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AALBORG UNIVERSITY
DENMARK

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PAPERS

The thesis is based on the following papers:

1. Dinesen PT, Joensen AM, Rix TA, Tjønneland A, Schmidt EB, Lundbye-Christensen S, Overvad K Effect of Dietary Intake of Saturated Fatty Acids on the Development of Atrial Fibrillation and the Effect of Replacement of Saturated With Monounsaturated and Polyunsaturated Fatty Acids. American Journal of Cardiology. 2017;120:1129–1132.

2. Dinesen PT, Rix TA, Joensen AM, Tjønneland A, Lundbye-Christensen S, Overvad K, Schmidt EB Adipose Tissue Content of Saturated Fatty Acids and Atrial Fibrillation: a Case-cohort Study. European Journal of Clinical Investigation. 2017;47(12):e12836.

3. Dinesen PT, Rix TA, Joensen AM, Dahm CC, Lundbye-Christensen S, Schmidt EB, Overvad K Patterns of Adipose Tissue Fatty Acids and the Risk of Atrial Fibrillation: a Case-cohort Study. Submitted.

ENGLISH SUMMARY

Atrial fibrillation (AF) is a common cardiac arrhythmia and the prevalence increases with age. AF can cause embolization of thrombi from the atria's to the brain and account for no less than 20% of strokes. The prevalence is increasing, thus, AF is a growing epidemic and a serious public health concern and there is a need for preventive initiatives. Previous studies have suggested that saturated fatty acids (SFA) may be associated with incident AF.

This thesis and related papers were based on the Danish cohort "Diet, Cancer and Health" including dietary information and adipose tissue biopsies from more than 57.000 participants. The a priori hypothesis was that SFA were associated with a higher incidence of AF and three studies were designed to explore this hypothesis.

First, we investigated if replacing dietary SFA with monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) was associated with a lower risk of AF using substitution models. Analyses found a statistically significant direct association for developing AF in men when replacing dietary SFA with total n-3 PUFA. In women and when substituting SFA with MUFA and other PUFA, we found a neutral association with AF.

Subsequently, we examined the association between adipose tissue content of SFA and the risk of developing AF in a case-cohort study with Cox proportional hazard regression. We found a neutral association between adipose tissue content of SFA and the risk of incident AF in both men and women.

Lastly, the third study explored the association between adipose tissue patterns of fatty acid identified by the statistical method treelet transform and the risk of developing AF. We found that an adipose tissue fatty acid pattern with SFA was associated with a statistically significant lower hazard of AF in women but not in men. In both men and women patterns with n-6 PUFA may be associated with a lower hazard of AF, while patterns with marine n-3 PUFA and n-9 fatty acids were associated with a lower hazard of AF in women.

DANSK RESUME

Atrieflimren (AF) er en hyppigt forekommende hjerterytmeforstyrrelse og hyppigheden stiger med alderen. AF kan forårsage dannelse af tromber i atrierne med efterfølgende risiko for embolisering til hjernen. AF er skyld i mere end 20% af apopleksi tilfælde. Grundet en stigende prævalens er der behov for nye profylaktiske tiltag. Tidligere studier har antydet en association mellem mættet fedt og udviklingen af AF.

Denne afhandling, og studierne den er baseret på, bygger på den danske kohorte "Kost, kræft og helbred". Kohorten indeholder information om kost og fedtvævsbiopsier fra mere end 57.000 deltagere. Arbejdshypotesen var, at mættet fedt er associeret med en øget risiko for udvikling af AF, og tre studier blev designet for at afsøge denne hypotese.

Det første studie undersøgte med Cox regression, hvordan substitution af mættet fedt i kosten med mono- og polyumættede fedtsyrer påvirkede associationen med AF. Vi fandt, at hvis mænd erstattede mættet fedt med omega-3 polyumættet fedt, var der en øget risiko for AF. Hos kvinder og ved substitution med andre mono- og polyumættede fedtsyrer var associationen neutral.

Det andet studie omhandlede associationen mellem fedtvævs indhold af mættet fedt og risikoen for AF i et case-kohorte studie med Cox regression. Vi fandt, at indholdet af mættet fedt i fedtvæv var neutralt associeret med risikoen for at udvikle AF hos både mænd og kvinder.

Det sidste studie undersøgte associationen mellem fedtvævs fedtsyremønstre identificeret med treelet transform og risikoen for at udvikle AF. Studiet viste, at hos kvinder var mønstre med mættet fedt, marine omega-3 polyumættede fedtsyrer og omega-9 fedtsyrer associerede med en lavere risiko for AF. Ved begge køn var der en mulig association mellem omega-6 polyumættede fedtsyrer og en lavere risiko for AF.

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The past 2.5 years as a PhD-student at AF Study Group, Department of Cardiology, Aalborg University Hospital has been a memorable time filled with great people, laughter and a steep learning curve. It has been an opportunity and a privilege to work in a highly professional research institution, where you not only get to grow as a researcher but also at person.

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I would also like to thank the rest of the colleagues in The Lipid Clinic for all the good times we have shared. The coffee breaks with nurses, secretaries and medical laboratory technicians were cozy and contributed to a friendly and helpful work environment. A special thanks to Britt Mejer Christensen for all your help with practical questions and issues.

The PhD office has been my second home for the past years and I owe a huge thanks to my fellow PhD-students and research year students. We have shared both serious and entertaining issues throughout the years. Despite all the talk about great and poor coffee, I will continue to be a tea kind of girl. A special thanks to Stine Krogh Venø, Anne Lasota and Christina Graversen – this PhD would not have been the same without you. You are not only great colleagues, but also close friends and I look forward to spending more time with you in the future.

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I would also like to thank my parents and my friends for their support throughout medical school, the first years as a young doctor and now through this PhD thesis.

Finally, to Christian - thank you for all your love, support and encouragement. You had me at hello and ever since I have felt incredibly fortunate to share my life with you.

Pia Dinesen
February, 2018

TABLE OF CONTENTS

ABBREVIATIONS

AF: Atrial fibrillation

AFL: Atrial flutter

CVD: Cardiovascular disease

FFQ: Food-frequency questionnaire

HR: Hazard ratios

MUFA: Monounsaturated fatty acids

PUFA: Polyunsaturated fatty acids

SFA: Saturated fatty acids

TT: Treelet transform

Chapter 1. INTRODUCTION

Atrial fibrillation (AF) is a common cardiac arrhythmia affecting 1-2% of the population. The prevalence increases with age reaching 5-15% at an age of >80 years (1). In 2010, the worldwide number of AF patients was believed to be 33.5 million (2). This number is estimated to rise substantially in the future due in part to the aging population and an increase in established risk factors for AF such as diabetes mellitus and obesity, as well as better treatment and survival of cardiovascular diseases with a predisposition for AF (1,3–5). AF is associated with higher mortality and morbidity (6,7) including a five-fold higher risk of stroke and a three-fold higher incidence of heart failure (8). Thus, AF is to be considered a growing epidemic and a serious public health concern (6) with an urgent need for preventive initiatives.

The association between dietary components and AF has been investigated previously. Thus, heavy alcohol drinking has been found to be associated with an higher risk of AF while a moderate alcohol intake does not affect the risk (9,10). Regarding fatty acids, some studies have explored the association between marine n-3 polyunsaturated fatty acids (PUFA) or fish intake and the risk of AF and reported inconsistent results (11–16). Finally, two previous studies have suggested that saturated fatty acids (SFA) may be related to the development of AF (17,18) with diverging results.

This thesis explores the association between SFA in the diet and in adipose tissue and the risk of developing AF, and how adipose tissue patterns of fatty acids is associated with the risk of incident AF.

