04</td>
</tr>
<tr>
<td>Sardine</td>
<td>0.02</td>
</tr>
<tr>
<td>Salmon</td>
<td>0.01-0.07</td>
</tr>
</tbody>
</table>

Modified from (Virtanen, Rissanen et al. 2007)
concentration of mercury in fish and marine animals depends on the size and age of the fish, their fat concentrations, as well as season and region. It has been reported that larger, long-living deep-water fish species and predatory species contain higher concentrations of methylmercury (Roman, Walsh et al. 2011).

### 2.4.2 Recommendation

EPA and DHA have been suggested to account for approximately 1% of total energy intake and 10% of the total omega-3 fatty acid intake (approximately 160 mg per day for general population) (Kris-Etherton, Penny M., Grieger et al. 2009). The 2015–2020 Dietary Guidelines for Americans recommended 2 servings of seafood per week, preferably oily fish (an average 250 mg per day EPA+DHA) for both healthy individuals and those with history CVD. Pregnant women are advised to take a higher amount of long-chain omega-3 PUFAs (at least 300 mg/day; 1/3 EPA+2/3 DHA) (Zhang, Fulgoni et al. 2018). According to the AHA guidelines (Lichtenstein, Appel et al. 2006), the general population is encouraged to consume more fish (at least twice per week, especially oily fish). The Finnish dietary guidelines recommend eating a variety of fish species 2-3 times per week, and that the intake of omega-3 PUFAs should be at least 1% of energy (National Nutrition Council 2014). However, there is no specific recommendation for intake of EPA+DHA for the general population (National Nutrition Council 2014). Some of the guidelines are presented in Table 3.

Moreover, 1000 mg EPA+DHA per day, which is equal to 6-7 servings of oily fish per week, is recommended as a form of secondary prevention among those with existing cardiovascular disturbances, especially in patients with recent heart failure and myocardial infarction (Siscovick, Barringer et al. 2017).

Although seafood-derived methylmercury can exert adverse effects on human health, the intake of fish (especially those with lower concentrations of mercury) one or two servings a week is still recommended (Zhang, Z., Fulgoni et al. 2018). The daily intake of methylmercury in US is about 2.4 µg from all sources (mainly from fish). According to the latest US Environmental Protection Agency guideline, the permissible tolerable weekly intake (PTWI) for methylmercury is approximately 1.3 µg/kg body weight (Zhang, Fulgoni et al. 2018). Children and pregnant women are advised to avoid/limit eating shark, swordfish, king mackerel and tilefish due to the high mercury concentration in these species (Rice 2004).

According to the Finnish Food Authority, children, young people and persons of fertile age should not eat large herring, salmon or trout caught from the Baltic Sea due to potentially high exposure to PCBs and dioxins or pike due to the potentially high methylmercury content more often than once or twice a month. Pregnant women and nursing mothers should avoid pike completely. In addition, people who daily eat fish from inland lakes are advised to reduce their intake of also other predatory fish, including large perch and pike perch (Finnish Food Authority 2019).
Different methods are available for the quantitation of omega-3 PUFAs including food frequency questionnaire, food records and biomarkers (tissue measurement and blood specimens) (Baylin, Campos 2006). Food frequency questionnaires and food records may not accurately estimate the intake of omega-3 PUFAs (Livingstone, Prentice et al. 1990, Black, Goldberg et al. 1991). For example, fish intake was overestimated by validated food frequency questionnaire in two Finnish studies, which were designed to cover long-term food consumption (Männistö, Virtanen et al. 1996, Paalanen, Männistö et al. 2006). On the other hand, food recording of a few days/weeks may not encompass the typical fish intake habits, if fish consumption during those days is less or more than usual.

The common biomarkers used in the epidemiological studies are omega-3 PUFA levels in blood fractions including plasma or serum (phospholipids, triglyceride, cholesterol ester), and cell membrane (especially erythrocyte membrane) (Baylin, Campos 2006). Omega-3 PUFA concentrations in plasma or serum indicate the recent intake of marine-derived omega-3 PUFAs (days/weeks). In contrast, omega-3 PUFAs content in erythrocyte membrane reflects longer-term dietary intake (weeks/months) (Cao, Schwichtenberg et al. 2006). The sum of EPA and DHA in erythrocyte membranes, which is known as the omega-3 index, is a more accurate indicator of
cardiovascular events, as compared to the traditional circulating risk factors (e.g. lipid profile) (Harris, Von Schacky 2004, Harris 2008). The normal ranges of the omega-3 PUFAs in plasma or serum have been not defined, due to various factors such as recent fish intake, geographic features, and genetic factors.

Another method for the assessment is the analysis of the composition of omega-3 PUFAs in adipose tissue; this value provides information about the regular intake (Tjønneland, Overvad et al. 1993). It reflects the dietary intake of omega-3 PUFAs during at least the last year (Arner, Bernard et al. 2011). The omega-3 concentration in adipose tissue is low and the sampling of a adipose tissue biopsy to allow the measurement of the fatty acid composition requires complicated and expensive methodologies (Hodson, Skeaff et al. 2008) and therefore adipose tissue is not a very useful biomarker of omega-3 PUFA status, especially for large studies.

Various biomarkers are available for the assessment of methylmercury. Total mercury levels in blood, hair or toenail are widely used biomarkers of mercury measurement. Hair and toenail mercury can be used as a long-term indicator of total dietary mercury intake (weeks/months), whereas blood levels reflect the recent mercury intake (days) (MacIntosh, Williams et al. 1997, Wilson, Suk 2002). Dietary assessment is another method to evaluate mercury exposure; however, it is not widely used since the mercury level in the dietary sources depends on the seafood species (Groth III 2010). Mercury can be also measured in breast milk (García-Esquinas, Pérez-Gómez et al. 2011), meconium placenta and umbilical cord (Gundacker, Fröhlich et al. 2010), but these methods are not widely used or appropriate in epidemiological studies. Moreover, urinary mercury mainly represents inorganic and elemental mercury from sources such as dental amalgam (Nicolae, Ames et al. 2013), therefore it is not a useful way to assess the burden of organic mercury (methylmercury).

### 2.4.4 Long-chain omega-3 PUFAs, mercury and chronic diseases

Numerous lines of evidence from experimental and epidemiological studies have shown the beneficial impact of higher intakes of fish and other seafood or long-chain omega-3 PUFAs on the prevention and progression of several disorders such as cardiovascular disease (primary and secondary prevention) (Harris, Dayspring et al. 2013, Elagizi, Lavie et al. 2018), inflammatory disorders (Calder 2015), some types of cancers (mainly breast, colorectal and prostate cancers) (Calviello, Serini et al. 2007, Bougnoux, Hajjaji et al. 2010, Gu, Suburu et al. 2013), neurological disorders (Haast, Kiliaan 2015) and diabetes (Shahidi, Ambigaipalan 2018). However, the findings of some recent clinical studies do not completely support these beneficial effects (Shahidi, Ambigaipalan 2018, Manson, Cook et al. 2019).

Although data regarding the health effects of mercury are still under investigation, it is recognized to be an environmental contaminant harmful to human health, particularly in children, pregnant women and elderly (Oken, Rifas-Shiman et al.
It has been demonstrated that mercury exerts a negative impact on the cardiovascular, immunologic, and neurocognitive and behavioral outcomes. Moreover, mercury, especially inorganic mercury salts, can cause nephrotoxicity and reproductive toxicity (Karagas, Choi et al. 2012).

2.4.5 Long-chain omega-3 PUFAs, mercury, CVD and CVD risk factors

The impact of omega-3 PUFAs, predominantly EPA and DHA, on the cardiovascular events has been extensively studied. In the early 1970s, it was observed that fish-eating populations such as Greenland Inuits had a lower risk of myocardial infarction and other coronary events (Bang, Dyerberg et al. 1980), results subsequently confirmed in Japanese and Alaskan populations (Davidson, Bulkow et al. 1993). This finding was supported by the results of various epidemiological and intervention studies that reported the beneficial impact of long-chain omega-3 PUFAs on the risk of fatal CHD, particularly SCD (Erkkilä, Lehto et al. 2003, Virtanen, Voutilainen et al. 2005, Turunen, Verkasalo et al. 2008, Mozaffarian, Wu 2011, Djoussé, Akinkuolie et al. 2012, Harris, Dayspring et al. 2013, Macchia, Grancelli et al. 2013, Siscovick, Barringer et al. 2017). In contrast, recent studies have yielded conflicting results (Aung, Halsey et al. 2018, Elagizi, Lavie et al. 2018, Manson, Cook et al. 2019). These conflicting results might be partially explained by the differences in the study designs and study populations. Moreover, the subjects in the recent trials had been mainly treated with state-of-the-art medication, and furthermore they may have had high exposures to EPA+DHA already before the trials through substantial fish intakes or have used fish oil, therefore these trials may have been underpowered to detect the beneficial effects on the CVD outcome (Mozaffarian, Wu 2011).

Many clinical and observational studies have detected an inverse association between a higher intake of long-chain omega-3 PUFAs and their objective biomarkers, and the risk of cardiovascular events via its favorable impact on well-established CVD risk factors including high triglyceride concentrations, high blood pressure, low HDL cholesterol, platelet aggregation and thrombus formation (this mainly with DPA), elevated levels of inflammatory markers, and increased oxidative stress (Mozaffarian, Wu 2011, AbuMweis, Jew et al. 2018). However, it would be important to find new mechanisms underlying the cardioprotective effect of long-chain omega-3 PUFAs.

In recent years, possible adverse effects of methylmercury exposure on the cardiovascular health has attracted some attention (Genchi, Sinicropi et al. 2017). It has been demonstrated that a higher concentration of methylmercury is directly associated with the risk of hypertension, CHD, myocardial infarction, cardiac arrhythmias, atherosclerosis and cardiovascular disease (Salonen, Seppänen et al. 1995, Salonen, Seppänen et al. 2000, Guallar, Sanz-Gallardo et al. 2002a, Virtanen, Voutilainen et al. 2005, Virtanen, Laukkanen et al. 2012). Mercury may also diminish the cardioprotective effects of long-chain omega-3 PUFAs (Guallar, Sanz-Gallardo et al. 2002b, Rissanen, Voutilainen et al. 2003, Virtanen, Voutilainen et al. 2005,
Virtanen, Laukkanen et al. 2012). Although many studies have shown the adverse cardiovascular effects of mercury, some studies did not support such adverse effects (Hallgren, Hallmans et al. 2001, Wennberg, Bergdahl et al. 2010, Mozaffarian, Shi et al. 2011, Mozaffarian, Shi et al. 2012). These contrary findings might be due to the study setting, population differences and different mercury measurements. In addition, in our data from KIHD, the average Hg levels were higher than in many other cohorts that have reported mercury exposure (Wennberg, Bergdahl et al. 2010, Mozaffarian, Shi et al. 2011), which may also have affected the associations.

The mechanisms underlying the potential adverse effects of methylmercury on the cardiovascular health are not fully understood; however, they can be partially explained by the role of methylmercury in increasing free radical stress, lipid peroxidation, reactive oxygen species, inflammation, thrombosis, and mitochondrial and immune dysfunction (Genchi, Sinicropi et al. 2017). In KIHD, a higher hair mercury content has been associated with elevated oxidation of low-density lipoprotein (LDL) and this has been directly associated with the risk of acute myocardial infarction and CVD (Salonen, Seppänen et al. 1995).

2.4.6 Long-chain omega-3 PUFAs, mercury and cardiac electrophysiology and performance

The long-chain omega-3 PUFAs and methylmercury have also been shown to be associated with some parameters of cardiac electrophysiology; however, the research underlying this area is not comprehensive. Very little is known regarding the association of the long-chain omega-3 PUFAs and methylmercury with abnormal parameters of cardiac electrophysiology and performance. For the purpose of this thesis, summaries of publications on the study questions in different population samples are presented in Table 4 to Table 10.

Long-chain omega-3 PUFAs, mercury and QT- and JT-intervals

There are few studies that have evaluated the association of long-chain omega-3 PUFA with the QT interval; their results are summarized in Table 4.

In a large population-based study in US, a higher intake of fish was associated with a significantly 46% lower likelihood of prolonged QTc among 5096 older men and women from the Cardiovascular Health Study (Mozaffarian, Prineas et al. 2006). In another large cross-sectional study among 3042 Greek men and women aged 18-89 years, those who consumed >300 g fish per week had 13.6% lower QTc as compared to the non-consumers. (Chrysohoou, Panagiotakos et al. 2007)

There are only two small cross-sectional studies that have assessed the association of the circulating long-chain omega-3 PUFAs with the QTc. Higher concentrations of the EPA+DHA in cholesteryl esters were not associated with the QTc duration among 53 healthy men and women (31.0 (5.6) years) (Brouwer, Zock et al. 2002). Similarly,
in a recent cross-sectional study, plasma EPA+DHA concentration and total seafood consumption were not associated with the QTc duration among 94 middle-aged seafood consumers (Miller, C., Karimi et al. 2018).

