DISSERTATION

Investigation on Polyunsaturated Fatty Acid Levels of Patients with and without Coronary Artery Disease and on the Impact of Statins on Fatty Acid Metabolism

Die Konzentration mehrfach ungesättigter Fettsäuren im Blut bei Patienten mit und ohne koronare Herzkrankheit und die Statinwirkung auf den Fettsäurestoffwechsel

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Table of contents

List of tables iii

List of figures iv

List of abbreviations v

Abstract 1

Zusammenfassung 2

1 Introduction 3

1.1 Classification of fatty acids 3

1.2 The fatty acid discovery process 4

1.3 Synthesis of fatty acids 5

1.4 Fatty acids and clinical disease 7

2 Methods 9

2.1 Research subjects 9

2.2 Sample collection and clinical data acquisition 9

2.3 Sample preparation and determination by GC 9

2.4 Statistics 11

3 Results 13

3.1 Patient clinical data and records 13

3.2 Comparative analysis of total fatty acids in patients with CAD and controls 13

3.3 Comparing fatty acid levels in patients with and without statin therapy 16

3.4 Fatty acid profile comparison between control and coronary artery disease patients not on statin therapy 17

4 Discussion 20

4.1 Relationship between n-3 PUFA and CAD 20

4.2 Effect of n-3 PUFA on triglycerides 22

4.3 Effect of statins on the distribution of n-6 PUFAs in CAD patients 22

4.4 Strengths and weaknesses of the study 23

5 Conclusions 25
6 Reference list 26
7 Statutory Declaration 33
8 Declaration of your own contribution to the publications 34
9 Printing copy of the publication 35
10 Curriculum vitae 50
11 Publication list 51
12 Acknowledgments 52

List of tables

Table 1 Patient’s Characteristics 13

List of figures

Figure 1. Elongation and desaturation pathways of n-3 PUFA and n-6 PUFA.....6
Figure 2. FA levels in blood from patients without and with CAD. 15
Figure 3. n-3 PUFAs and dyslipidemia. 16
Figure 4. Correlation of FAs and statin treatment. 17
Figure 5. Correlation of FAs in control and CAD patient subgroups without statin treatment. 19

List of abbreviations

AA	Arachidonic acid
AdA	Adrenic acid
AMI	Acute myocardial infarction
CAD	Coronary artery disease
CVD	Cardiovascular disease
DGLA	Dihomo-gamma-linolenic acid
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
D5D	Delta-5 desaturase
EFAs	Essential fatty acids
EPA	Eicosapentaenoic acid
FAs	Fatty acids
FADS	Fatty acid desaturases
GC	Gas chromatography
HDL	High-density lipoprotein
KHK	Koronare Herzkrankheit
LA	Linoleic acid
LCFAs	Long-chain fatty acids
LDL	Low-density lipoprotein
MCFAs	Medium- chain fatty acids
MUFAs	Monounsaturated fatty acids
N-3 PUFAs	N-3 polyunsaturated fatty acids
N-6 PUFAs	N-6 polyunsaturated fatty acids
PUFAs	Polyunsaturated fatty acids

SCFAs	Short-chain fatty acids
SFAs	Saturated fatty acids
TG	Triacylglycerol
VLDL	Very low-density lipoprotein
VLCFAs	Very long- chain fatty acids

Abstract

Coronary artery disease (CAD) is a major health issue that contributes significantly to the global mortality rate. The use of statins, a type of medication commonly used to lower cholesterol levels, has been shown to reduce the risk of morbidity and death in individuals with CAD. Despite the beneficial effects of statin therapy, some patients with CAD continue to experience cardiovascular events. Research has suggested that addition of n-3 PUFA supplementation to statin therapy may help to further lower the residual risk of cardiovascular complications in these patients. There are potential benefits of n-3 PUFAs supplementation for reducing residual risk in CAD patients treated with statins. However, there is a limited understanding of the distribution of these fatty acids in this patient population and of the impact of statins on fatty acid metabolism. In this study, the composition of fatty acids (FAs) in blood samples was analyzed to determine the levels of n-3 polyunsaturated fatty acids (n-3 PUFAs) in patients with and without CAD. A total of 273 patients undergoing cardiac catheterization were included in this study and this cohort was stratified into two groups: those with catheter-proven relevant CAD (n=192) and those without (n=81). Blood samples were analyzed for their fatty acid content using gas chromatography. Results showed that patients with CAD had a higher ratio of dihomo-gamma-linolenic acid (DGLA) to arachidonic acid (AA) and a higher delta-5 desaturase index (D5D index), indicating increased AA formation from precursors. Additionally, CAD patients had significantly lower levels of n-6 PUFAs and n-3 PUFAs, particularly eicosapentaenoic acid (EPA), in the blood. In this study a negative association between n-3 PUFAs, particularly EPA and DHA, and triglycerides levels was demonstrated.