Chapter 2. BACKGROUND

2.1. ATRIAL FIBRILLATION

In patients with AF, the electric activity of the atria is irregular, which leads to a disturbed coordination of impulses from the atria to the ventricles. This, in turn, leads to an irregular and often rapid heart rhythm.

The electrocardiogram defining AF has three characteristics (1,7):

1. The RR intervals are absolutely irregular.
2. There are no distinct P waves.
3. The interval between atrial activation (if visible) is usually variable and >300 beats per minute.

An example of a section of an electrocardiogram from a patient with AF is shown in Figure 1.

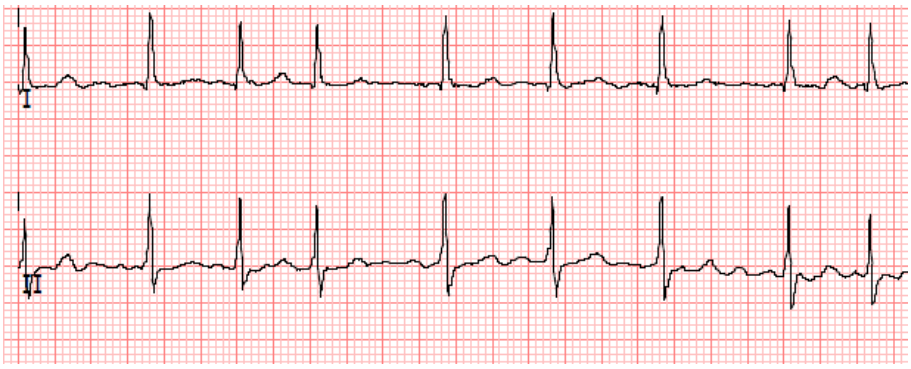


Figure 1. A section of an electrocardiogram showing characteristic AF.

AF is divided in to four types according to the duration and clinical presentation of the arrhythmia (1):

1. *Paroxysmal AF:* Self-terminating within 7 days.
2. *Persistent AF:* If the AF episode lasts more than 7 days or requires termination by cardioversion.
3. *Long-standing persistent AF:* AF lasting for more than one year and a rhythm control strategy has been chosen.

4. *Permanent AF:* The arrhythmia is accepted by the patient and further attempts to achieve rhythm control is not pursued.

AF is a progressive disease and the pathophysiology contains at least three components: initiation, maintenance and progression to longer-lasting forms of AF. Each episode of AF is initiated by a trigger acting on a vulnerable substrate. Ectopic firing is often located in the pulmonary veins and can initiate a re-entry arrhythmia in a susceptible substrate caused by electrical or structural remodeling of the atria, which in turn can maintain the arrhythmia (19,20). Atrial fibrosis affects atrial electrophysiology and can cause ectopic firing and conduction-slowing (21) favouring re-entry arrhythmias where the atria has to have regained excitability. The last-mentioned is also favoured by atrial enlargement (22). AF itself can cause remodeling of the atria, both structural and electrical, which makes the atria more vulnerable, thus reinforcing the arrhythmia (19,22,23).
Progression is caused by remodeling due to the arrhythmia and due to aging and other heart diseases e.g. hypertension or heart failure (20).

The patients may experience symptoms such as palpitations, dyspnoea and fatigue, often leading to a lower quality of life (24). Furthermore, AF can facilitate the formation of thrombi in the atria which can cause embolization to the brain giving rise to an ischemic stroke. AF accounts for no less than 20% of all strokes and the sequelae after a stroke tend to be more severe in patients with AF (25,26). Depending on patients' risk profile for stroke, patients may require lifelong anticoagulant treatment in the form of vitamin K antagonist or non-vitamin K antagonist oral anticoagulants, which may have serious adverse effects such as bleeding episodes (1). Also, untreated AF is associated with an increased risk of heart failure (27).

Risk factors for developing AF include male sex (7), hypertension (7,28), age (29), smoking (30), obesity (7,31,32), previous myocardial infarction (3), diabetes mellitus (33–35), heart failure (3,6,7), chronic kidney disease (6,36) and heredity (6,7,20).

AF is closely related to the less common arrhythmia atrial flutter (AFL), which is a more organized re-entry tachycardia with regular rapid activation of the atria. Symptoms and complications are similar to AF and the conditions often coexist.

2.2. FATTY ACIDS

Fatty acids are classified according to the number and location of double bonds in the carbon chain. SFA have no double bonds. Monounsaturated fatty acids (MUFA) have one double bond and PUFA have two or more double bonds in the carbon chain (Figure 2). The MUFA and PUFA are in addition named e.g. n-3, n-6 or n-9 referring to whether the first double bond is placed at carbon atom number 3, 6 or 9 counted from the methyl end of the chain. Thus, a specific fatty acid, e.g linoleic acid is characterized as shown in Figure 2, where C:18:2, n-6 refers to a fatty acid with 18 carbon atoms and two double bonds, where the first is placed at carbon atom number 6. Cis and trans are terms that refer to the configuration around the double bond. Most naturally occurring fatty acids are in the cis configuration, while trans fatty acids are derived from meat and dairy products from ruminants, where the fatty acids are produced by the action of bacteria in the ruminant stomach (37), or are products of industrial processing.

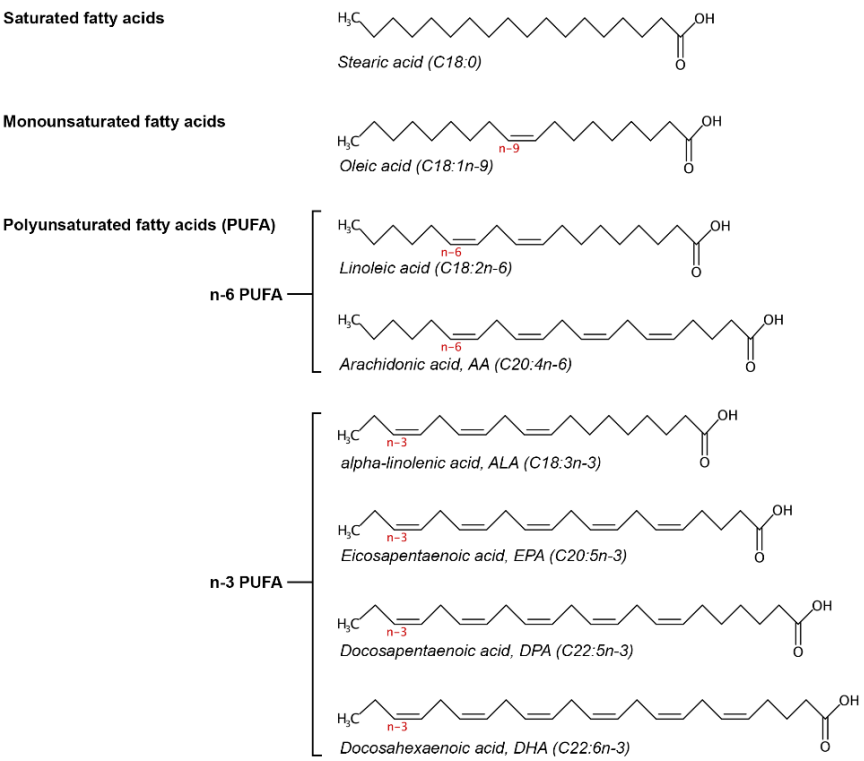


Figure 2. The classification of fatty acids. *Reproduced with permission from A. Gammelmark, Dissertation 2016: Marine n-3 Fatty Acids and Genetic Variants in the 5-Lipoxygenase Pathway. Copyright: Anders Gammelmark.*

The body's endogenous exposure to SFA is determined by the dietary intake and de novo lipogenesis from ingested carbohydrates and proteins (38–42). Dietary sources for the main SFA are shown in Table 1.