Only two randomized controlled trials (RCT) have investigated the effect of fish oil supplementation on the QTc duration. No effect on the QTc was found in a small RCT among 42 healthy middle-aged men and women after 12-week supplementation with 3.5 g/d of fish oil (Geelen, Brouwer et al. 2002). Moreover, fish oil supplementation did not alter the QTc duration in 206 haemodialysis patients among those who received 1.7 g/d fish oil for 12 weeks (Kirkegaard, Svensson et al. 2012).

Data regarding the association of methylmercury and QTc is very limited. There is only one study that has evaluated the association of blood methylmercury and QTc; it failed to find any association (Miller, C., Karimi et al. 2018). In addition, the association between the long-chain omega-3 PUFAs, mercury and the JT-interval has not been examined.
Table 4. Summary of the previous studies on the association of long-chain omega-3 PUFAs and mercury with QT- and JT-interval

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study setting</th>
<th>Study population</th>
<th>Exposure</th>
<th>Exposure assessment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brouwer et al. 2002 (Brouwer, Zock et al. 2002)</td>
<td>Cross-sectional</td>
<td>53 men and women mean age 31.0±5.6 years, healthy</td>
<td>EPA, DHA</td>
<td>24-hour dietary recall/Serum cholesteryl esters</td>
<td>No association between EPA and DHA in cholesteryl esters with the duration of QT-interval</td>
</tr>
<tr>
<td>Geelen et al. 2012 (Geelen, Brouwer et al. 2002)</td>
<td>RCT</td>
<td>42 men and women age 50-70 years, healthy</td>
<td>1.5g/day fish oil vs. sunflower oil for 12 weeks</td>
<td>24-hour dietary recall/Serum cholesteryl esters</td>
<td>No effect on QT interval</td>
</tr>
<tr>
<td>Mozaffarian et al. 2006 (Mozaffarian, Prineas et al. 2006)</td>
<td>Cross-sectional</td>
<td>5096 men and women age ≥65 years, healthy</td>
<td>Tuna or other broiled or baked fish intake</td>
<td>Food frequency questionnaire</td>
<td>Lower likelihood of prolonged QT interval among those with higher fish intake.</td>
</tr>
<tr>
<td>Chrysousou et al. 2007 (Chrysousou, Panagiotakos et al. 2007)</td>
<td>Cross-sectional</td>
<td>3042 men and women age ≥18 years, healthy</td>
<td>Fish intake</td>
<td>Food frequency questionnaire</td>
<td>Lower QTc and had lower likelihood of having prolonged QT-interval among those with fish intake ≥300 g/week</td>
</tr>
<tr>
<td>Kirkegaard et al. 2012 (Kirkegaard, Svensson et al. 2012)</td>
<td>RCT</td>
<td>172 men women age 64-71 years, haemodialysis patients</td>
<td>1.7g/day fish oil vs. olive oil for 12 weeks</td>
<td>24-hour dietary recall/Serum cholesteryl esters</td>
<td>No effect on QTc duration</td>
</tr>
<tr>
<td>Miller et al. 2018 (Miller, C., Karimi et al. 2018)</td>
<td>Cross-sectional</td>
<td>94 men and women mean age 48.9 years, healthy</td>
<td>plasma EPA+DHA, blood methylmercury, different types of fish intake</td>
<td>Food frequency questionnaire/plasma</td>
<td>No association between EPA+DHA, methylmercury, and total fish intake and QTc</td>
</tr>
</tbody>
</table>

CVD, cardiovascular disease; EPA, Eicosapentaenoic Acid; DHA, Docosahexaenoic Acid; QTc, heart-rate corrected QT duration; RCT, Randomized control trial; PUFA, Polyunsaturated fatty acids; ECG, Electrocardiogram.
Long-chain omega-3 PUFAs, mercury and exercise cardiac power

There is no prior data regarding the association of long-chain omega-3 PUFA and mercury with the ECP; however, few supplementation studies have been conducted to evaluate the impact of long-chain omega-3 PUFAs on the VO₂max and peak SBP during exercise, but with conflicting results. The results of these RCTs are summarized in Table 5.

In a few small RCTs, fish oil (EPA+DHA) supplementation did not significantly influence the VO₂max level in normal-weight sedentary men (Boss, Lecoultre et al. 2010), overweight and obese middle-aged and older men and women (DeFina, Marcoux et al. 2010), healthy untrained men (Kawabata, Neya et al. 2014), male athletes (Raastad, Hastmark et al. 1997, Peoples, McLennan et al. 2008, Lewis, Radonic et al. 2015). Two RCTs indicated the beneficial impact of the fish oil supplementation on the VO₂max level among overweight women (Haghravan, Keshavarz et al. 2016) and endurance-trained athletes (Żebrowska, Mizia-Stec et al. 2015).

Data on the association of long-chain omega-3 PUFAs with peak SBP during exercise is very limited. In one small RCT, consumption of DHA-rich meals led to a lower systemic vascular resistance and to a smaller increase in SBP during exercise in healthy young men compared to the control meal (high-oleic sunflower oil) (Rontoyanni, Hall et al. 2012), while two other studies did not find any effect of fish oil supplementation on exercise-induced blood pressure among well-trained men (Peoples, McLennan et al. 2008) and men with a history of myocardial infarction (O’Keefe, Abuissa et al. 2006), compared to those eating the control meal (olive oil and corn + olive oil, respectively). Different study populations, the dose of fish oil, the duration of the RCT and the type of control meal can partially explain the inconsistent results regarding the effect of long-chain omega-3 PUFAs with VO₂max and SBP during exercise.

No epidemiological studies have evaluated the association of methylmercury on VO₂max and peak SBP during exercise.
<table>
<thead>
<tr>
<th>Authors</th>
<th>Study population</th>
<th>Exposure</th>
<th>Exposure assessment</th>
<th>Control</th>
<th>Duration</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vo2max</strong></td>
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</tr>
<tr>
<td>Raastad et al. 1997</td>
<td>28 men age 18-35 years, soccer players</td>
<td>5.2g/day of fish oil</td>
<td>Erythrocyte cell membrane</td>
<td>Corn oil</td>
<td>10 weeks</td>
<td>No effect on Vo2max</td>
</tr>
<tr>
<td>(Raastad, Hastmark et al. 1997)</td>
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</tr>
<tr>
<td>Peoples et al. 2008</td>
<td>16 men mean age 27±2.7 years, well-trained cyclists</td>
<td>3.2g/day fish oil</td>
<td>Erythrocyte cell membrane</td>
<td>Olive oil</td>
<td>8 weeks</td>
<td>No effect on Vo2max or SBP during exercise</td>
</tr>
<tr>
<td>(Peoples, McLennan et al. 2008)</td>
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</tr>
<tr>
<td>DeFinas et al. 2010</td>
<td>128 men and women age 30-60, overweight and obese</td>
<td>3g/day fish oil</td>
<td>Serum cholesterly esters</td>
<td>Soybean + corn oil</td>
<td>24 weeks</td>
<td>No effect on Vo2max</td>
</tr>
<tr>
<td>(DeFinas, Marcoux et al. 2010)</td>
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<tr>
<td>Boss et al. 2010</td>
<td>16 men age 22-25 years, normal-weight sedentary</td>
<td>1.8/day fish oil</td>
<td>Plasma concentration</td>
<td>Olive oil</td>
<td>10 days</td>
<td>No effect on Vo2max</td>
</tr>
<tr>
<td>(Boss, Lecouttre et al. 2010)</td>
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<tr>
<td>Kawabata et al. 2014</td>
<td>20 men mean age 23±1 years, healthy and untrained</td>
<td>3.6 g/day of EPA-rich fish oil</td>
<td>Erythrocyte cell membrane</td>
<td>A medium-chain triglyceride</td>
<td>8 weeks</td>
<td>No effect on Vo2max</td>
</tr>
<tr>
<td>(Kawabata, Neya et al. 2014)</td>
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<tr>
<td>Lewis et al. 2015</td>
<td>30 men mean age 25±4.6 years, athletes</td>
<td>375 mg EPA + 230 mg DPA + 510 mg DHA/day fish oil</td>
<td>Plasma concentration</td>
<td>Olive oil</td>
<td>3 weeks</td>
<td>No effect on Vo2max</td>
</tr>
<tr>
<td>(Lewis, Radonic et al. 2015)</td>
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<tr>
<td>Study Authors and Year</td>
<td>Study Design</td>
<td>Participants</td>
<td>Intervention</td>
<td>Outcome(s)</td>
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<tr>
<td>Zebrowska et al. 2015</td>
<td>13 men mean age 23.1±5.4, elite cyclists</td>
<td>2.6g/day (fish oil + vitamin E)</td>
<td>Serum concentration</td>
<td>Olive oil</td>
<td>3 weeks</td>
<td>Increased Vo2max</td>
</tr>
<tr>
<td>Haghravan et al. 2016</td>
<td>50 women age 20-45 years, overweight</td>
<td>0.9/day fish oil</td>
<td>Plasma concentration</td>
<td>Olive oil</td>
<td>8 weeks</td>
<td>Increased Vo2max</td>
</tr>
</tbody>
</table>

**SBP during Exercise**

<table>
<thead>
<tr>
<th>Study Authors and Year</th>
<th>Study Design</th>
<th>Participants</th>
<th>Intervention</th>
<th>Outcome(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O'Keefe et al. 2006</td>
<td>18 men mean age 67.8±6.5 years, with documented CHD</td>
<td>0.81 mg/day fish oil</td>
<td>Serum concentration</td>
<td>Corn + olive oil</td>
</tr>
<tr>
<td>Rontoyanni et al. 2012</td>
<td>22 men mean age 23±3.6 years, healthy</td>
<td>4.7 g/day EPA or DHA</td>
<td>Plasma concentration</td>
<td>High-oleic sunflower oil</td>
</tr>
</tbody>
</table>

BP, Blood pressure; CHD, Coronary heart disease; EPA, Eicosapentaenoic Acid; DHA, Docosahexaenoic Acid; DPA, Docosapentaenoic Acid; HR, heart rate; RCT, Randomized controlled trial; PUFA, Polyunsaturated fatty acids; SBP, Systolic blood pressure.
Long-chain omega-3 PUFAs, mercury and heart rate

Several clinical and epidemiological studies have assessed the association between long-chain omega-3 PUFAs and resting HR. The results of a few observational studies have shown that a higher intake of long-chain omega-3 PUFAs is associated with the lower resting HR (Table 6). In a collaborative cross-sectional study from United Kingdom and France, a higher intake of fish was associated with decreased resting HR in 9758 middle-aged healthy men without a history of CHD (Dallongeville, Yarnell et al. 2003). This finding was later confirmed with a large epidemiological study among healthy population from US (Mozaffarian, Prineas et al. 2006, Mozaffarian, Gottdiener et al. 2006) and a cross-sectional study among among 181 Canadian adults (Valera, Dewailly et al. 2011a).

Table 6. Summary of the previous cross-sectional studies on the association of omega-3 PUFAs with resting HR

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study population</th>
<th>Exposure assessment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dallongeville et al. 2003 (Dallongeville, Yarnell et al. 2003)</td>
<td>9758 men age 50-59 years, without CHD</td>
<td>Fish intake vs. non-intake</td>
<td>Decreased resting HR</td>
</tr>
<tr>
<td>Mozaffarian et al. 2006 (Mozaffarian, Prineas et al. 2006)</td>
<td>5,096 men and women age≥65 years</td>
<td>Dietary fish and omega-3 fatty acid intake (intake of tuna or other broiled or baked fish)</td>
<td>Higher intake of tuna or other broiled or baked fish was associated with the lower resting HR</td>
</tr>
<tr>
<td>Mozaffarian et al. 2006 (Mozaffarian, Gottdiener et al. 2006)</td>
<td>5,073 men and women age≥65 years</td>
<td>Intake of tuna or other broiled or baked fish versus fried fish</td>
<td>Higher intake of tuna or other broiled or baked fish was associated with reduced resting HR</td>
</tr>
<tr>
<td>Valera et al. 2011 (Valera, Dewailly et al. 2011a)</td>
<td>181 men and women age ≥40 years</td>
<td>EPA, DHA</td>
<td>Decreased resting HR, but only among women</td>
</tr>
</tbody>
</table>

CHD, Coronary heart disease; EPA, Eicosapentaenoic Acid; DHA, Docosahexaenoic Acid; DPA, Docosapentaenoic Acid; HR, heart rate.