Composition	**Name**	**Dietary Sources** (18,43,44)
C12:0	Lauric acid	Dairy products, palm oil, sunflower oil
C14:0	Myristic acid	Dairy products, sunflower oil
C16:0	Palmitic acid	Meat, dairy products and palm oil
C18:0	Stearic acid	Bovine meat, dairy products and palm oil
C20:0	Arachidic acid	Peanuts and canola oil
C22:0	Behenic acid	Peanuts and canola oil
C24:0	Lignoceric acid	Peanuts and canola oil

Table 1. Number of carbon atoms, name and main dietary sources of saturated fatty acids.

In the de novo lipogenesis pathway fatty acids are formed when acetyl-coenzyme A are polymerized. The major product of this polymerization is C16:0 (palmitic acid), whereas C14:0 (myristic acid) is a minor product of the synthesis. Furthermore, C16:0 can be processed by elongation and/or desaturation to other fatty acids e.g. the MUFA C18:1 n-9 (oleic acid) (41).

2.3. SATURATED FATTY ACIDS AND ATRIAL FIBRILLATION

Recently, Fretts et al. (18) investigated the association between plasma phospholipid SFA and the risk of developing AF in American adults (both Caucasians and Blacks). The participants were ≥65 years of age without cardiovascular disease at baseline and participated in the Cardiovascular Health Study. Plasma levels of C16:0 to 24:0 were expressed as weight percentage of total phospholipid fatty acids analysed, while information on AF was based on annual electrocardiograms or hospital discharge diagnoses. The cohort for this analysis consisted of 2,899 participants of whom 707 developed AF during a median follow-up of 11.2 years. The authors concluded that higher circulating plasma levels of C16:0 were associated with a higher risk of AF when comparing the highest with the lowest quartile with a HR of 1.48 (95% CI 1.18 to 1.86) in the multivariate model and the trend was statistically significant. In contrast, circulating levels of C18:0 to 24:0 were associated with a lower

risk of incident AF (p-value ≤0.01) when comparing the second to the fourth quartiles with the first quartile. When investigating combined circulating levels of C16:0 to C24:0 there was, however, no statistically significant association with the risk of developing AF.

The association between SFA in the diet and AF has been investigated in the Women's Health Study, which was established to investigate the effect of low-dose aspirin, ß-carotene, and vitamin E in the primary prevention of cardiovascular disease (CVD) and cancer in American, female health care professionals (17). Dietary data from a baseline food-frequency questionnaire (FFQ) were used to investigate the risk of AF when energy from dietary carbohydrates was replaced by energy from MUFA or SFA. The participating 33,665 women were ≥45 years of age and free of CVD at baseline. Information on AF was obtained from questionnaires answered by participants and from medical records. The study included 1,441 cases of AF during a median of 19.2 years of follow-up. The authors found that a 5% higher intake of energy from SFA replacing carbohydrates was associated with a higher risk of persistent and chronic AF with a HR of 1.47 (95% CI 1.04 to 2.09) in a multivariate analysis. The risk of developing paroxysmal AF was not statistically significant with a HR of 0.85 (95% CI 0.66 to 1.08). The p-value for the difference between developing paroxysmal or persistent/chronic AF was 0.01. If energy from MUFA replaced carbohydrates, there was a statistically significant lower risk of persistent and chronic AF with a HR of 0.67 (95% CI 0.46 to 0.98), while the HR for development of paroxysmal AF was 1.03 (95% CI 0.78 to 1.34) in the multivariate model. The p-value for the difference between developing paroxysmal and persistent/chronic AF was 0.07. Thus, the authors suggested that dietary fatty acids might prevent AF progression rather than preventing the initiation of the arrhythmia. The study also investigated the association between quintiles of SFA intake in energy percentages and the risk of incident AF with the first quintile as the reference. The authors did not find a statistically significant association between dietary intake of SFA and the risk of paroxysmal AF. However, there was a statistically significant positive association between SFA intake and persistent/chronic AF comparing the fifth quintile and the first quintile. This model does not account for substitution aspects. In substitution a dietary component is replaced by another, while keeping energy intake constant e.g. having a higher intake of SFA and a concomitantly lower intake of carbohydrates.

The mechanism(s) by which SFA may affect the risk of incident AF is not fully established. However, it has been suggested that C16:0 ceramide accumulation in cells may increase the permeability of the mitochondrial

membrane to proteins, leading to apoptosis (45). Subsequently, apoptosis may impair intermyocyte coupling due to the formation of fibrosis, which has been shown to cause a substrate for AF initiation in dogs (19,46,47). Also in dogs, Soloff (48) have shown that rapid infusion of C16:0 and C18:0 may cause arrhythmias - primarily conduction defects, while slow infusion of C16:0 and C18:0 and rapid infusion of MUFA and PUFA did not provoke arrhythmias.

Chapter 3. AIMS AND HYPOTHESES

The overall aim of this thesis was to evaluate the potential association between SFA and the risk of developing AF. The a priori hypothesis was that SFA were associated with a higher incidence of AF. The following three studies were designed to explore this hypothesis.

Study 1
Aim: To investigate how dietary substitution of SFA with MUFA or PUFA is associated with the risk of incident AF.
Hypothesis: Replacing dietary SFA with unsaturated fatty acids reduce the risk of AF.

Study 2
Aim: To examine the association between adipose tissue content of SFA and the risk of AF.
Hypothesis: Adipose tissue content of SFA are associated with a higher risk of AF.

Study 3
Aim: To explore the association between adipose tissue patterns of fatty acids and the risk of developing AF.
Hypothesis: Various patterns of fatty acids in adipose tissue are differently associated with the risk of incident AF.

Chapter 4. METHODS

4.1. STUDY POPULATION

This thesis was based on the Danish cohort "Diet, Cancer and Health" which was established with the purpose of exploring the association between dietary habits in a middle-aged population and the future risk of developing cancer. To be eligible for the cohort, participants had to be born in Denmark and not registered with a diagnosis of cancer in the Danish Cancer Registry at the time of invitation. A total of 160,725 Danish men and women aged 50-64 years, living in the urban areas of Copenhagen and Aarhus municipality received a written invitation between December 1993 and May 1997. If subjects had not responded within three weeks a first reminder was sent. If they did not reply to this within another three weeks a second reminder was sent. After this no further attempts were made to include the participant. A total of 57,053 individuals (29,875 women and 27,178 men) accepted the invitation and provided written informed consent including permission for prospective data collection using their social security number and national registries. Information on participants' dietary habits and lifestyle factors were obtained from questionnaires and interviews at a baseline visit to one of the study centres. At the baseline visit participants had anthropometric measurements done and biological material was sampled (49).

A randomly drawn subcohort of 3,500 participants was used in studies with case-cohort designs e.g. when using adipose tissue samples (Study 2 and 3 in this thesis).

Participants with a delayed baseline registration of cancer in the Danish Cancer Registry were excluded from the cohort and in the studies included in this thesis, we excluded participants with a diagnosis of thyroid disease and known AF and/or AFL before baseline. Follow-up was until July 31st 2013, a diagnosis of AF/AFL in the Danish National Patient Register, death or emigration, whichever came first.

The relevant ethics committees (project number: N-20160001) and the Danish Data Protection Agency (project number: 2015-186) approved the study.

4.2. FOOD-FREQUENCY QUESTIONNAIRES

Prior to the baseline visit at a study centre the participants had completed a detailed, semiquantitative 192-item FFQ. The FFQ was developed for this cohort according to traditional Danish dietary habits. The FFQ was found to be a useful tool for categorizing individuals according to their intake of energy and nutrients when validated against a 7-day weighed dietary record (50).

Participants were asked to report their average intake of different foods and beverages within the last 12 months in categories ranging from never up to ≥8 times a day. Intake of fatty acids such as SFA in g/day was calculated using the software FoodCalc (Center for Applied Computer Science, University of Copenhagen, DK; http://www.ibt.ku.dk/jesper/foodCalc) using standardized recipes and portion sizes for Danish dishes. In order not to overestimate the intake of specific dietary items, the questions on frequency of specific items were supplemented by questions regarding the intake of food groups such as vegetables – cooked or raw, meat and total fruits. Afterwards, the intake of the specific items was weighted according to the answers on intake of food groups (49–51) ensuring that there was concordance between e.g. number of carrots eaten and vegetables as a food group.

4.3. ADIPOSE TISSUE

All participants in the cohort had an adipose tissue biopsy taken at baseline.

The adipose tissue biopsy was taken from the buttocks according to the method described by Beynen and Katan (52). In brief, the participant lied face down on an examination couch and the skin on the upper half of the buttocks was disinfected. The participants were instructed to tense his/hers muscles and a skinfold from the upper outer quadrant was held with one hand. This location was chosen to minimize the risk of damaging the ischiatic nerve (52). A luer lock system (Terumo, Terumo Corp, Tokyo, Japan) with a needle, a venoject multisample luer adaptor and an evacuated blood tube was inserted in an angle of about 45˚ with the other hand. After the procedure, the samples were flushed with nitrogen and stored at -150°C until analysis. Prior to analysis, the biopsies were thawed and a small amount of adipose tissue was removed and prewarmed at 50°C for 10 min. Still at 50°C, heptane was used to dissolve the fat and the fatty acids were transesterified for 2 min with 2 mol/L KOH, according to the International Union of Pure and Applied Chemistry Standard Methods for the Analysis of Oils, Fats, and

Derivatives. The fatty acid composition was determined by gas chromatography using a Varian 3900 GC with a CP-8400 autosampler (Varian, Middleburg, The Netherlands) equipped with a flame ionization detector. Constant flow, split injection mode and a CP-sil 88 60m x 0.25-mm internal diameter capillary column and temperature programming from 90°C to 210°C were used. Helium was used as the carrier gas. Commercially available standards (Nu-Chek-Prep, Inc, Elysian, MN, US) were used to identify the individual fatty acids (45,53). Results for each fatty acid were given as percentages of total fatty acids.

4.4. OUTCOME

The outcome in the studies was incident AF and/or AFL during the follow-up period for participants in the "Diet, Cancer and Health" cohort. Follow-up was from baseline until a diagnosis of AF/AFL in the Danish National Patient Register, death, emigration, or July 31st 2013, whichever came first. For study participants their social security number was linked to the Danish National Patient Register where information on AF status was retrieved. Since 1977 hospital discharge diagnoses have been recorded in the Danish National Patient Register and from 1995 diagnoses from out-patient and emergency rooms visits have been recorded as well. Information regarding incident cases of AF and/or AFL was obtained using the 8th International Classification of Diseases (ICD-8, code 427.4 in the international version and 427.93/427.94 in the Danish version) until the end of 1993. Afterwards, the 10th Revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) (code I.48.9x) was used. A previous validation study (54) found a positive predictive value of the combined diagnosis of AF/AFL in this cohort to be 92.6% (95% CI 88.8% to 95.2%).

4.5. STATISTICS

We used time-to-event analysis with the combined diagnosis of AF and AFL being the event of interest. We employed Cox proportional hazard regression analysis with age as the underlying time axis and delayed entry to adjust optimally for age. Patients were considered at risk from baseline until the end of follow-up on July 31st 2013, a diagnosis of AF/AFL in the Danish National Patient Register, death or emigration, whichever came first. Due to age being the underlying time axis we adjusted all models for baseline age to ensure that the age of exposure data were comparable.

Study 1 was a cohort design and the whole cohort was used in Cox proportional hazards regression analysis. We used substitution models

where total fat intake, total energy intake, and energy from protein and carbohydrates were kept constant (55). The regressions were set up in such a way that we compared the rate of AF between subjects having statistically replaced SFA with either MUFA or PUFA.

Study 2 and 3 were based on fatty acid composition of adipose tissue and we therefore used a case-cohort design and weighted Cox proportional hazards regression. Adipose tissue composition was available for cases and all members of the subcohort. Weights were assigned to all participants according to the method described by Kalbfleisch and Lawless (56). The weight was one for all cases and biopsies from noncases in the randomly drawn subcohort were weighed as (number of noncases in the cohort)/(number of noncases in the subcohort).

In Study 3, we examined patterns of fatty acids in adipose tissue using the statistical method treelet transform (TT) (57). TT is a data-driven dimension-reducing method that produce a number of factors, which account for much of the variation in the dataset. TT forms sparse patterns/factors of adipose tissue fatty acids where not all fatty acids contribute to every factor in contrast to the more widely used principal component analysis (58), thus, TT is considered simpler to interpret. When creating factors, multidimensional data becomes weighted averages for each individual.

The output of TT is a dendrogram/cluster tree. The branches of the tree contains the related groups of variables. To extract factors/patterns the tree has to be cut. The optimal level at which to cut was determined by cross-validation with a range of factors as described by Gorst-Rasmussen (59). Scores for each case and participants in the subcohort were generated from the factors according to the individual's fatty acid profile. Afterwards, quintiles for factors were used as exposure variables in weighted Cox proportional hazards regression.

Analyses were stratified by sex in all three studies. Model assumptions for Cox proportional hazards regression were assessed with plots of Schoenfeld residuals versus age and were not violated (data not shown). A two-tailed p-value <0.05 was considered statistically significant. The latest available version of Stata statistical software (versions 14 and 15; StataCorp LP, College Station, TX, US) was used.

Models

As mentioned in "Chapter 2. Background" there are several known risk factors for AF. From the existing literature we a priori chose potential confounders and risk factors to adjust for in the analyses. The same three models apply for all three studies.

Model 1: Minimally adjusted

Categorical variable:

- Baseline age in quintiles

This was done to ensure that the age of exposure data were comparable when age was used as the time axis. This can be exemplified by considering two persons of the same sex who entered the study on the same date at 50 and 55 years of age, respectively. To compare their hazard of AF at 56 years of age, exposure data would be 6 years for the first participant and 1 year for the second participant. Thus, we adjusted for baseline age.

Model 1A: Adjusted for Model 1 and for lifestyle factors.

Categorical variables:

- Years in school (≤7 years, 8 to 10, ≥10 years)
- Smoking (never, former, <15, 15 to 25, >25 g/day)

Continuous variables were adjusted for as restricted cubic splines with five knots positioned as described by Harrell (60).

- Body mass index (kg/m^2)
- Waist circumference (cm)
- Alcohol intake (g/day)

By adjusting for lifestyle factors the risk of potential confounding was reduced.

Model 2: Adjusted for Model 1A and for comorbidities registered at baseline. Disease data were retrieved from national registries.

Categorical variables:

- History of hypertension and/or treatment for hypertension (yes/no)

- History of hypercholesterolemia and/or cholesterol-lowering treatment (yes/no)
- Angina pectoris (yes/no)
- Heart failure (yes/no)
- Previous myocardial infarction (yes/no)
- Diabetes mellitus (yes/no)
- Renal disease (yes/no)

The comorbidities are strong risk factors for developing AF, hence they should be included in the model in order to avoid bias. Adjusting for these comorbidities could, however, cause bias by being potential intermediate variables. Hereby risk factors for the comorbidities become confounders. For the comorbidities included in this study this might be a minor problem, as most of the risk factors for developing the listed comorbidities are common to those of AF

Chapter 5. STUDIES

A flowchart of the participants in the "Diet, Cancer and Health" cohort and the three studies are shown in Figure 3.