The impact of long-chain omega-3 PUFAs on the resting HR have been mainly evaluated in some RCTs; these have reported a beneficial impact of long-chain omega-3 PUFAs supplementation (Grimsgaard, Bonaa et al. 1997, Mori, Burke et al. 2000, Prabodh Shah, Ichiuji et al. 2007, Hansen, A., Olson et al. 2014, Logan, Spriet 2015) and DHA-rich fish oil (Stark, Holub 2004, Theobald, Goodall et al. 2007) on the
resting HR among healthy population. However, a few RCTs did not find support for these kinds of beneficial effects (Geelen, Zock et al. 2003, Walser, Stebbins 2008, Noreen, Brandauer 2012, Cottin, Alsaleh et al. 2016). Details of these RCTs are presented in Table 7.
Table 7. Summary of the previous randomised control trials on the effect of omega-3 PUFAs supplementation on resting HR among healthy populations

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study population</th>
<th>Exposure</th>
<th>Exposure assessment</th>
<th>Control</th>
<th>Duration</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grimsgaard et al. 1997 (Grimsgaard, Bonaa et al. 1997)</td>
<td>234 men age 36-56</td>
<td>3.8 EPA, 3.6 DHA g/day</td>
<td>Ethyl ester concentration</td>
<td>Corn oil</td>
<td>7 weeks</td>
<td>Decreased resting HR only by DHA</td>
</tr>
<tr>
<td>Mori et al. 2000 (Mori, Burke et al. 2000)</td>
<td>56 men and women age 50-59</td>
<td>4.0g/day purified EPA or DHA</td>
<td>Plasma phospholipid concentration</td>
<td>Olive oil</td>
<td>6 weeks</td>
<td>Decreased resting HR only by DHA</td>
</tr>
<tr>
<td>Geelen et al. 2003 (Geelen, Zock et al. 2003)</td>
<td>84 men and women age 50-70</td>
<td>3.5g/day fish oil</td>
<td>Serum cholesteryl esters</td>
<td>sunflower oil</td>
<td>12 weeks</td>
<td>No effect</td>
</tr>
<tr>
<td>Stark and Holub, 2004 (Stark, Holub 2004)</td>
<td>64 women age 45-70</td>
<td>2.8g/day DHA-rich fish oil</td>
<td>Serum phospholipid concentration</td>
<td>mixture of corn and soy oil</td>
<td>4 weeks</td>
<td>Decreased resting HR</td>
</tr>
<tr>
<td>Shah et al. 2007 (Prabodh Shah, Ichiuji et al. 2007)</td>
<td>26 men and women age 26-41</td>
<td>1.0g/day fish oil</td>
<td>Serum phospholipid concentration</td>
<td>Corn oil</td>
<td>2 weeks</td>
<td>Decreased resting HR</td>
</tr>
<tr>
<td>Theobald et al. 2007 (Theobald, Goodall et al. 2007)</td>
<td>38 men and women age 40–65</td>
<td>0.7g/day DHA-rich fish oil</td>
<td>Erythrocyte cell membrane</td>
<td>Olive oil</td>
<td>12 weeks</td>
<td>Decreased resting HR</td>
</tr>
<tr>
<td>Walser et al. 2008 (Walser, Stebbins 2008)</td>
<td>21 men and women age 20-48</td>
<td>5g/day fish oil</td>
<td>Serum phospholipid concentration</td>
<td>Safflower</td>
<td>6 weeks</td>
<td>No effect</td>
</tr>
<tr>
<td>Noreen et al. 2012 (Noreen, Brandauer 2012)</td>
<td>40 men age 19-55</td>
<td>2.4g/day fish oil</td>
<td>Serum fatty acids composition</td>
<td>Safflower</td>
<td>6 weeks</td>
<td>No effect</td>
</tr>
<tr>
<td>Hansen et al. 2014 (Hansen, Olson et al. 2014)</td>
<td>95 men age 21-60</td>
<td>Salmon 3 times/week</td>
<td>multiple-day food record</td>
<td>Chicken, pork or beef</td>
<td>23 weeks</td>
<td>Decreased resting HR</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Intervention</td>
<td>Outcome Measure</td>
<td>Type of Oil</td>
<td>Duration</td>
<td>Effect</td>
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<tr>
<td>Logan and Spriet, 2015 (Logan, Spriet 2015)</td>
<td>24 women age 60-76</td>
<td>3g/day fish oil</td>
<td>Serum phospholipid concentration</td>
<td>Olive oil</td>
<td>12 weeks</td>
<td>Decreased resting HR</td>
</tr>
<tr>
<td>Cottin et al. 2016 (Cottin, Alsaleh et al. 2016)</td>
<td>30 men age 18-45</td>
<td>3g/day fish oil</td>
<td>Erythrocyte cell membrane</td>
<td>Olive oil</td>
<td>6 weeks</td>
<td>No effect</td>
</tr>
</tbody>
</table>

EPA, Eicosapentaenoic Acid; DHA, Docosahexaenoic Acid; HR, heart rate; RCT, Randomized controlled trial.
Very little is known regarding the impact of long-chain omega-3 PUFAs on the peak HR during exercise as well as on the HR recovery after exercise. It has been reported that fish oil supplementation did not alter HR during submaximal exercise in 26 healthy men (Macartney, Hingley et al. 2014), as well as in football players (Buckley, Burgess et al. 2009), in men with healed myocardial infarctions (O’Keefe, Abuissa et al. 2006), and in patients with history of coronary artery disease (Vacek, Harris et al. 1989). In contrast, there is only one small RCT which has reported that fish oil supplementation decreased peak HR during exercise test among well-trained men (Peoples, McLennan et al. 2008), which might be related to the difference in the control diets and study populations (Table 8).

Rather few small supplementation studies have examined the impact of long-chain omega-3 PUFA supplementation on the HR recovery after exercise and they have reported a faster HR recovery after an exercise test by fish oil supplementation (Macartney, Hingley et al. 2014, O’Keefe Jr, Abuissa et al. 2006) (Table 8). In one cross-sectional study among 992 patients with stable coronary artery disease from US, long-chain omega-3 PUFA (DHA + EPA) concentrations were strongly associated with a better HR recovery (Moyers, Farzaneh-Far et al. 2011). The conflicting results regarding the impact of long-chain omega-3 PUFAs and HR, especially in RCTs, can be partially explained by the differences in the study setting, study population, dosage or length of supplementation period or due to the use of antiarrhythmic and other cardioprotective medications.
Table 8. Summary of the previous randomised control trials on the effect of omega-3 PUFAs supplementation on with peak HR during exercise and HR recovery

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study population</th>
<th>Exposure</th>
<th>Exposure assessment</th>
<th>Control</th>
<th>Duration</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacek et al. 1989 (Vacek, Harris et al. 1989)</td>
<td>8 patients with history coronary artery disease, mean age 54±5</td>
<td>10g/d fish oil</td>
<td>-</td>
<td>Vegetable oil</td>
<td>6 weeks</td>
<td>No effect on peak HR</td>
</tr>
<tr>
<td>O’Keefe et al. 2006 (O’Keefe, Abuissa et al. 2006)</td>
<td>18 men with documented CHD, mean age 68±6</td>
<td>0.81g/d fish oil</td>
<td>Serum concentration</td>
<td>Corn and olive oils</td>
<td>16 weeks</td>
<td>No effect on peak HR, faster HR recovery</td>
</tr>
<tr>
<td>Peoples et al. 2008 (Peoples, McLennan et al. 2008)</td>
<td>16 Well-trained men, mean age 27±3</td>
<td>8g/d fish oil</td>
<td>Erythrocyte cell membrane</td>
<td>olive oil</td>
<td>8 weeks</td>
<td>Decreased peak HR</td>
</tr>
<tr>
<td>Buckley et al. 2009 (Buckley, Burgess et al. 2009)</td>
<td>25 men, football players, mean age 22±1, 23±1</td>
<td>6g/d DHA-rich fish oil</td>
<td>Erythrocyte cell membrane</td>
<td>Sunflower oil</td>
<td>5 weeks</td>
<td>No effect on peak HR</td>
</tr>
<tr>
<td>Macartney et al. 2014 (Macartney, Hingley et al. 2014)</td>
<td>26 physically fit men, mean age 24±7, 28±5</td>
<td>0.7g/d fish oil</td>
<td>Erythrocyte cell membrane</td>
<td>Soya bean oil</td>
<td>4 weeks</td>
<td>No effect on peak HR, faster HR recovery</td>
</tr>
</tbody>
</table>

EPA, Eicosapentaenoic Acid; DHA, Docosahexaenoic Acid; HR, heart rate; RCT, Randomized control trial.
Data on the association of mercury on HR is limited. According to the three cross-sectional studies conducted in healthy adults, blood and hair mercury concentrations were not associated with the resting HR (Choi, Weihe et al. 2009, Valera, Dewailly et al. 2011, Valera, Dewailly et al. 2011b). This finding is in line with the result of one 14-week intervention study that blood methylmercury from bigeye tuna and swordfish did not alter the resting HR among 54 healthy Japanese adults (mean age 25.2 ± 4.1) (Yaginuma-Sakurai, Murata et al. 2010). In contrast, according to the finding of one study, a higher blood methylmercury concentration was associated with increased resting HR among adults (Valera, Dewailly et al. 2013) (Table 9).

Table 9. Summary of the previous cross-sectional studies on the association of mercury exposure with resting HR

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study population</th>
<th>Exposure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choi et al. 2009 (Choi, Weihe et al. 2009)</td>
<td>70 men from Faroe Islands mean age 54.8 ± 9.4</td>
<td>Blood, toenail and hair mercury</td>
<td>No association</td>
</tr>
<tr>
<td>Valera et al. 2011 (Valera, Dewailly et al. 2011)</td>
<td>791 Canadian adults age 34–36 years</td>
<td>Hair and blood mercury</td>
<td>No association</td>
</tr>
<tr>
<td>Valera et al. 2011 (Valera, Dewailly et al. 2011b)</td>
<td>180 Canadian adults age≥ 18 years and 101 teenagers age 12-17 years</td>
<td>Hair and blood mercury</td>
<td>No association</td>
</tr>
<tr>
<td>Valera et al. 2013 (Valera, Dewailly et al. 2013)</td>
<td>313 Canadian adults age≥18 years</td>
<td>Blood mercury</td>
<td>Increased</td>
</tr>
</tbody>
</table>

BP, Blood pressure; CHD, Coronary heart disease; EPA, Eicosapentaenoic Acid; DHA, Docosahexaenoic Acid; DPA, Docosapentaenoic Acid; HR, heart rate; HRV, Heart rate variability; RCT, Randomized control trial.
Long-chain omega-3 PUFAs, mercury and exercise-induced myocardial ischemia

One small RCT has been conducted to evaluate the effect of long-chain omega-3 PUFA supplementation on the myocardial ischemia during exercise (Table 10). In a double-blind, crossover RCT among 8 patients with stable CHD, dietary supplementation with fish oil for 12 weeks did not alter the parameters of myocardial ischemia during exercise, as compared to placebo (Mehta, Lopez et al. 1988). The study of Moyers et al. (2011) is the only population-based study conducted in the US, which has assessed the association of blood long-chain omega-3 PUFA concentrations and exercise-induced ischemia among 992 patients with stable coronary artery disease but they failed to find an association (Table 10) (Moyers, Farzaneh-Far et al. 2011). No prior experimental, clinical or observational study data is available regarding the possible impact of mercury exposure on the occurrence of exercise-induced myocardial ischemia.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study setting</th>
<th>Study population</th>
<th>Exposure</th>
<th>Exposure assessment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mehta et al. 1988 (Mehta,</td>
<td>RCT</td>
<td>8 patients with</td>
<td>3.2g EPA+2.2g DHA/d vs.</td>
<td>-</td>
<td>No change in subjective or objective parameters of myocardial ischemia</td>
</tr>
<tr>
<td>Lopez et al. 1988)</td>
<td></td>
<td>stable coronary</td>
<td>placebo for 12 weeks</td>
<td></td>
<td>during exercise by short-term dietary supplementation with omega-3 PUFA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>artery disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moyers et al. 2011 (Moyers,</td>
<td>Cross-</td>
<td>992 subjects with</td>
<td>EPA+DHA Serum concentration</td>
<td>Better HR recovery,</td>
<td></td>
</tr>
<tr>
<td>Farzaneh-Far et al. 2011)</td>
<td>sectional</td>
<td>stable coronary</td>
<td></td>
<td>exercise capacity and</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>artery disease</td>
<td></td>
<td>exercise time. No</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>association with</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>exercise-induced</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ischemia</td>
<td></td>
</tr>
</tbody>
</table>

CHD, Coronary heart disease; EPA, Eicosapentaenoic Acid; DHA, Docosahexaenoic Acid; HR, heart rate; RCT, Randomized control trial; PUFA, Polyunsaturated fatty acids.
Mechanisms

Although the exact mechanistic pathway, which could adequately explain the association between long-chain omega-3 PUFAs and ECG parameters is not fully clarified, the possible mechanisms underlying such associations can be partially explained by the favorable impact of these fatty acids on the cardiac endothelial function (McLennan, Abeywardena 2005, London, Albert et al. 2007). It has been demonstrated that long-chain omega-3 PUFAs influence membrane ion channel activity, cellular ion concentrations, autonomic tone, and β-adrenergic and other receptors (McLennan, Abeywardena 2005, London, Albert et al. 2007). The intakes of EPA and DHA are related to increased endothelium-derived vasodilators including nitric oxide, 3-series prostacyclin, and endothelium-dependent hyperpolarizing factor (Kinlay, Creager et al. 2001, Cottin, Sanders et al. 2011), which are predictors of endothelial dysfunction (Takase, Uehata et al. 1998).

The mechanism underlying the negative impact of mercury in the electrical and mechanical activities of the heart is still being investigated; however, it might be due to the impacts of mercury in cardiac endothelial dysfunction through increased formation of free radical stress and inflammatory responses (InSug, Datar et al. 1997), inactivation of antioxidant compounds (Naganuma, Koyama et al. 1980) and promotion of lipid peroxidation (Salonen, Seppänen et al. 1995, Cuvin-Aralar, Furness 1991), which are related to the cardiac function (Dhalla, Temsah et al. 2000, Steer, Millgård et al. 2002, Korantzopoulos, Kolettis et al. 2003). Moreover, mercury may influence negatively the autonomic nervous system (Milioni, Nagy et al. 2017), which regulates many cardiac functions, for example HR (Cheng, Yang 2005).
3 AIMS OF THE STUDY

The aim of this doctoral thesis was to elucidate potential mechanisms that could at least partly explain how the long-chain omega-3 PUFAs and mercury exposure affect the risk of CVD. The purpose was to investigate the associations of serum long-chain omega-3 PUFAs (EPA+DPA+DHA) and the hair mercury concentration, objective biomarkers of exposures, with the parameters of cardiac electrophysiology and performance in a population-based sample of eastern Finnish middle-aged and older men aged 42–60 years.

The specific aims of this doctoral thesis were:

1. To assess the association of the serum long-chain omega-3 PUFA levels and hair mercury concentrations with QTc and JTC. A secondary objective was to investigate prospectively the association of serum long-chain omega-3 PUFAs, hair mercury, QTc and JTC with risk of SCD (Study I),

2. To evaluate the serum long-chain omega-3 PUFAs and hair mercury concentrations with ECP and its components, VO_{2}max and maximal SBP during exercise (Study II),

3. To examine the association between serum long-chain omega-3 PUFAs levels and hair mercury concentrations with a resting HR, peak HR during exercise and HR recovery after exercise (Study III),

4. To determine the association between the serum long-chain omega-3 PUFAs levels and hair mercury concentrations with the occurrence of exercise-induced myocardial ischemia, among men with or without a history of CHD. (Study IV).
4 SUBJECTS AND METHODS

4.1 STUDY POPULATION

This doctoral thesis used the data from the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD). The KIHD is an ongoing population-based study examining the risk factors for CVD, atherosclerosis, and related outcomes in men from Kuopio and the surrounding communities in eastern Finland (Salonen. 1988). The baseline examinations were carried out between March 1984 and December 1989. The study sample consisted of all men aged 42, 48, 54, or 60 years at the time of the baseline examinations and who were living in the city of Kuopio or surrounding rural communities (age-stratified random sample). Of the 3235 applicable men, 2682 (82.9%) joined the study and participated in the baseline examinations. Data at baseline was collected by questionnaires and in the examinations done by a study nurse and a physician at a study visit (please see below for more details).

The baseline data was used for this doctoral thesis. In Study I, men with a history of CHD at baseline (n=730) and those who had wide QRS complex (QRS≥120 ms) (bundle branch block, n=429) were excluded from analyses. Of the remaining men, complete data was available for 1411 men. In Study II, we excluded men with history of CHD at baseline (n=677). Of the remaining men, 1672 men with exercise test data were included in the final analysis. In Study III, we excluded participants with a history of CHD (n=1016) and those using beta-blockers (n=135). We also excluded one participant with resting HR outside of the nomogram range (HR<30 or HR>130 beats/min). After the exclusions, the final study population included 1008 men with complete data. In Study IV, 2199 men with complete data were included in the analysis. The study protocol was approved by the Research Ethics Committee of the University of Kuopio. All subjects gave their written informed consent for participation. The details of the study population used in each original study are presented in Table 11.
Table 11. Summary of the study design, participants, exposure variables, outcomes of interests in study I-IV.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>Outcome of interest</th>
<th>Exclusion criteria</th>
<th>Number of participants</th>
</tr>
</thead>
</table>
| I     | • Cross-sectional  
        • Prospective | • Heart rate-corrected  
                        • QT- and JT-intervals  
                        • Risk of sudden cardiac power | • QRS≥120 ms (n=429)  
                        • Missing values of exposures and outcome (n=112)  
                        • History of CHD (n=730) | 1411                   |
| II    | • Cross-sectional | • Exercise cardiac power  
                        • VO₂max  
                        • Maximal SBP during exercise | • Missing values of exposures and outcomes (n=333)  
                        • History of CHD (n=677) | 1672                   |
| III   | • Cross-sectional | • Resting heart rate  
                        • Maximal heart rate during exercise  
                        • Heart rate recovery after exercise | • Missing value of exposures and outcome (n=522)  
                        • History of CHD (n=1016)  
                        • Using beta-blockers (n=135)  
                        • Resting HR outside of the nomogram range (<30 or>130 beats/min) (n=1) | 1008                   |
| IV    | • Cross-sectional | • Exercise-induced myocardial ischemia | • Missing value of exposures and outcomes (n=483) | 2199                   |

CHD, coronary heart disease; HR, heart rate; SBP, systolic blood pressure.
4.2 DETERMINATION OF SERUM FATTY ACIDS AND MERCURY

Venous blood and hair samples were obtained between 8 A.M. and 10.00 A.M at the baseline examinations after having abstained from ingesting alcohol for 3 days, smoking for 12 hours, and eating for 12 hours. After the subject had rested in the supine position for 30 minutes, blood was drawn with Terumo Venoject VT-100PZ vacuum (Terumo Corp., Tokyo). No tourniquet was used for blood sampling. A study nurse cut a hair sample of a pre-specified length from the scalp hair of the subjects for mercury measurements, which weighed about 40 mg (Salonen, Seppanen et al. 1995). Hair samples were processed in a random order in the Department of Chemistry of the University of Kuopio and were measured by a chemist.

Serum fatty acids were measured in 1991 from samples that had been stored at -80°C until analysis, in one gas chromatographic run without pre-separation, as described previously (Laaksonen, Lakka et al. 2002). Serum fatty acids were extracted with chloroform-methanol. The chloroform phase was evaporated and treated with sodium methoxide, which methylated esterified fatty acids. Quantification was carried out with reference standards (Check Prep Inc., Elysian, MN). Each analyte had its own individual reference standard, and there was also an internal standard, eicosan. 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 and in the data analyses, the proportion of one fatty acid from the total serum fatty acids was calculated. The coefficient of variation was 9.4% for EPA (20:5n-3), 12.7% for docosapentaenoic acid (DPA, 22:5n-3) and 11.9% for DHA (22:5n-3). In the determination pf the serum total long-chain omega-3 PUFA concentrations, we used the sum of EPA, DPA and DHA levels. The repeatability of the fatty acid measurements was assessed in a subsample of 739 generally healthy men, for whom data was available both from the baseline examinations and from examinations conducted 4 years later. The correlation coefficients were 0.47 for EPA, 0.54 for DPA and 0.56 for DHA (Laaksonen, Lakka et al. 2002).

Hair mercury was determined between May 1992 and August 1993. Hair mercury was detected by flow injection analysis-cold vapor atomic absorption spectrometry and amalgamation (Salonen, Seppänen et al. 1995). The coefficient of variation was between 7.7% and 8.6%. Repeat hair samples were collected from 21 subjects between 4 to 9 years (mean, 6 years) after the baseline examination as a kind of survey for tracking of hair mercury values over time. The Pearson correlation coefficient between the original and the repeat measurement was 0.91.
4.3 ASSESSMENT OF CARDIAC FUNCTION PARAMETERS

All electrocardiographic intervals and amplitudes were measured automatically from standard 12-lead electrocardiographic recording (Furberg, Manolio et al. 1992). Paper speed was 50 mm/s. A maximal symptom-limited exercise tolerance test was performed between 8:00 AM and 10:00 AM using an electrically braked cycle ergometer (Lakka, Venalainen et al. 1994). The tests were supervised by an experienced physician with the assistance of an experienced nurse. In the men examined before June 1986, the testing protocol comprised a 3-minute warm-up at 50 W followed by a step-by-step increase in workload by 20 W/min. The remaining men were tested with a linear increase in the workload at 20 W/min. An electrocardiography (ECG) recording was printed every 30 seconds during exercise. The Mason-Likar lead system was used, including leads VI, V5, and aVF. An ECG recording, which was coded manually by a cardiologist, was printed every 30 seconds based on averages of 6-second intervals during exercise.

4.3.1 QT- and JT-intervals

The QT-interval was measured from the onset of the QRS complex to the end of the T-wave; the T-wave was characterized as the intersection of the isoelectric line and the tangent of the maximal slope on the downward limb of the T-wave (Statters, Malik et al. 1994). Because of the strong correlation between the QT-interval and heart rate, heart rate-corrected QT- and JT-intervals were calculated by using the Bazett’s formula, which is the most commonly used QT correction formula (Ahnve 1985). QTc = QT/√RR (RR = the interval between 3 consecutive R-waves in the ECG) (Stramba-Badiale, Karnad et al. 2018); and JTc = QTc-QRS.

4.3.2 Exercise cardiac power and its components

ECP was measured by the ratio of measured VO₂max with peak SBP (Kurl, Laukkanen et al. 2005). VO₂max was defined as the highest value for or the plateau of oxygen uptake in a respiratory gas analyser (Lakka, Venalainen et al. 1994). Blood pressure was measured every 2 minutes both manually and automatically during exercise until the test was stopped and every 2 minutes after the exercise. After the exercise test, each participant remained seated on the bicycle for 8 minutes. The highest SBP achieved during the exercise test was defined as the maximum exercise SBP. For safety reasons, all tests were supervised by an experienced physician with the assistance of an experienced nurse. ECG was recorded continuously with the Kone 620 electrocardiograph (Kone, Turku, Finland) (Laukkanen, Mäkkikallio et al. 2010, Kurl, Laukkanen et al. 2001).

4.3.3 Heart rate

HR was recorded from an ECG at rest, at the end of each 60-s interval during the exercise test, at peak exercise and during recovery. Resting HR was expressed as the
lowest HR value, whether measured in a supine position before the test (about 5 min rest before the test) or while sitting on a bicycle at the initiation of the test, whichever gave the lowest reading. Exercise ECGs were coded manually by one cardiologist at the initiation of the test for 8 minutes. During recovery, the workload was set to 0 watts and subjects could continue pedalling at a self-chosen frequency as desired. No predefined pedalling frequency was used during recovery. HR recovery was defined a priori as the reduction in HR from HR peak to HR at 2 min after the exercise test to maximize the number of subjects included in the analyses, because values of HR at 1 min after the exercise test were not available for all men.

4.3.4 Myocardial ischemia

Myocardial ischemia during exercise and after 5 min of recovery was defined as ischemia in the ECG without typical chest pain indicating CHD. The criteria for ischemia in ECG during exercise and recovery were horizontal or down-sloping ST depression ≥1.0 mm at 80 ms after J point or any ST depression of >1.0 mm at 80 ms after J point (Salonen, Nyyssonen et al. 1992).

4.3.5 Sudden cardiac death

All deaths that occurred by the end of 2013 were checked from the hospital documents, health center wards, and death certificates. The sources of information were interviews, hospital documents, death certificates, autopsy reports, and medico-legal reports. 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 paramedical staff. All the documents related to the death were scrupulously cross-checked 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 as a SCD when it occurred either within 1 h after the onset of an abrupt change in symptoms or within 24 h after the 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 considered as a SCD. Follow-up time was calculated from the baseline to the date of SCD, other death or the end of follow-up in 31.12.2013, whichever came first.

4.4 OTHER MEASUREMENTS

Intakes of foods and nutrients were assessed at the time of the baseline blood sampling. Subjects used household measures to record quantitatively their food intake over a 4-day period as instructed by a nutritionist who also crosschecked the completed food intake records at the study visit. The intakes of food and nutrients
were estimated using the NUTRICA® software (version 2.5; Social Insurance Institution, Turku, Finland), which includes a comprehensive database of 1300 food items and dishes and 30 nutrients. The data bank of Nutrica is compiled using mainly Finnish values for the nutrient composition of foods devised by the Social Insurance Institution, Turku, Finland). Smoking habits (current/previous smoking, number of cigarettes per day), history of CVD events (myocardial infarction, angina pectoris, other ischemic heart disease), and medication use were recorded using a self-administered questionnaire, checked by the interviewer and the physician. Anthropometry parameters (height, weight, body mass index, body circumferences (waist and hip)) were measured by a study nurse and BMI was calculated as weight (kg)/height (m2) (Salonen 1988). Physical activity was evaluated based on the 12-month leisure-time physical activity questionnaire and expressed as kcal/day. The subjects were asked to record the frequency (number of sessions per month), average duration (hours and minutes per session), and intensity (scored as 0 for recreational activity, 1 for conditioning activity, 2 for brisk conditioning activity, and 3 for competitive, strenuous exercise) for each activity performed (Lakka, Venalainen et al. 1994).