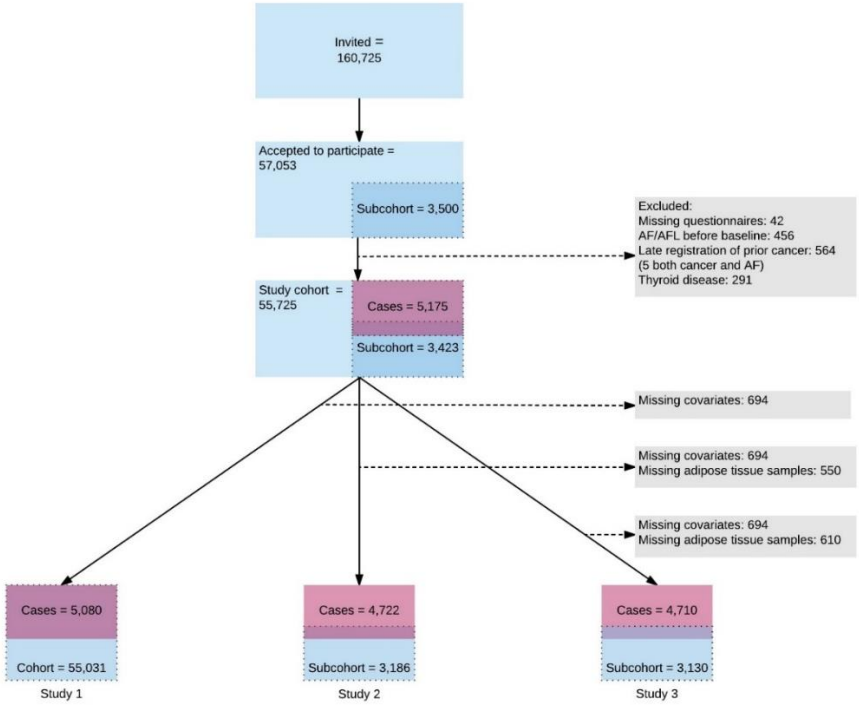


Figure 3. Flowchart of the cohort and overview of the three studies.
Note: More adipose tissue samples are missing in Study 3 than Study 2 because Study 2 only included the major SFA while Study 3 also involved fatty acids found in smaller concentrations in adipose tissue.

5.1. STUDY 1

Aim

In Study 1, we wanted to explore how substituting dietary SFA (C12:0-24:0) with MUFA or PUFA affected the risk of developing AF in men and women. The a priori hypothesis was that replacing SFA with MUFA or PUFA would lower the risk of AF.

Study design

A cohort study where participants in the "Diet, Cancer and Health" study were followed using data on AF status from the Danish National Patient Register.

Methods

Data on dietary intake of fatty acids originated from the 192-item FFQ answered by all participants at baseline. If data on covariates were missing participants were excluded. The intake of fatty acids in g/day was calculated using standardized recipes and portion sizes for Danish dishes.

Data were analysed by Cox proportional hazards regression with age as the time axis. All models were adjusted for age at baseline in quintiles to ensure that the age of exposure data were comparable. Substitution models kept total fat intake, total energy intake, and energy from protein and carbohydrates constant (55). Then, we statistically substituted 1 g/day of SFA with 1 g/day of MUFA, PUFA, total n-3 PUFA, total n-6 PUFA, and the major dietary n-6 PUFA linoleic acid.

Main results from the study

A total of 5,080 cases of AF occurred during a median follow-up of 17 years. AF occurred more often in men than in women (3,119 cases/7.8 per 1000 person-years vs. 1,961 cases/4.2 per 1000 person-years, respectively).

Men had a HR of 1.08 (95% CI 1.02 to 1.14) when replacing 1 g/day of SFA with 1 g/day of total n-3 PUFA, while no statistically significant association was found when substituting SFA with MUFA, total PUFA or n-6 PUFA. In women, no statistically significant associations when substituting SFA with PUFA or MUFA could be demonstrated.

Strengths and limitations

The study had several strengths: If a person were to lower the intake of SFA without losing weight then dietary SFA would have to be replaced by something else e.g. the use of vegetable oils instead of butter when preparing food. Thus, substitution models simulates the way we eat and ensures a clear interpretation of the results. We used national registries on vital and disease status, which provided a long and almost complete follow-up. We used a validated FFQ (50,51) and the positive predictive value for the diagnosis of AF was high in the cohort (54). Furthermore, due to the large number of cases we were able to investigate the association for men and women separately.

The study also had limitations: Study participants were all Caucasians and middle-aged at inclusion, thus the results may not be valid in other race or age groups. Unfortunately, we only had information on diet at baseline and a change in the participant's diet during the follow-up period could potentially affect the risk of developing AF. Due to the participants being middle-aged they might, however, have a more stable diet than e.g. younger individuals. We adjusted for known confounders for AF registered at baseline which did not alter the results but there is always the possibility of residual confounding e.g. from hereditary factors which we did not have any information on. We were only able to identify cases of AF diagnosed in hospitals and some patients might have been treated by a private practising cardiologist or general practitioners. Also, patients with minor symptoms might not have sought medical help. Finally, as this was an observational study we were not able to establish causality, only suggest associations.

Main conclusions from the study

In contrast to the a priori hypothesis, we found that men had a higher hazard of AF when replacing dietary SFA with total n-3 PUFA. In women and when substituting SFA with MUFA, total PUFA, or n-6 PUFA we could not demonstrate a lower hazard of AF as hypothesized.

5.2. STUDY 2

Aim

The aim of the study was to investigate the association between adipose tissue content of total SFA including the major SFA C14:0, C16:0 and C18:0 and the risk of developing AF. Our hypothesis was that a high adipose tissue content of SFA was associated with development of AF.

Study design

The study was a case-cohort study based on the "Diet, Cancer and Health" cohort.

Methods

Adipose tissue composition was available for all cases and participants in the randomly drawn subcohort and was analysed as described in section "4.3. Adipose Tissue". Information on AF status was found in national registries. We calculated hazard ratios (HR) for the exposure in quintiles using weighted Cox proportional hazards regression with age as the time axis and the first quintile as the reference. To examine SFA as a continuous variable we used restricted cubic splines with three knots in order to see a potential non-linear association (60).

Radar plots provided the possibility of visually exploring underlying dietary patterns associated with higher levels of SFA in adipose tissue (61).

We calculated the Pearson correlation coefficients for adipose tissue content of SFA and the dietary intake of SFA adjusted for total energy intake in men and women separately.

Main results from the study

A total of 4,722 cases of AF occurred during a median follow-up of 14.6 years.

A statistically significant association between adipose tissue content of SFA in quintiles and the rate of AF could not be established in either men or women in any of the three models.

When examining dose-response relationship we found a slightly inverse U-shaped curve in men and a slightly U-shaped curve in women in all

three models. None of these associations were, however, statistically significant.

The radar plots illustrated that a diet rich in SFA was associated with higher levels of SFA in adipose tissue, while a low content of SFA in adipose tissue was associated with a diet usually considered healthier in both men and women.

The Pearson correlations coefficients adjusted for total energy intake relating dietary intake of SFA to their content in adipose tissue were low ($r = 0.17$ for men and $r = 0.14$ for women).

Strengths and limitations

The study had some strengths: We had a large number of cases which made it possible to investigate the association for men and women separately. The use of national registries provided us with long and almost complete follow-up and the diagnosis of AF in this cohort has been validated previously (54). We used adipose tissue, which is rarely available in other large cohort studies, as an objective marker of the body's exposure to SFA.