For lipids and lipoproteins, fresh serum samples were separated by using ultracentrifugation (with a Kontron TGA-65 ultra-centrifuge) at 20°C for 10 minutes. Resting blood pressure was measured by a study nurse with a random-zero mercury sphygmomanometer. The measuring protocol included, after a supine rest of 5 minutes, three measurements in the supine, one in the standing, and two in the sitting position with 5-minute intervals. The mean of all six systolic pressure values was used in the present analyses as the systolic blood pressure and the mean of all six diastolic measurements as the diastolic blood pressure. Hypertension diagnosis was defined as systolic/diastolic blood pressure >140/90 mmHg and was checked twice at the study visit, or as a clinical diagnosis of hypertension or the use of hypertension medication. Diabetes was defined as a self-reported physician diagnosis of type 2 diabetes and/or fasting blood glucose concentration ≥6.7 mmol/L at the study visit. CHD at baseline was defined either as a history of myocardial infarction or angina pectoris, angina pectoris on effort, the use of nitroglycerin for chest pain once a week or more frequently or chest pain as a cause of stopping the baseline exercise stress test.

4.5 DATA ANALYSES

The baseline characteristics of the KIHD study population were described using means with standard deviation for continuous variables and chi-square test for categorical variables (Studies I – IV). In all studies, correlations between individual long-chain omega-3 PUFAs were evaluated by the calculation of Spearman correlation coefficients. The selection of potential confounders was based on established risk factors for CHD, previously published associations in the KIHD study, or on associations with exposures or outcomes in the analyses. Missing values
in covariates were replaced by population means (<0.5% of the values). Those covariates (e.g. dietary factors) that did not alter the associations were not included in the final models. All P-values were two-sided (α=0.05). The mean values of outcome in the quartiles of the long-chain omega-3 PUFAs and hair mercury were analysed using analysis of covariance (ANCOVA), adjusted for potential confounders (Study I – III). Logistic regression models were used to estimate odds ratios (OR) for the odds of prolonged QT- and JT-intervals (Study I) and exercise-induced myocardial ischemia (Study IV) in exposure quartiles, with the lowest category as the reference.

In Study I, a multivariable Cox proportional hazards regression model was used to evaluate the hazard ratio (HR) of incident SCD in tertiles of the long-chain omega-3 PUFA concentrations, QTc and JTc. It is noteworthy that tertiles were used because of the lower number of events in these analyses than in the other analyses. Further adjustments for QTc or JTc were done in order to check whether adjusting for QTc or JTc would attenuate the previously observed associations of the levels of serum of long-chain omega-3 PUFA and hair mercury with the risk of incident SCD (Virtanen, Laukkanen et al. 2012) (Study I). Statistical significance of the interactions on a multiplicative scale was assessed by stratified analysis with hair mercury divided by the median (Study I & II) and with CHD status (Study IV) and likelihood ratio tests with a cross-product term. For assessing the clinical significance, we calculated effect sizes based on the Cohen’s d index (the difference between the group means divided by the standard deviation of the comparison category) (Ferguson 2009) (Study II & III). The specific statistical analysis methods and models for each study of this doctoral thesis are presented in Table 12. Statistical analyses were performed with SPSS software version 21 (Study I & II) and 23 (Study III & IV) for Windows (IBM Corp., Armonk, New York, USA).
<table>
<thead>
<tr>
<th>Study</th>
<th>Outcome</th>
<th>Statistical methods</th>
<th>Adjusted covariates in final model*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>QTc and JTC</td>
<td>ANCOVA</td>
<td>Age, examination year, BMI, type 2 diabetes, smoking status, leisure-time physical activity, education, income, treated hypertension, alcohol intake and energy intake.</td>
</tr>
<tr>
<td></td>
<td>Prolonged QTc and JTC**</td>
<td>Logistic regression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sudden cardiac death</td>
<td>Cox regression</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Exercise cardiac power</td>
<td>ANCOVA</td>
<td>Age, examination year, BMI, type 2 diabetes, smoking status, leisure-time physical activity, education, income, treated hypertension, bronchial asthma, LDL, HDL, CRP and intakes of energy, carbohydrates and alcohol.</td>
</tr>
<tr>
<td></td>
<td>VO₂max</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maximum SBP during exercise</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Resting HR</td>
<td>ANCOVA</td>
<td>Age, examination year, BMI, type 2 diabetes, smoking status, leisure-time physical activity, education, income, treated hypertension, fasting blood glucose, alcohol intake and energy intake.</td>
</tr>
<tr>
<td></td>
<td>Maximum HR during Exercise</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HR recovery after exercise</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>exercise-induced myocardial ischemia</td>
<td>Logistic regression</td>
<td>Age, examination year, BMI, type 2 diabetes, smoking status, leisure-time physical activity, treated hypertension, alcohol intake and energy intake.</td>
</tr>
</tbody>
</table>

ANCOVA, analysis of covariance; BMI, body mass index; CRP, C-reactive protein; HDL, high density lipoprotein cholesterol; HR, heart rate; LDL, low density lipoprotein cholesterol; PUFA, Polyunsaturated fatty acids; SBP, systolic blood pressure.

* Additional adjustments for other covariates did not appreciably change the associations (<5% change in estimates).

** We used the 95th percentile of the distribution of QTc and JTC to define abnormal QTc (≥445.67 ms) and JTC (≥343.83 ms).
5 RESULTS

5.1 BASELINE CHARACTERISTICS

The baseline characteristics of the KIHD cohort in Study I-IV are presented in Table 13. The characteristics of the study populations in the four studies are generally similar, which meant that data from the baseline examinations (1984 to 1989) could be used in all these studies. The mean age of the participants ranged from 51.4 (SD 5.7) to 52.4 (SD 5.3) years.

The mean intakes were as follows; total fat 38.7 (SD 5.6)-39.0 (SD 5.5) E%; saturated fat, 18.2 (SD 3.8)-18.4 (SD 4.3) E%; monounsaturated fat, 11.7 (SD 2.1)-11.8 (SD 2.1) E%; PUFAs, 4.5 (SD 1.4) E% and Trans-fat, 1.0 (SD 0.4) E%, (values from studies I-IV, respectively). The mean intakes of protein ranged between 15.7 (SD 2.4) and 15.7 (SD 2.5) E%, for carbohydrates, the range was between 42.6 (SD 6.2) to 43.4 (SD 6.6) E%. The mean fish intake ranged between 45.4 (SD 54.1) and 46.0 (SD 53.3) g/day in studies I-IV. The mean (minimum-maximum) serum concentrations of EPA, DPA and DHA, as a percentage of all serum fatty acids, were approximately 1.70% (1.67-1.69%), 0.60% (0.55-0.56%) and 2.5% (2.48-2.64%), respectively, and the mean (minimum-maximum) hair mercury concentration was approximately 1.9 µg/g (1.88-1.94 µg/g) in studies I-IV. The correlation coefficients of the individual long-chain omega-3 PUFAs with each other and with hair mercury were 0.71 for EPA and DHA, 0.56 for EPA and DPA, 0.50 for DHA and DPA, 0.35 for EPA and mercury, 0.30 for DHA and mercury and 0.18 for DPA and mercury.

Men with higher serum total long-chain omega-3 PUFA concentrations were more likely to have a higher education, a higher body mass index (BMI), more leisure time physical activity, greater alcohol intake, elevated serum HDL cholesterol levels and higher hair mercury concentrations. They also had lower serum triglyceride levels and a lower total energy intake, as compared to the men with lower serum total long-chain omega-3 PUFAs concentrations. In Study IV, generally similar associations were observed among men with or without a history of CHD, although a higher serum total long-chain omega-3 PUFA concentration was associated with less smoking only among those without a history of CHD.

Men with higher hair mercury concentrations were older, less educated and less physically active, were more likely to be current smokers and had lower triglyceride concentrations. They also had higher BMI, greater alcohol consumption, and elevated concentrations of serum HDL and LDL cholesterol and long-chain omega-3 PUFAs.
Table 13. The baseline characteristics of the KIHD study cohort, according to the studies of this thesis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Study I (n=1411)</th>
<th>Study II (n=1672)</th>
<th>Study III (n=1008)</th>
<th>Study IV (n=2199)</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51.9 (5.4)</td>
<td>52.3 (5.3)</td>
<td>51.4 (5.7)</td>
<td>52.4 (5.3)</td>
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<tr>
<td>Education (years)</td>
<td>9.1 (3.6)</td>
<td>9.0 (3.6)</td>
<td>9.1 (3.6)</td>
<td>9.0 (3.6)</td>
</tr>
<tr>
<td>Body mass index (kg/m2)</td>
<td>26.7 (3.4)</td>
<td>26.5 (3.3)</td>
<td>26.9 (3.6)</td>
<td>26.8 (3.5)</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>30.6%</td>
<td>29.5%</td>
<td>31.0%</td>
<td>29.5%</td>
</tr>
<tr>
<td>Physical activity (kcal/day)</td>
<td>136.1 (170.7)</td>
<td>138.4 (170)</td>
<td>140.5 (163.2)</td>
<td>138.3 (169.1)</td>
</tr>
<tr>
<td>Serum EPA (E%)</td>
<td>1.69 (0.92)</td>
<td>1.67 (0.89)</td>
<td>1.69 (0.92)</td>
<td>1.67 (0.92)</td>
</tr>
<tr>
<td>Serum DPA (E%)</td>
<td>0.55 (0.10)</td>
<td>0.56 (0.10)</td>
<td>0.55 (0.10)</td>
<td>0.55 (0.10)</td>
</tr>
<tr>
<td>Serum DHA (E%)</td>
<td>2.64 (0.74)</td>
<td>2.48 (0.71)</td>
<td>2.48 (0.73)</td>
<td>2.46 (0.74)</td>
</tr>
<tr>
<td>Hair mercury (µg/g)</td>
<td>1.90 (2.0)</td>
<td>1.94 (1.99)</td>
<td>1.88 (2.06)</td>
<td>1.91 (1.96)</td>
</tr>
<tr>
<td>Serum total long-chain omega-3 PUFA (E%)</td>
<td>4.70 (1.61)</td>
<td>4.72 (1.60)</td>
<td>4.71 (1.57)</td>
<td>4.68 (1.62)</td>
</tr>
<tr>
<td>Serum triglyceride (mmol/l)</td>
<td>1.25 (0.75)</td>
<td>1.24 (0.75)</td>
<td>1.22 (0.79)</td>
<td>1.27 (0.75)</td>
</tr>
<tr>
<td>Serum HDL (mmol/l)</td>
<td>1.29 (0.29)</td>
<td>1.30 (0.29)</td>
<td>1.32 (0.30)</td>
<td>1.30 (0.29)</td>
</tr>
<tr>
<td>Serum LDL (mmol/l)</td>
<td>4.02 (0.98)</td>
<td>4.00 (0.98)</td>
<td>3.85 (0.93)</td>
<td>4.00 (0.97)</td>
</tr>
<tr>
<td>Total fat (E%)</td>
<td>38.8 (5.5)</td>
<td>38.7 (5.6)</td>
<td>39.0 (5.5)</td>
<td>38.7 (5.6)</td>
</tr>
<tr>
<td>Saturated fat (E%)</td>
<td>18.3 (3.7)</td>
<td>18.2 (3.8)</td>
<td>18.4 (3.7)</td>
<td>18.3 (3.8)</td>
</tr>
<tr>
<td>Monounsaturated fat (E%)</td>
<td>11.7 (2.1)</td>
<td>11.7 (2.2)</td>
<td>11.8 (2.1)</td>
<td>11.7 (2.2)</td>
</tr>
<tr>
<td>PUFA (E%)</td>
<td>4.5 (1.4)</td>
<td>4.5 (1.4)</td>
<td>4.5 (1.4)</td>
<td>4.5 (1.4)</td>
</tr>
<tr>
<td>Trans fat (E%)</td>
<td>1.0 (0.4)</td>
<td>1.0 (0.4)</td>
<td>1.0 (0.4)</td>
<td>1.0 (0.4)</td>
</tr>
<tr>
<td>Carbohydrate (E%)</td>
<td>43.3 (6.5)</td>
<td>43.4 (6.6)</td>
<td>42.6 (6.2)</td>
<td>43.1 (6.5)</td>
</tr>
<tr>
<td>Protein (E%)</td>
<td>15.7 (2.4)</td>
<td>15.7 (2.5)</td>
<td>15.7 (2.4)</td>
<td>15.7 (2.4)</td>
</tr>
<tr>
<td>Total energy (kcal/d)</td>
<td>2400 (625)</td>
<td>2388 (625)</td>
<td>2422 (646)</td>
<td>2380 (625)</td>
</tr>
<tr>
<td>Fish intake (g/day)</td>
<td>45.8 (54.0)</td>
<td>45.9 (54.4)</td>
<td>46.0 (53.3)</td>
<td>45.4 (54.1)</td>
</tr>
<tr>
<td>Alcohol intake (g/week)</td>
<td>72.3 (114.4)</td>
<td>72.5 (110.1)</td>
<td>73.8 (112.5)</td>
<td>74.0 (117.4)</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>4.5</td>
<td>4.4</td>
<td>3.8</td>
<td>4.6</td>
</tr>
<tr>
<td>Treated hypertension (%)</td>
<td>55.4</td>
<td>55.7</td>
<td>48.0</td>
<td>56.1</td>
</tr>
</tbody>
</table>

Results are presented as mean (SD) for continuous variables and as proportions for categorical data: EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; HDL, high density lipoprotein; LDL, low density lipoprotein; E%, percent of energy. Dietary intakes of foods and nutrients were assessed by 4-days food records.
5.2 **STUDY I: QT- AND JT-INTERVALS, SUDDEN CARDIAC DEATH**

5.2.1 **Association with serum long-chain omega-3 PUFA**

In this cross-sectional study of 1411 middle-aged and older men, the mean durations of QTc and JTc were 416 (21.8) ms and 314 (22.3) ms, respectively. In all, 104 men (7.4%) had prolonged QTc and 101 (7.2%) had prolonged JTc. In the multivariable-adjusted model, higher serum total long-chain omega-3 PUFA concentrations (EPA+DPA+DHA) were inversely associated with the QTc and JTc [the mean difference 3.2 ms (95% CI 0.003 – 6.4 ms, *P*-trend=0.02 across quartiles) for QTc and 4.6 ms (95% CI 1.3 – 7.8 ms, *P*-trend=0.002) for JTc] (Figure 5&6, respectively). When the fatty acids were investigated individually, generally similar inverse associations with the QTc and JTc were observed with EPA, DPA and DHA (Figure 5&6). The men in the highest quartile of serum total long-chain omega-3 PUFAs had 46% lower odds for displaying a prolonged QTc (95% CI -2 – 72%, *P*-trend=0.04) and 43% lower odds for a prolonged JTc (95% CI -6 – 69%, *P*-trend=0.08) compared with the subjects in the lowest quartile, after adjustments for potential confounders. The associations were generally similar when we evaluated the individual long-chain omega-3 PUFAs. The associations between DHA and JTc, and the association between DPA and QTc were borderline significant.

During the mean follow-up of 22.9 years, 85 SCD events occurred (6.0%). A higher serum total long-chain omega-3 PUFA level was associated with a lower risk of SCD [multivariable-adjusted extreme tertile HR=0.50 (95% CI 0.29 to 0.86; *P*-trend=0.02)]. A similar association was observed with DHA [multivariable-adjusted extreme tertile HR=0.45 (95% CI 0.25 to 0.81; *P*-trend=0.007)], but the associations with EPA [multivariable-adjusted extreme tertile HR=0.72 (95% CI 0.41 to 1.25; *P*-trend=0.21)] and DPA [multivariable-adjusted extreme tertile HR=0.71 (95% CI 0.44 to 1.21; *P*-trend=0.20)] were not statistically significant. Furthermore, adjustment for QTc or JTc had no appreciable impact on these associations.
5.2.2 Associations with hair mercury concentration

The hair mercury concentration was not associated with the QTc and JTc (the mean difference between extreme quartiles of hair mercury concentration was 1.9 ms (95% CI -1.4 – 5.2 ms, P-trend=0.18) for QTc and 2.8 ms (95% CI -0.6 – 6.1 ms, P-trend=0.09) for JTc) (Figure 5&6), but it slightly attenuated the association of serum long-chain omega-3 PUFAs with the QTc and JTc, although the interaction was not statistically significant (P for interaction >0.26). 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). We did not detect any association between hair mercury concentration and on either the odds of prolonged QTc and JTc or the risk of SCD. 

![Image](image-url)

Figure 5&6. Mean QTc/JTc in quartiles of serum long-chain omega-3 PUFAs and hair mercury. Black bars represent the 1st quartile, deep grey bars represent the 2nd quartile, light grey bars represent the 3rd quartile and white bars represent the 4th quartile. The values are adjusted for age, examination year, BMI, diabetes, smoking, leisure-time physical activity, education, income, treated hypertension, and intakes of energy and alcohol. P-values are for the test of trend across exposure quartiles. BMI, body mass index; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid
5.3 **STUDY II: EXERCISE CARDIAC POWER, VO₂MAX AND MAXIMAL SBP DURING EXERCISE**

5.3.1 **Association with serum long-chain omega-3 PUFA**

The mean (SD) values were 12.46 (3.07) mL/mmHg for ECP, 2545 (559) mL/min for VO₂max and 206.6 (26.5) mmHg for maximal SBP during exercise. Men with a higher serum total long-chain omega-3 PUFA concentration had higher ECP and VO₂max [the mean difference 0.42 mL/mmHg (95% CI 0.04-0.80, P-trend=0.04) for ECP and 83 mL/min (95% CI 15-152, P-trend=0.02) for VO₂max]. (Figure 7&9). Serum long-chain omega-3 PUFA levels were not associated with the maximal SBP during exercise [the mean difference 0.9 mmHg (95% CI -2.8 – 4.5, P-trend=0.69)] (Figure 8). When the fatty acids were investigated individually, in general, similar associations were observed with EPA, DPA and DHA (Figure 7-9).

5.3.2 **Association with hair mercury concentration**

The hair mercury concentration was not associated with ECP, VO₂max and maximal SBP during exercise (Figure 7-9). However, a higher hair mercury concentration modestly attenuated the associations of the long-chain omega-3 PUFA levels with ECP and VO₂max. When we stratified the analyses according to the hair mercury median (1.30 μg/g), the direct association between serum long-chain omega-3 PUFA and ECP was observed only among those with a low mercury content [the mean difference 0.61 mL/mmHg (95% CI 0.03-1.18, P-trend=0.03)]. Moreover, the association between the long-chain omega-3 PUFA levels with VO₂max was stronger in those men with a low mercury content.
Figure 7-9. Exercise cardiac power, VO2max and maximal SBP during exercise in quartiles of serum long-chain omega-3 PUFAs and hair mercury.
Black bars represent the 1st quartile, deep grey bars represent the 2nd quartile, light grey bars represent the 3rd quartile and white bars represent the 4th quartile. The values are adjusted for age, examination year, BMI, smoking, leisure-time physical activity, energy intake, carbohydrate intake, alcohol intake, drug for hypertension, C-reactive protein, and LDL and HDL cholesterol concentrations.
P-values are for the tests of trend across exposure quartiles.
BMI, body mass index; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; HDL, high density lipoprotein; LDL, low density lipoprotein; SBP, systolic blood pressure.
5.4 STUDY III: RESTING HEART RATE, PEAK HEART RATE DURING EXERCISE AND HEART RATE RECOVERY

5.4.1 Association with serum long-chain omega-3 PUFA

The mean values for resting HR, peak HR during the exercise test and HR recovery 2 min after the exercise test were 68.2 beats/min, 164.8 beats/min and 40.6 beats/min, respectively. After adjustment for the potential confounders, higher serum total long-chain omega-3 PUFA concentration was associated with a lower resting HR [the mean difference -2.17 beats/min (95% CI -4.10, -0.24 beats/min, P-trend=0.02)] (Figure 10). Generally, similar inverse associations were observed with EPA, DPA and DHA, although the association with EPA appeared slightly stronger than those with DPA and DHA (Figure 10). No associations were observed with peak HR during exercise or HR recovery after exercise, except for a borderline statistically significant association between serum DPA and a better HR recovery (Figure 11)

5.4.2 Association with hair mercury concentration

The hair mercury concentration was not associated with resting HR and HR recovery after exercise [the mean difference -1.24 beats/min (95% CI -3.24, 0.76 beats/min, P-trend=0.10) for resting HR and 0.98 beats/min (95% CI 0.98 -1.11, 3.07 beats/min, P-trend=0.21) for HR recovery] (Figure 10). A higher hair mercury content was associated with a lower peak HR during the exercise test after adjustment for age and examination year [the mean difference -3.55 beats/min (95% CI -6.25, -0.85, P-trend=0.001)]. However, further adjustment for potential confounders attenuated the association [the mean difference -1.55 beats/min (95% CI -1.55 -4.22, 1.11 beats/min, P-trend=0.10)] (Figure 11).
Figure 10-12. Resting HR, peak HR during exercise, HR recovery after exercise in quartiles of serum long-chain omega-3 PUFAs and hair mercury. Black bars represent the 1st quartile, deep grey bars represent the 2nd quartile, light grey bars represent the 3rd quartile and white bars represent the 4th quartile. The mean distributions are adjusted for age, examination year, BMI, diabetes, hypertension, smoking, education, income, leisure-time physical activity, intake of alcohol, energy intake, and blood glucose.
P-values are for the tests of trend across exposure quartiles. BMI, body mass index; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; HR, heart rate; PUFA, polyunsaturated fatty acid.
5.5 STUDY IV: EXERCISE-INDUCED MYOCARDIAL ISCHEMIA

5.5.1 Association with serum long-chain omega-3 PUFA

Among 2199 men, 1857 (84.4%) were free of CHD and 342 (15.6%) had a history of CHD. Exercise-induced myocardial ischemia was found in 623 (28.3%) of the 2199 men, in 295 (86.3%) of the men with prior CHD as compared to 328 (17.6%) of the men without prior CHD. The mean (SD) serum total levels of long-chain omega-3 PUFAs were 4.68 (1.62) % in the entire population, 4.68 (1.77) % in men with a history of CHD and 4.67 (1.59) % in men without CHD.

In the entire population, the men in the highest serum total long-chain omega-3 PUFA quartile had a 33% lower odds for exercise-induced myocardial ischemia (multivariable-adjusted OR for extreme-quartile difference 0.67, 95% CI 0.51–0.87, \( P \)-trend=0.006) (Table 14). In general, similar inverse associations were observed with EPA, DPA and DHA (Figure 13).

When stratified by the history of CHD, the serum total long-chain omega-3 PUFA concentration was associated with 90% (OR for extreme-quartile difference 0.10, 95% CI 0.03–0.39, \( P \)-trend<0.001) lower odds of exercise-induced myocardial ischemia among the men with a history of CHD (Figure 13), but no statistically significant associations were observed among the men without CHD history (OR in the highest quartile 0.80, 95% CI 0.57–1.12, \( P \)-trend=0.17) (\( P \)-interaction=0.01) (Figure 13).

5.5.2 Association with hair mercury concentration

In the entire population, a higher hair mercury concentration was associated with 62% increased odds for exercise-induced myocardial ischemia (OR for extreme-quartile difference 1.62, 95% CI 1.22–2.14, \( P \)-trend=0.002) (Table 14). When we stratified the analyses based on the CHD history, we did not detect any statistically significant associations among those with or without a history of CHD (\( P \) for interaction=0.24) (Figure 13).
Table 14. Odds ratios for exercise-induced myocardial ischemia in quartiles of serum long-chain omega-3 polyunsaturated fatty acids and hair mercury among men*

<table>
<thead>
<tr>
<th></th>
<th>Quartile</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>P for trend</th>
</tr>
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<tr>
<td></td>
<td>1 (n =549)</td>
<td>2 (n=550)</td>
<td>3 (n =550)</td>
<td>4 (n =550)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA+DPA+DHA, %</td>
<td>&lt;3.61</td>
<td>3.61-4.35</td>
<td>4.36-5.33</td>
<td>&gt;5.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivariable-adjusted model</td>
<td>1 (reference group)</td>
<td>0.74 (0.56 to 0.97)</td>
<td>0.70 (0.53 to 0.91)</td>
<td>0.67 (0.51 to 0.87)</td>
<td></td>
<td>0.006</td>
</tr>
<tr>
<td>EPA, %</td>
<td>&lt;1.09</td>
<td>1.09-1.46</td>
<td>1.47-1.97</td>
<td>&gt;1.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivariable-adjusted model</td>
<td>1 (reference group)</td>
<td>0.70 (0.54 to 0.92)</td>
<td>0.77 (0.59 to 1.01)</td>
<td>0.69 (0.53 to 0.91)</td>
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<tr>
<td>DPA, %</td>
<td>&lt;0.48</td>
<td>0.48-0.54</td>
<td>0.55-0.61</td>
<td>&gt;0.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivariable-adjusted model</td>
<td>1 (reference group)</td>
<td>0.67 (0.52 to 0.88)</td>
<td>0.70 (0.53 to 0.91)</td>
<td>0.71 (0.54 to 0.93)</td>
<td></td>
<td>0.03</td>
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<tr>
<td>DHA, %</td>
<td>&lt;1.95</td>
<td>1.95-2.36</td>
<td>2.37-2.84</td>
<td>&gt;2.84</td>
<td></td>
<td></td>
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<tr>
<td>Multivariable-adjusted model</td>
<td>1 (reference group)</td>
<td>0.70 (0.53 to 0.92)</td>
<td>0.81 (0.62 to 1.05)</td>
<td>0.70 (0.53 to 0.92)</td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Hair mercury, µg/g</td>
<td>&lt;0.70</td>
<td>0.70-1.39</td>
<td>1.40-2.71</td>
<td>&gt;2.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivariable-adjusted model</td>
<td>1 (reference group)</td>
<td>1.29 (0.97 to 1.72)</td>
<td>1.27 (0.96 to 1.69)</td>
<td>1.62 (1.22 to 2.14)</td>
<td></td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Values are odds ratios (95% confidence interval).
Multivariable-adjusted model: Age, examination year, body mass index, diabetes, smoking, leisure-time physical activity, treated hypertension, alcohol intake and energy intake.
Subgroup