However, there were some limitations: The adipose tissue content of other SFA such as C12:0, C20:0 and C22:0 was too low to meaningfully examine their potential association with AF. The content of SFA was reported as percentages of total fatty acids, thus depending on the content of other fatty acids, which may be considered a limitation. The adipose tissue biopsy was only taken at baseline so adipose tissue composition might have changed if the participants had altered lifestyle and dietary habits during the follow-up period. Furthermore, we used subcutaneous adipose tissue from the buttocks, which may contain more MUFA and less SFA than subcutaneous abdominal adipose tissue (62–64). Also, the low correlation coefficients relating dietary SFA intake to SFA content in adipose tissue suggest that adipose tissue content of AF is not a good objective marker of the long-term dietary intake of SFA. All participants were Caucasians and middle-aged at enrolment so the results may not apply for other race or age groups.

Main conclusions from the study

In this study we did not confirm the hypothesis of a direct association between adipose tissue content of SFA and the risk of AF. In contrast, our findings suggest a neutral association between adipose tissue content of SFA and the risk of developing AF.

5.3. STUDY 3

Aim

To explore the association between fatty acid patterns in adipose tissue and the risk of incident AF. Our hypothesis was that certain patterns of fatty acids would be associated with AF. Especially, if a pattern containing SFA was identified by TT, this would be associated with an increased risk of incident AF.

Study design

The study was a case-cohort study.

Methods

Adipose tissue composition was available for all cases and the randomly drawn subcohort and was analysed as described in section "4.3. Adipose Tissue". Information on AF status was obtained from national registries. We performed TT as described in section "4.5. Statistics". Quintiles of factors were used as the exposure variables in weighted Cox proportional hazards regression with the first quintile as the reference.

Main results from the study

During a median follow-up of 14.6 years, 4,710 participants developed AF.

We identified seven factors from the dendrogram containing eight fatty acids at the most and they explained 66% of the fatty acid variance in the subcohort.

TT1 contained SFA, while TT2 consisted of trans fatty acids and TT3 consisted of marine n-3 PUFA. TT4 contained n-6 PUFA (except linoleic acid), TT5 contained n-9 fatty acids, while TT6 consisted of minor products of stearoyl-CoA desaturase and TT7 consisted of the plant-based n-3 PUFA α-linolenic acid and the n-6 PUFA linoleic acid.

For men in TT1, there were a neutral to weakly negative association for AF with HR = 0.82 (95% CI 0.65 to 1.02) in the fifth quintile compared to the first quintile, while for women the HR was 0.76 (95% CI 0.58 to 0.99), suggesting a potentially lower hazard of AF associated with a pattern with SFA.

Patterns with marine n-3 PUFA (TT3) and n-9 fatty acids (TT5) were associated with a lower hazard of AF in women, but not in men. Patterns with n-6 PUFA (TT4) was associated with a lower hazard of AF in both women and men.

Strengths and limitations

This study had several strengths: We studied the naturally occurring adipose tissue fatty acid patterns and their association with the hazard of developing AF. We did so in a large cohort with a long and almost complete follow-up for both AF and vital status. The diagnoses of AF in this cohort had previously been validated with a high positive predictive value (54). If AF cases were misclassified this would unlikely be associated with the exposure and would therefore lead to an underestimation of a potential association.

The study also had limitations: Adipose tissue samples were obtained once, so a change in the participants' dietary habits, lifestyle and metabolism could change the composition of fatty acids in adipose tissue and thereby the patterns found by TT, which this study would be unable to capture. All study participants were Caucasians and 50-64 years old at enrolment so the results may not be valid in other race or age groups. Also, there is always a possibility of residual confounding.

Main conclusions from the study

We found that a pattern with SFA may be associated with a lower hazard of AF in women. A priori we had expected that patterns with SFA would be associated with a higher hazard of AF. In both men and women patterns with n-6 PUFA may be associated with a lower hazard of AF and finally, patterns with marine n-3 PUFA and n-9 fatty acids were associated with a lower hazard of AF in women.

Chapter 6. DISCUSSION

The aim of this thesis was to explore the association between substitution of dietary fatty acids and adipose tissue content of SFA and development of AF as well as the association between adipose tissue patterns of fatty acids and the risk of incident AF. The a priori hypothesis was that SFA would be associated with a higher risk of developing AF. The studies were based on the large Danish cohort "Diet, Cancer and Health" that included more than 57,000 participants with information on dietary intake of SFA and adipose tissue biopsies available for fatty acid composition analysis as a biomarker of SFA exposure.

In Study 1, we examined how replacement of dietary SFA with either MUFA or PUFA was associated with the risk of AF in men and women. In study 2, we explored the association between adipose tissue content of the major SFA and the risk of incident AF. Adipose tissue content of fatty acids is considered an objective long-term marker of both the dietary intake and metabolism within the previous 1-3 years and of the de novo lipogenesis within the body (62,64–66). In Study 3, we identified patterns of fatty acids in adipose tissue by TT and estimated the risk of AF associated with each pattern.

The mainly neutral findings for the association between SFA and incident AF presented in this thesis and associated papers (45,67) cannot be directly compared to the existing literature due to different analytical materials and methods. Table 2 provides a summary of the three studies presented in this thesis compared to the papers by Fretts et al. (18) and Chiuve et al. (17).

	Study design	Cases of AF (Participants)	Sex Age	Exclusion criteria	Follow-up period	Exposure	Fatty acids
Study 1 (67)	Cohort Study	5,080 (57,053)	Both 50-64 years	Cancer Thyroid disease AF/AFL	17 years	FFQ and substitution of SFA	Combined C12:0-C24:0 substituted with MUFA and PUFA
Study 2 (45)	Case-cohort Study	4,722 (57,053)	As Study 1	As Study 1	14.6 years	Adipose tissue content of SFA	Combined C14:0-C18:0
Study 3	As Study 2	4,710 (57,053)	As Study 1	As Study 1	As Study 2	Adipose tissue patterns of fatty acids	All measured fatty acids in adipose tissue
Fretts et al. (18)	Cohort Study	707 (2,899)	Both ≥65 years	Coronary heart disease AF	11.2 years	Plasma phospholipid SFA	C16:0-C24:0, both combined and individually
Chiuve et al. (17)	Cohort Study	1,441 (33,665)	Women ≥45 years	Cardiovascular disease Cancer Chronic diseases* AF	19.2 years	FFQ and substitution carbohydrates Quintiles of SFA	Carbohydrates substituted with SFA and MUFA Combined C4:0-C14:0 (quintiles) C16:0 and C18:0 individually (quintiles)

Table 2. Characteristics of the studies presented in this thesis and the previously published papers. AF, atrial fibrillation. AFL, atrial flutter. FFQ, food-frequency questionnaire. SFA, saturated fatty acids. MUFA, monounsaturated fatty acids. PUFA, polyunsaturated fatty acids. *Not specified which diseases this was, the authors adjusted the results for hypertension, diabetes mellitus and hypercholesterolemia.

In contrast to our hypothesis, the main finding of Study 1 (67) was a moderately higher risk of AF with a HR of 1.08 (95% CI 1.02 to 1.14) when 1 g/day of dietary SFA was replaced by n-3 PUFA in men. For women there were no association between replacing SFA with n-3 PUFA and incident AF (HR 0.99, 95% CI 0.90 to 1.08). It is unknown why an association was only seen in men but it might be due to differences in sex hormones, which may affect metabolic processes involved in the processing of fatty acids. It would have been of interest to separate the marine n-3 PUFA and the plant-based n-3 PUFA α-linolenic acid, but this was unfortunately not possible since the median consumption of total n-3 PUFA was 2.4 g/day of which the intake of marine n-3 PUFA was only 0.6 g/day. Several studies examining the effect of dietary marine n-3 PUFA and the risk of AF has been published previously. In this cohort, Rix et al. (11) reported a U-shaped association between the intake of marine n-3 PUFA and the risk of incident AF and found that the lowest risk of AF was close to the median intake of marine n-3 PUFA (0.6 g/day) in the population with higher risks observed with both very low and high intakes of marine n-3 PUFA. In another large study, Mozaffarian et al. (13) investigated the association between fish intake and the risk of AF in a population-based cohort of 4,815 adults including 980 cases of AF and found that fish intake ≥1 time a week was associated with a significantly lower risk of AF. Studies on dietary intake of α-linolenic acid and the risk of AF have not demonstrated any associations (68).