**EPA + DPA + DHA**
- All participants
- Participants with history of CHD
- Participants without history of CHD

**EPA**
- All participants
- Participants with history of CHD
- Participants without history of CHD

**DPA**
- All participants
- Participants with history of CHD
- Participants without history of CHD

**DHA**
- All participants
- Participants with history of CHD
- Participants without history of CHD

**MERCURY**
- All participants
- Participants with history of CHD
- Participants without history of CHD

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Odds Ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All participants</td>
<td>0.92 (0.83 - 1.01)</td>
<td>0.09</td>
</tr>
<tr>
<td>Participants with history of CHD</td>
<td>0.62 (0.47 - 0.83)</td>
<td>0.001</td>
</tr>
<tr>
<td>Participants without history of CHD</td>
<td>0.95 (0.84 - 1.07)</td>
<td>0.39</td>
</tr>
<tr>
<td>All participants</td>
<td>0.95 (0.86 - 1.04)</td>
<td>0.27</td>
</tr>
<tr>
<td>Participants with history of CHD</td>
<td>0.65 (0.49 - 0.86)</td>
<td>0.002</td>
</tr>
<tr>
<td>Participants without history of CHD</td>
<td>0.97 (0.85 - 1.09)</td>
<td>0.59</td>
</tr>
<tr>
<td>All participants</td>
<td>0.91 (0.82 - 1.01)</td>
<td>0.06</td>
</tr>
<tr>
<td>Participants with history of CHD</td>
<td>0.68 (0.49 - 0.96)</td>
<td>0.03</td>
</tr>
<tr>
<td>Participants without history of CHD</td>
<td>0.94 (0.83 - 1.07)</td>
<td>0.34</td>
</tr>
<tr>
<td>All participants</td>
<td>0.90 (0.81 - 0.99)</td>
<td>0.03</td>
</tr>
<tr>
<td>Participants with history of CHD</td>
<td>0.61 (0.45 - 0.93)</td>
<td>0.001</td>
</tr>
<tr>
<td>Participants without history of CHD</td>
<td>0.93 (0.82 - 1.06)</td>
<td>0.27</td>
</tr>
<tr>
<td>All participants</td>
<td>1.18 (1.08 - 1.30)</td>
<td>0.001</td>
</tr>
<tr>
<td>Participants with history of CHD</td>
<td>0.88 (0.63 - 1.23)</td>
<td>0.45</td>
</tr>
<tr>
<td>Participants without history of CHD</td>
<td>1.06 (0.94 - 1.19)</td>
<td>0.37</td>
</tr>
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Figure 13. Multivariable adjusted odds of exercise-induced myocardial ischemia in 1 SD change in the serum long-chain omega-3 PUFAs and hair mercury. The model is adjusted for age, examination year, BMI, diabetes, treated hypertension, smoking, leisure-time physical activity, intake of alcohol and energy intake.

CHD, coronary heart disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid.
6 DISCUSSION

This doctoral thesis examined the association of serum long-chain omega-3 PUFAs and hair mercury concentrations, biomarkers of fish intake, with cardiac electrophysiology and performance parameters, specifically, QTc and JTc (Study I), exercise cardiac power and its components (Study II), HR at rest, peak HR during exercise and HR recovery after exercise (Study III) and exercise-induced myocardial ischemia (Study IV).

6.1 STUDY I

In this study, higher serum long-chain omega-3 PUFA concentrations were inversely associated with the QTc, JTc and with the likelihood of prolonged QTc and JTc. In contrast, the hair mercury concentration was not associated with the QTc, JTc; however, it slightly attenuated the associations of the long-chain omega-3 PUFAs.

Rather few studies have assessed the association of long-chain omega-3 PUFA levels with the QT interval and these have reported inconclusive outcomes. The results of the present study are in agreement with the findings of two previous cross-sectional studies, that a higher intake of fish and long-chain omega-3 PUFAs was associated with a lower likelihood of prolonged QTc among men and women aged ≥65 years (Mozaffarian, Prineas et al. 2006), and among American men and women aged 18-89 years (Chrysohoou, Panagiotakos et al. 2007). In contrast, no association between circulating levels of long-chain omega-3 PUFAs and QTc was found in two small cross-sectional studies conducted in American healthy populations (Brouwer, Zock et al. 2002, Miller, C., Karimi et al. 2018). Similarly, fish oil supplementation for 12 weeks did not alter the QTc value among a healthy population from the US (Geelen, Brouwer et al. 2002) and Danish haemodialysis patients (Kirkegaard, Svensson et al. 2012). However, the small size of these RCTs makes it difficult to draw firm conclusions on the effectiveness of fish oil supplementation in lowering the QTc. This is the first study that has evaluated the association between the long-chain omega-3 PUFAs and JT-interval.

QTc and JTc on the ECG trace reflect the duration of ventricular repolarization (Rautaharju, Surawicz et al. 2009); therefore, the inverse association between the serum levels of long-chain omega-3 PUFAs and QTc and JTc may be explained by the role of these fatty acids in the modulation of myocyte electrophysiology function (e.g. reduction in the activity of membrane sodium channels and in the modulation of the activity of the membrane L-type calcium channel), which are involved in the ventricular repolarization process (Xiao, Kang et al. 1995, Hallaq, Smith et al. 1992).

The lack of association between the hair mercury concentration and QTc found here is in line with the finding of the one and only cross-sectional study that failed to
report any association between the methylmercury concentration and QTc among middle-aged healthy men and women (Miller, Karimi et al. 2018). At present, this is the only study that has evaluated the association between the mercury concentration and JT-interval.

In the current study, higher serum long-chain omega-3 PUFA concentrations were inversely associated with the risk of SCD, which supports the previous findings from KIHD (Virtanen, Laukkanen et al. 2012) and other studies (Mozaffarian, Wu 2011). We also observed direct 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 (Zhang, Post et al. 2011). Several different pathways have been established to account for the beneficial impact of long-chain omega-3 PUFAs on the risk of SCD including anti-arrhythmic, anti-hypertensive, anti-thrombotic and anti-inflammatory properties, and beneficial effects related to endothelial and autonomic function and lipoprotein metabolism (Mozaffarian, Wu 2011). However, in the present study, the inverse association of the long-chain omega-3 PUFA with the SCD risk was not explained by the prevention of prolonged ventricular repolarization since adjustments for QTc or JTc did not attenuate the associations between the serum long-chain omega-3 PUFA concentrations and the risk of SCD.

6.2 STUDY II

At present, this is the first study which has evaluated the association of serum long chain omega-3 PUFAs and hair mercury concentrations with the ECP. In this study, the serum long-chain omega-3 PUFA concentration was associated with higher ECP and VO2max, but not with maximal SBP during exercise. The hair mercury concentration was not associated with ECP and its components. A higher hair mercury concentration slightly attenuated the associations of the long-chain omega-3 PUFA with VO2max and ECP.

Rather few supplemental studies have examined whether there is a link between long-chain omega-3 PUFAs and VO2max, but with inconsistent findings. In a 3-week RCT, 13 elite male Polish cyclists were allocated to either 1.3 g/twice a day fish oil supplementation (660 mg EPA, 440 mg DHA, 200 mg other fatty acids and 13.4 mg vitamin E) or a placebo. A higher post-intervention VO2max level was observed after fish oil supplementation as compared with placebo (Żebrowska, Mizia-Stec et al. 2015). This effect was also observed in another RCT where fish oil supplementation (0.9 g/day EPA+DHA) with lifestyle modification for 12 weeks improved VO2max among overweight adult Iranian women (Haghavara, Keshavarz et al. 2016). However, other RCTs among normal-weight sedentary men from Switzerland (Boss, Lecoultre et al. 2010), and in obese middle-aged and older American men and women (DeFina, Marcoux et al. 2010) as well as in male athletes (Raastad, Hastmark et al. 1997, Peoples, McLennan et al. 2008, Kawabata, Neya et al. 2014, Lewis, Radonic et
al. 2015) have not found support for this beneficial impact. The conflicting results regarding the impact of long-chain omega-3 PUFAs on the VO\textsubscript{max} in RCTs may be partially explained by the differences in the study setting, study populations, dosage or length of supplementation period.

The mechanism underlying the direct association between long-chain omega-3 PUFAs and exercise capacity, ECP and VO\textsubscript{max}, might be due to the beneficial impact of these fatty acids on the functions of the vascular endothelial, such as improvements in vascular reactivity, increased production of endogenous antioxidant enzymes and decreased inflammatory cytokines, and bioavailability of endothelial nitric oxide (Wu, Meininger 2002, Khan, Elherik et al. 2003, Mickleborough 2013), which are strongly associated with a better exercise capacity.

In the current study, hair mercury attenuated the associations of the long-chain omega-3 PUFAs with VO\textsubscript{max} and ECP. This attenuation may be at least partially explained by the role of mercury in the endothelial dysfunction by a reduction in nitric oxide bioavailability and nitric oxide synthase expression (Furieri, Galán et al. 2011).

In the present study, long-chain omega-3 PUFAs were not associated with the SBP during exercise. The intake of long-chain omega-3 PUFAs has been previously reported to be associated with lower resting SBP in KIH\textsuperscript{D} (Virtanen, Nyantika et al. 2012, Nyantika, Tuomainen et al. 2015) and other study populations (Cabo, Alonso et al. 2012, Miller, Van Elswyk et al. 2014). However, data is very limited regarding the association of long-chain omega-3 PUFAs and exercise-induced SBP. In one RCT conducted in 22 young healthy men, high-fat meal (high-oleic sunflower oil enriched with 4.7 g of either EPA or DHA) decreased the systemic vascular resistance and slightly increased the SBP during exercise compared to the control group who only received high levels of oleic sunflower oil (Rontoyanni, Hall et al. 2012). In contrast, in another RCT, 0.81g/day EPA+DHA for two sequential 4-month periods did not influence the SBP during exercise in 18 older men with the history of CHD (O’Keefe, Abuissa et al. 2006).

It has been reported that the hair mercury concentration is directly associated with higher blood pressure (Pedersen, Jørgensen et al. 2005, Fillion, Mergler et al. 2006, Choi, Weihe et al. 2009, Bautista, Stein et al. 2009, Valera, Dewailly et al. 2009, Yorifuji, Tsuda et al. 2010, Hong, Cho et al. 2013); however, in the present study, no association was observed between hair mercury concentration and SBP during exercise. The reason for the lack of associations between serum long-chain omega-3 PUFAs and hair mercury concentrations with SBP during exercise may be due to the role of the hemodynamic response to exercise which is not considered in SBP at rest (McHam, Marwick et al. 1999).
6.3 STUDY III

The primary finding of the present study suggested that a higher serum long-chain omega-3 PUFA concentration was associated with lower resting HR. No associations were observed with peak HR during exercise or HR recovery after exercise. The hair mercury content was associated with lower peak HR and it only slightly attenuated the associations of the serum long-chain omega-3 PUFAs.

The inverse association between long-chain omega-3 PUFAs and resting HR in the present study has been previously observed in many clinical and epidemiological studies. In a large cross-sectional study examining a total of 9758 healthy Irish and French men aged 50-59 years, fish consumers had lower resting HR as compared to non-consumers (Dallongeville, Yarnell et al. 2003). In the two cross-sectional studies from the Cardiovascular Health Study among healthy older men and women from US (aged ≥65 years), a higher intake of tuna or other broiled or baked fish, but not fried fish, was associated with a lower resting HR (Mozaffarian, Prineas et al. 2006, Mozaffarian, Gottdiener et al. 2006). According to the result of another cross-sectional study conducted in 181 adults (aged ≥40 years), circulating long-chain omega-3 PUFAs, mainly EPA, were associated with the lower resting HR, but exclusively among Canadian women (Valera, Dewailly et al. 2011a).

According to the latest meta-analysis of 51 randomized RCTs with approximately 3000 participants with different health statuses, fish oil significantly reduced resting HR by 2.2 beats/min as compared to the placebo, especially in RCTs with DHA supplementation (Hidayat, Yang et al. 2018). The result of this meta-analysis is in line with another meta-analysis including 30 RCTs conducted by Mozaffarian et al. that pointed to a slight significant reduction in resting HR (1.6 beats/min) attributable to fish oil supplementation, as compared to placebo (Mozaffarian, Geelen et al. 2005).

In the current study, EPA had a slightly stronger association with resting HR than the other long-chain omega-3 PUFAs. These findings differ from two RCTs that showed that DHA, but not EPA, was significantly associated with a lower resting HR. In a 7-week RCT, a 2.2 beats/min reduction in resting HR was observed due to 4g/day DHA supplementation, but not with EPA supplementation, among 234 healthy men aged 36-56 years (Grimsgaard, Bonaa et al. 1997). Similarly, in a 6-week RCT conducted in 59 overweight, mildly hyperlipidaemic men aged 50-59 years, 4g/day DHA reduced the HR by 3.5 beats/min; however, no difference was observed in resting HR due to EPA supplementation (Mori, Burke et al. 2000).

Different study populations and different study settings might be one explanation for these conflicting results. It has been demonstrated that DHA has a greater impact on the prevention of ischemia-induced arrhythmia (McLennan, Peter, Howe et al. 1996). Moreover, EPA and DHA exert different effects on the function of membrane ion channels in relation to the cardiac rhythm. EPA seems to be a more effective
inhibitor of the voltage-gated Na+, which is related to the risk of cardiac arrhythmias (George 2005, Li, Sun et al. 2008).

The mechanism explaining the association of the concentrations of long-chain omega-3 PUFAs and methylmercury with peak HR during exercise and HR recovery is unclear. At present, this is the first population-based study to have investigated these associations. Only a few RCTs have evaluated the effect of fish oil supplementation on the peak HR during exercise and their findings have been inconclusive. In agreement with the results emerging from the present study, fish oil supplementation had no impact on peak HR during exercise among healthy adult Australian men (Hingley, Macartney et al. 2014, Macartney, Hingley et al. 2014), Australian football players (Buckley, Burgess et al. 2009), or older American men with a history of CHD (Vacek, Harris et al. 1989, O’Keefe, Abuissa et al. 2006). In contrast, in an 8-week RCT conducted in well-trained Australian men, 8g/day EPA+DHA reduced the peak HR during exercise (Peoples, McLennan et al. 2008). This inconsistent result might be explained partially by the role genetic factors in the peak HR during exercise (Chomistek, Chasman et al. 2013, Sarzynski, Ghosh et al. 2017). Another explanation might be also the differences in the study setting, dosage or length of supplementation period.

According to the two supplementation studies, long-chain fish oil supplementation positively influenced the HR recovery after exercise among older American men with documented CHD (O’Keefe, Abuissa et al. 2006) and physically fit young Australian men (Macartney, Hingley et al. 2014). This finding was also observed in one cross-sectional study that noted that the circulating long-chain omega-3 PUFA concentration was associated with a lower risk of impaired HR recovery among participants with stable coronary artery disease from US (Moyers, Farzaneh-Far et al. 2011). In the present study, only serum DPA was associated with a better HR recovery after exercise. The mechanism underlying this finding is beyond the scope of the current study; however, one reason might be that there is a stronger association between DPA and some inflammatory markers, especially interleukin-6, which are directly associated with post-exercise HR recovery (Edwards, Burns et al. 2006, Tang, Dewland et al. 2009).

Very little prior information is available regarding the impact of mercury on the resting and exercise-induced HR. In line with the findings of the present study, in a 14-week RCT among 44 healthy Japanese adults, the hair mercury concentration, acquired by the consumption of bigeye tuna and swordfish, did not alter the resting HR (Yaginuma-Sakurai, Murata et al. 2010). According to the three cross-sectional studies, blood, hair and toenail mercury concentrations were not associated with the resting HR among adults (Choi, Weihe et al. 2009, Valera, Dewailly et al. 2011, Valera, Dewailly et al. 2011b) and teenagers (Valera, Dewailly et al. 2011b). In contrast, in one cross-sectional-study conducted in Canadian adults, a higher blood methylmercury concentration (mean 15.4 μg/L) was associated with resting HR
being increased by 6.9 beats/min (Valera, Dewailly et al. 2013). For example, these conflicting results might be since different methods were applied for measuring the exposure to mercury and the different mercury concentrations. The present study is the first observational study that has evaluated the association of methylmercury concentrations and peak HR during exercise and HR recovery after exercise. Although a higher mercury concentration was associated with the lower peak HR; no association was observed between the hair mercury concentration and HR recovery after exercise. There is no clear mechanism to explain how mercury could affect peak HR, but not resting HR or HR recovery.

6.4 STUDY IV

In this study, higher serum long-chain omega-3 PUFA concentrations were associated with lower odds for exercise-induced myocardial ischemia, mainly among those individuals with a history of CHD. Furthermore, a direct association was observed between the hair mercury concentration and the occurrence of exercise-induced myocardial ischemia in the entire study population.

There is very limited knowledge regarding the association of long-chain omega-3 PUFAs and the risk of myocardial ischemia during exercise. In contrast to the finding of the present study, in a 12-week RCT among 8 American patients with stable CHD, dietary supplementation with fish oil there was no change in the parameters of myocardial ischemia during exercise (e.g. exercise-induced ST-segment depression and onset of angina) (Mehta, Lopez et al. 1988). This lack of association was confirmed by the findings from one cross-sectional study that the blood long-chain omega-3 PUFA concentration was not associated with the exercise-induced ischemia among American patients with stable coronary artery disease (Moyers, Farzaneh-Far et al. 2011). No prior experimental, clinical or observational data is available regarding the possible impact of mercury on the occurrence of exercise-induced myocardial ischemia.

Coronary blood flow needs to be increased during physical exercise/stress to meet myocardial oxygen demand (Duncker, Bache 2008, Joyner, Casey 2015). Vessel narrowing and plaque formation in the arteries reduce the coronary blood flow which leads to an imbalance in the myocardial oxygen supply, and consequently to exercise-induced myocardial ischemia (Hashmi, Al-Salam 2015). The inverse association between serum long-chain omega-3 PUFA concentrations and the odds for exercise-induced myocardial ischemia, especially among those with history of CHD, might be due to the beneficial impact that long-chain omega-3 PUFAs exert on the coronary vasodilator reserve, which could lead to improved blood flow during exercise.

This is the first study which has evaluated the association of mercury concentration and myocardial ischemia. A direct association was observed between
hair mercury concentration and the odds for exercise-induced myocardial ischemia in the entire population. The mechanism underlying this association might be due to the role of mercury to evoke an endothelial dysfunction and to promote the formation of atherosclerotic plaques by increasing oxidative stress, which seems to be an important factor underpinning the progression of myocardial ischemia (Roman, Walsh et al. 2011)
6.5 STRENGTHS AND LIMITATIONS OF THIS STUDY

The study population in this project was relatively large and we gathered high-quality data, including data about the ECG parameters, which made possible an extensive adjustment for potential confounding factors. This meant that the analyses were more powerful and thus the results are more reliable. The other strengths of this project include the use of serum long-chain omega-3 PUFAs and hair mercury instead of dietary measurements, which both are regarded as established biomarkers for intake (Hodson, Skeaff et al. 2008, Roman, Walsh et al. 2011).

Because serum fatty acids and hair mercury are objective biomarkers for exposure, their use minimize the bias by misclassification that would reduce the associations towards the null. Unfortunately, we did not have access to omega-3 PUFA concentrations in erythrocyte membrane or adipose tissue, which reflect the longer-term dietary intake (Cao, Schwichtenberg et al. 2006). Furthermore, although on average the hair mercury measurements were made 6 years apart, they showed a high correlation (r=0.91); the 4-year correlations in the serum long-chain omega-3 PUFA measurements were more modest, ranging from 0.47 to 0.56. This may reflect biological variability in the serum fatty acids concentrations or differences in the intake of the long-chain omega-3 PUFAs or to laboratory error. In addition, the coefficients of variation in the measurement of the serum long-chain omega-3 PUFA were rather large. However, as these kinds of bias are most likely random, they would again attenuate the true associations. The blood samples were kept frozen at -80°C before analysis, which prevented degradation of the fatty acids.

Potential limitations of this project include the observational study design, so no conclusions can be drawn about causality. Another 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.

Furthermore, the average hair mercury concentrations are somewhat higher in the KIHD cohort than have been reported in other study populations (Mozaffarian, Shi et al. 2011, Wennberg, Bergdahl et al. 2010). Therefore, the results of the present project may not be generalizable to study populations with lower average mercury exposures.

Specifically, in Study I, using heart rate-corrected QT- and JT-intervals and excluding participants with prolonged QRS complex likely reduced the inter-individual variability, which improved the sensitivity to detect associations between the fatty acids and hair mercury and ventricular repolarization. In the analyses with incident SCD, the long follow-up time may have attenuated the associations, which were based only on a single exposure assessment at baseline. Moreover, the relatively small number of SCD events may have limited the power to identify statistically significant associations with SCD risk.
A specific strength of the Study II was using the VO₂max, the golden standard for measuring cardiorespiratory fitness (Laukkanen, Kurl et al. 2002), and ECP, which provides information about the differences in cardiovascular resistance and cardiac afterload (Kurl, Laukkanen et al. 2005).
The following conclusions can be deduced from this population study on the association of serum long-chain omega-3 PUFA levels and hair mercury concentrations with specific parameters of cardiac functions:

1. Higher circulating concentrations of the long-chain omega-3 PUFAs, mainly a marker of fish intake in this study population, were positively associated with ventricular repolarization, as measured by QTc and JTc. In contrast, the hair mercury concentration had no association with these parameters. However, the inverse association between the long-chain omega-3 PUFAs and the risk of SCD was not explained by the prevention of prolonged ventricular repolarization.

2. Higher circulating concentrations of the long-chain omega-3 PUFAs were associated with higher ECP and VO2max. The mercury concentration modestly attenuated these associations. Since low VO2 and low ECP are considered as risk factors for CVD, these results could partially explain how the intake of fish, especially fish with a low mercury content, may reduce the risk of adverse cardiac events.

3. Higher serum long-chain omega-3 PUFA concentrations were inversely associated with HR at rest. However, this kind of beneficial association was not observed with peak HR during exercise and HR recovery after exercise. Since a higher resting HR is a well-known risk factor of CVD, this result could partially explain how long-chain omega-3 PUFAs may reduce the risk of CVD. Moreover, the hair mercury content was associated with a lower peak HR and it only slightly attenuated the associations of the serum long-chain omega-3 PUFAs, therefore consumption of fish with a lower mercury content is recommended.

4. Higher circulating concentrations of the long-chain omega-3 PUFAs were inversely associated with the occurrence of exercise-induced myocardial ischemia among those individuals with a history of CHD, but not among those with no such history. The hair mercury concentration was associated with a higher occurrence of exercise-induced myocardial ischemia. This finding supported the hypothesis that fatty fish, especially fatty fish with a low mercury content, exert a cardioprotective effect.
RECOMMENDATIONS

In recent years, the provision of adequate and cost-effective care to combat the increasing prevalence of CVD in societies has received growing attention from national and local governments and international organizations, as well as from the general public. It is well-established that dietary factors play an important role in the development and prevention of CVD. With respect to the dietary factors, the seafood-derived long-chain omega-3 PUFAs have been shown to possess cardioprotective properties. This has been the impetus to clarify the biological mechanisms that account for the cardiovascular benefits associated with the intake of the long-chain omega-3 PUFAs.

In this thesis, higher serum concentrations of the long-chain omega-3 PUFAs were beneficially related to some specific parameters of cardiac electrophysiology and performance. However, hair mercury concentration generally attenuated these cardioprotective associations. This thesis reveals new mechanisms to explain the positive associations of long-chain omega-3 PUFAs with specific parameters of cardiac functions (including ECP and its components, peak HR during exercise and HR recovery after exercise, and exercise-induced myocardial ischemia), whereby intakes of long-chain omega-3 PUFAs and fish may lower the risk of CVD and consequently benefit public health. With respect to the prevention of CVD, guidelines should continue to recommend the intake of fatty fish, which are high in long-chain omega-3 polyunsaturated fatty acids but low in methylmercury. Further investigations among women and other populations will be needed to confirm the generalizability of the findings emerging from this thesis. In addition, the cellular mechanisms underlying the associations of the long-chain omega-3 PUFAs and methylmercury with cardiac functions are still not fully known and should be clarified.

The findings of this thesis generally supported the results of observational studies that have mainly demonstrated the cardioprotective properties of fish intake. In contrast, fish oil trials, especially recent ones, have not confirmed these beneficial effects, perhaps due to the low dose of supplementation and the short follow-up time. Moreover, the subjects in these recent trials had often been receiving state-of-the-art medication, and they may have had high exposure to EPA+DHA already before the trials through higher fish intake or fish oil use, and therefore these trials may have been underpowered to find beneficial effects on CVD outcome. Further studies, among different study populations, are clearly warranted to examine the effect of long-chain omega-3 PUFAs on the risk of CVD.

In addition to long-chain omega-3 PUFAs and methylmercury, fish contain many other beneficial components such as vitamin D, protein, and selenium, as well as
other harmful environmental contaminants such as polychlorinated biphenyls, polybrominated diphenyl ethers, dioxins, and chlorinated pesticides. Fish contain very low levels of saturated or trans fatty acids. Further studies should be carried out to investigate the independent and joint effects of these other, potentially health-related, components.

All in all, in agreement with the existing recommendations, the consumption of fatty fish, especially fish which are high in long-chain omega-3 polyunsaturated fatty acids but low in methylmercury, is recommended for the prevention of CVD.
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Fish-derived long-chain omega-3 polyunsaturated fatty acids and methylmercury are associated with risk of cardiovascular disease. However, the mechanism underlying these associations are not completely known. In this doctoral thesis, higher concentrations of the serum long-chain omega-3 polyunsaturated fatty acids were found to have beneficial associations with cardiac functions. However, methylmercury diminished these benefits. These findings reveal potential new mechanisms whereby intake of fish, especially fish that have high content of the long-chain omega-3 polyunsaturated fatty acids but low methylmercury content, may improve cardiac health.