A study by Chiuve et al. (17) (Table 2) found that replacing 5% of dietary energy from carbohydrates with SFA was associated with a higher risk of persistent and chronic AF in women (33,665 women including 1441 cases of AF) with a HR of 1.47 (95% CI 1.04 to 2.09). If MUFA replaced energy from carbohydrates a lower risk of persistent and chronic AF with a HR of 0.67 (95% CI 0.46 to 0.98) was found. The results can, however, not be directly compared to ours since we substituted SFA with unsaturated fatty acids and not with carbohydrates. When we substituted SFA with MUFA we found a neutral association with AF for both men and women in all three models. This was somewhat unexpected since the large Prevención con Dieta Mediterránea (PREDIMED) trial previously reported that supplementing a Mediterranean diet with extra-virgin olive oil significantly reduced the risk of incident AF (69). An explanation for this could be that a traditional Danish diet does not resemble the Mediterranean diet and that the MUFA Danes consume may not come from pure vegetable oils but from other sources such as mayonnaises and other dressings, which in turn may be associated with a diet considered unhealthy.

Chiuve et al. (17) also investigated the association between quintiles of SFA intake in energy percentages and the risk of incident AF using the first quintile as the reference. This model does not incorporate substitution and can therefore not be directly compared to Study 1. The authors did not find an association between dietary intake of SFA in quintiles and the risk of paroxysmal AF where none of the individual HR were statistically significant and the p-value for trend was >0.05. When looking at the HR for persistent/chronic AF there was a statistically significant higher hazard of AF in the fifth quintile compared to the first quintile and a significant trend for a higher risk of AF ($p \leq 0.03$), suggesting a higher risk of developing more sustained forms of AF with a higher intake of C4:0 to C10:0, C12:0 and C14:0 as well as of C16:0. Intake of C18:0 was on the other hand not associated with any forms of AF development.

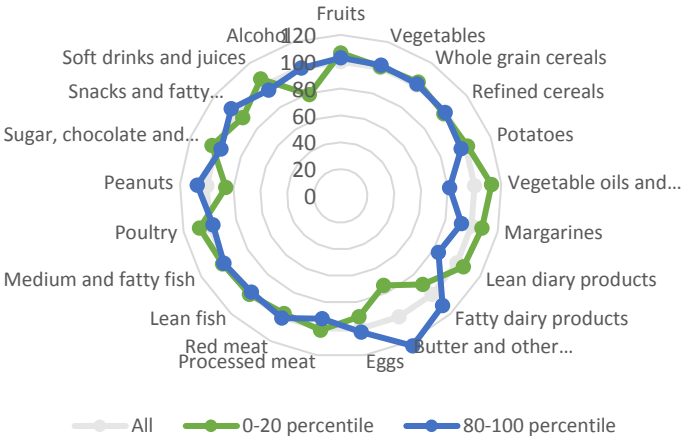
The main findings of Study 2 (45), where we examined adipose tissue composition of fatty acids, did not confirm our a priori hypothesis of an association between adipose tissue content of SFA and a higher risk of incident AF. In contrast, we found neutral associations both when investigating the SFA content in quintiles and as a continuous variable for both men and women. The study included adipose tissue content of the major SFA C14:0, C16:0 and C18:0. We chose not to investigate the association between adipose tissue content of individual SFA and the risk of AF since dietary SFA originates from shared food sources (Table 1) (44).

We calculated Pearson correlation coefficients (adjusted for total energy intake) between dietary intake of the major SFA and their adipose tissue content of these. For men we found $r = 0.17$ and for women $r = 0.14$. Thus, the adipose tissue content of SFA was not a good objective measure of the dietary intake of SFA in this cohort. In a validation study prior to establishment of the "Diet, Cancer and Health" cohort the Pearson correlation coefficient for adipose tissue content of SFA and another FFQ than used in this study was 0.27 in men and 0.23 in women (70). These and our findings were in line with previous studies. Thus, Baylin and Campos (71) also reported low correlation coefficients for SFA in the diet and in adipose tissue and argued that this was probably due to de novo lipogenesis, which is in line with the interpretation by other authors (18). Thus, German and Dillard (44) proposed that dietary intake of fatty acids determines less than 25% of the interpersonal variance in adipose tissue composition. In contrast, adipose tissue may serve as a more accurate biomarker for odd numbered SFA, such as C15:0 and C17:0 that cannot by endogenously synthesized (71). It has been suggested that de novo lipogenesis of SFA is sparse when consuming sufficient energy and

>25% of energy is from fat (64), which was the case in this cohort where the median energy intake from fat was >30% in both sexes. Although not a good objective measure of the dietary intake of SFA in this cohort, adipose tissue is a measure of the body's endogenous exposure to SFA and is determined by dietary intake of fatty acids as well as de novo lipogenesis from ingested carbohydrates and proteins (38–42).

The association between content of SFA in adipose tissue and dietary SFA is illustrated by radar plots (61) in Figure 4.

Men



Women

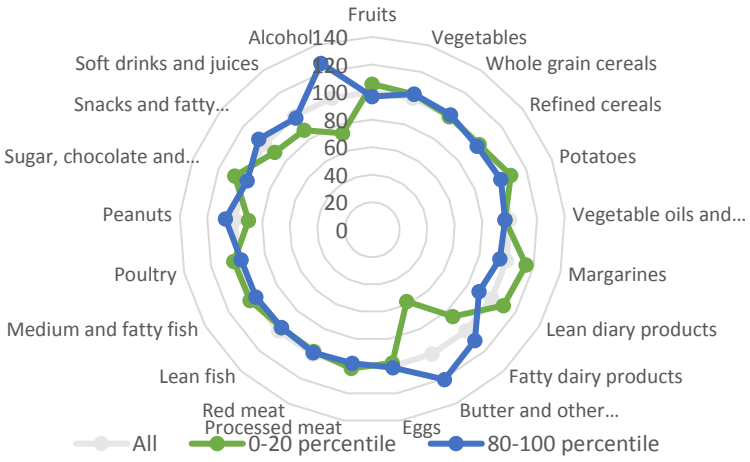


Figure 4. Radar plots. The median intake of selected food groups for participants in the lowest and highest quintiles of adipose tissue content of total SFA. The median in each group was indexed according to the overall median (grey line). Median intake in the lowest quintile (green line) and the highest quintile (blue line). *Reproduced with permission from Dinesen PT, Rix TA, Joensen AM, Tjønneland A, Lundbye-Christensen S, Overvad K, et al. Adipose Tissue Content of Saturated Fatty Acids and Atrial Fibrillation: a Case-cohort Study. Eur J Clin*

Invest. 2017;47(12):e12836. Copyright: Stichting European Society for Clinical Investigation Journal Foundation.

Radar plots (Figure 4) evaluates the association between dietary patterns illustrated by median intake of food groups and adipose tissue content of SFA in percent. Patterns illustrate differences in the underlying dietary patterns for participants with high and low adipose tissue content of SFA and the interpretation is based on knowledge of dietary sources of SFA. In both men and women there appeared to be an association between a diet rich in SFA and the content of SFA in adipose tissue. Furthermore, for women there appeared to be an association between consumption of alcohol and adipose tissue content of SFA. Alcoholic beverages is not a major source of SFA but dietary patterns rich in SFA can be associated with a higher consumption of alcohol.

Fretts et al. (18) (Table 2) used plasma phospholipids instead of adipose tissue as a biomarker of the body's exposure to SFA. Their results showed that higher circulating plasma levels of C16:0 were associated with a higher risk of AF when comparing the highest with the lowest quartile. Higher circulating levels of C18:0 to C24:0 were associated with a lower risk of incident AF when comparing the highest with the lowest quartile. When investigating C16:0 to C24:0 the association with incident AF was neutral. This suggests that chain length may be of importance for the effect of SFA on incident AF. The results, however, cannot be directly compared to ours as plasma phospholipids is a more short-term biomarker than adipose tissue used in our study (62,64,72).

Regarding circulating levels of SFA, we investigated the association between baseline serum content of SFA (C16:0 and C18:0) and the prevalence of AF in the Norwegian Omega-3 fatty acids in Elderly patients with Myocardial Infarction cohort (73) in an unpublished conference abstract. These analyses consisted of 534 individuals aged ≥70 years of which 67 had a diagnosis of AF at baseline. We found that there might be a protective association between serum content of SFA and the prevalence of AF with an odds ratio of 0.64 (95% CI 0.31 to 1.30) for the fourth quartile compared with the first quartile but confidence intervals were wide, possibly due to the relatively low number of cases.

Exploring patterns of fatty acids in adipose tissue (as done in Study 3), may provide new important information on how fatty acid patterns is associated with the risk of incident AF because individual fatty acids are highly correlated as a result of shared dietary sources and metabolic pathways (74). By using the statistical method TT, patterns were formed based on correlations between variables. A fatty acid is only contained in

one pattern and therefore TT is considered easier to interpret than the more widely used principal component analysis (58). Fatty acid patterns identified in Study 3 were characterized by the chemical structure of the fatty acids, which was also the case in a previous study using principal component analysis (75).

A priori, it was expected that a pattern containing SFA would be positively associated with AF. The analyses did not confirm this hypothesis in men, where we found a neutral to weak negative association with AF, with a HR of 0.82 (95% CI 0.65 to 1.02) between the highest quintile and the lowest quintile. In women, a pattern with SFA was associated with a statistically significant lower hazard of AF in quintile 5 compared to the first quintile with a HR of 0.76 (95% CI 0.58 to 0.99). As previously mentioned, the association between SFA and the risk of developing AF has shown conflicting results (17,18,45,67,76).

An inverse association between a marine n-3 PUFA pattern and AF was only seen in women. Similarly, previous studies on adipose tissue marine n-3 PUFA and the risk of AF have shown inconsistent results. A previous study on the subcohort used in Study 3 of this thesis, showed that marine n-3 PUFA in adipose tissue had a monotonic, negative dose-response trend suggestive of a negative association between marine n-3 PUFA and development of AF, although the association did not reach statistical significance (77). Another biomarker of seafood consumption is circulating marine n-3 PUFA, and its association with AF has previously shown conflicting results. Thus, Gronroos et al. (12) did not find an association between plasma levels of eicosapentaenoic acid and docosahexaenoic acid and incident AF, while Virtanen et al. (15) found that higher levels of marine n-3 PUFA in serum may protect against AF.

A pattern consisting of the plant-based α-linolenic acid and the n-6 PUFA linoleic acid was neutrally associated with the hazard of AF in both sexes. Previous studies on dietary intake and circulating levels of α-linolenic acid and the risk of AF have not established an association (15,68) and notably a combined intake of arachidonic and linoleic acid was not associated with AF in the large Framingham Heart Study (78).

We did not identify published studies regarding the association between adipose tissue patterns of trans fatty acids, n-6 PUFA (except linoleic acid) or n-9 fatty acids, and the risk of incident AF.

As seen in Table 2, the papers behind this thesis combines SFA as the exposure, while the other studies have explored individual SFA and the risk of incident AF. Due to the shared dietary sources (Table 1) and

metabolic pathways of SFA, individual SFA as exposure may introduce problems (44). As an example, C16:0 and C18:0 share dietary sources. Thus, when exploring e.g. C16:0 as an individual exposure there could be a positive association with AF, but due to C16:0 and C18:0 being highly correlated there is the risk of collinearity. Adjusting for C18:0 would not be an optimal solution, as the interpretation would be unclear and it would not be possible to solely consume C16:0 and not C18:0 due to the shared dietary sources.

The studies by Fretts et al. (18) and Chiuve et al. (17) excluded participants with coronary heart disease and CVD at baseline, while we adjusted for previous myocardial infarction, and angina pectoris because myocardial infarction has been found to be positively associated with incident AF (28,79). As mentioned previously, adjustment for comorbidities may cause bias. Some participants will develop CVD during follow-up, which was adjusted for as a time-varying covariate by Chiuve et al. (17). We did not adjust the results using time-varying covariates because suffering from an acute myocardial infarction or other CVD during follow-up may alter the participants' diet and therefore also the exposure. If exposure information was updated after the event, time-varying covariates could have been a possibility as both information on exposure and covariates would then have been updated (80,81). However, this would change the research question. Chiuve et al. (17) administered a second FFQ and updated the exposure data, which attenuated the association between SFA and the risk of persistent/chronic AF, which was no longer statistically significant when substituting energy from carbohydrates with energy from SFA.

We did not have information on plasma concentrations of SFA in this cohort, otherwise it would have been of interest to explore the association between plasma concentrations of SFA and the hazard of AF and compare the results to those reported by Fretts et al. (18). Finally, Chiuve et al. (17) found that the effect of SFA differed between paroxysmal and chronic AF. However, the analytical method used by Chiuve et al. (17) does not answer this question sufficiently as their analyses does not reflect the association between SFA and progression but solely which diagnoses the patient receive within two years of the initial diagnosis. In our cohort, the diagnoses of all AF types and AFL were combined as one outcome variable. If we wanted to investigate progression from paroxysmal AF to longer-lasting forms this would require registration of subtypes and the use of a so-called multistate model.

Chapter 7. CONCLUSIONS AND PERSPECTIVES

This thesis examined the association between dietary intake and adipose tissue content of SFA, as well as how patterns of fatty acids in adipose tissue was associated with incident AF.

We found that replacing dietary SFA with n-3 PUFA was associated with a higher the risk of AF in men, while the association was neutral in women. Substituting SFA with MUFA, total PUFA, n-6 PUFA or the major n-6 PUFA linoleic acid was not associated with the risk of incident AF.

Next, we explored if adipose tissue content of SFA was associated with the development of AF. Results showed a neutral association between adipose tissue content of SFA and the risk of AF in both men and women.

Lastly, we identified patterns of fatty acids in adipose tissue and explored their association with AF. A pattern with SFA was associated with a lower hazard of AF in women, while the association was neutral in men. Patterns with marine n-3 PUFA and n-9 fatty acids were both associated with a lower hazard of AF in women and a pattern with n-6 PUFA (except linoleic acid) was associated with a lower hazard of AF in both men and women.

AF remains a socio-economic burden to society and can have serious consequences for the affected individuals and their families, therefore preventive initiatives are essential. The mainly neutral findings regarding SFA and AF presented in this thesis expand the findings from previous studies, suggesting that SFA is not associated with incident AF. However, SFA may have other adverse effects on CVD that needs to be taken into consideration regarding the overall health of patients (82).

The existing literature on SFA and AF has explored diverse aspects including dietary intake of SFA and substitution of fatty acids and carbohydrates with SFA, circulating levels of SFA, adipose tissue content of SFA and adipose tissue patterns with SFA and none of these large observational studies have suggested a major clinically important association between SFA and incident AF. Thus, the need for additional observational studies seems limited.

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