Our results support a role of increased EPA levels for cardio protection. Our findings also suggest that statins have a direct impact on the metabolism of precursor n-6 fatty acids by promoting their conversion to AA, which is associated with an increased CAD risk.

Zusammenfassung

Die koronare Herzkrankheit (KHK) betrifft Millionen Menschen weltweit und trägt erheblich zur globalen Mortalität bei. Statine, welche häufig zur Senkung des Cholesterinspiegels eingesetzt werden, reduzieren das Risiko der Morbidität und der Mortalität bei KHK-Patienten. Trotz der positiven Effekte der Statintherapie haben KHK-Patienten weiterhin ein erhöhtes Risiko für kardiovaskuläre Ereignisse. Zusätzlich zur Statintherapie ist die Supplementation von n-3 polyungesättigten Fettsäuren (n-3 PUFAs) eine vielversprechende Option, um das verbliebene Risiko für kardiovaskuläre Ereignisse bei KHK-Patienten weiter zu verringern. Trotz des therapeutischen Potenzials von n-3 PUFAs, gibt es bisher nur wenige Untersuchungen zum Gehalt von n-3 PUFAs im Blut bei KHK-Patienten. Ebenso ist der Einfluss von Statinen auf den Fettsäurestoffwechsel in dieser Patientengruppe unzureichend untersucht.

In dieser Studie wurden die Fettsäurekonzentrationen in Blutproben von KHK-Patienten analysiert, um genauere Informationen zur n-3 PUFA-Konzentration zu erhalten. Insgesamt wurden 273 Patienten in die Studie eingeschlossen, bei denen eine indizierte Herzkatheteruntersuchung erfolgte. Sie wurden in zwei Gruppen eingeteilt: Zum einen Patienten mit in der Koronarangiographie diagnostizierter KHK (n = 192), zum andern Patienten ohne Nachweis einer KHK in der Herzkatheteruntersuchung (n = 81). Gewonnene Blutproben wurden mittels Gaschromatographie analysiert. Die Ergebnisse zeigten, dass KHK-Patienten ein erhöhtes Verhältnis von Dihomo-Gamma-Linolensäure (DGLA) zu Arachidonsäure (AA) sowie einen erhöhten Delta-5-Desaturase-Index (D5D-Index) aufwiesen. Dieses Ergebnis deutet auf eine gesteigerte AA-Synthese aus Vorläufersubstanzen hin. Darüber hinaus hatten Patienten mit diagnostizierter KHK signifikant niedrigere n-6 PUFA und n-3 PUFA Konzentrationen (insbesondere Eicosapentaensäure (EPA) Konzentrationen) im Blut als Patienten ohne Nachweis einer KHK. In Kombination mit klinischen Parametern zeigte sich in dieser Untersuchung ein negativer Zusammenhang zwischen n-3 PUFA-Konzentration und Triglyceridspiegeln im Blut. Die in dieser Arbeit beschriebenen Ergebnisse deuteten auf einen kardioprotektiven Effekt erhöhter EPA-Konzentrationen im Blut hin und hebt die Bedeutung von n-3 PUFAs und deren therapeutisches Potential hervor.

1 Introduction

Lipids are a diverse group of organic compounds that are mainly composed of three major component molecules: fatty acids, glycerol, and phospholipids (de Carvalho & Caramujo, 2018). Fatty acids are long-chain carboxylic acids with a hydrophobic (water-repelling) tail and a hydrophilic (water-attracting) head (Wiktorowska-Owczarek *et al*, 2015). They are the main building blocks of fats and oils. Glycerol is a three-carbon alcohol that serves as a backbone for the formation of lipids such as triglycerides and phospholipids (Kim *et al*, 2020). Phospholipids are a type of lipid that has a polar head group and two nonpolar fatty acid chains, making them important components of cell membranes (Schroeder *et al*, 1976). It is known that lipids display multiple biological effects, including cell signalling, membrane function and integrity, alveolar function, and water retention in the skin and eyes (Cockcroft, 2021; Imokawa *et al*, 1989). Fatty acids play a crucial role for the structure numerous biological lipid substances. This is why research focusses on a more comprehensive understanding of cellular and tissue-level processes. Firstly, phospholipids constitute the main component of cell membranes and are comprised of fatty acids (Dyall *et al*, 2022). Secondly, it is essential for lipid storage and energy homeostasis that triacylglycerol (TG) is hydrolysed into non-esterified free fatty acids and glycerol (Alves-Bezerra & Cohen, 2017). Thirdly, fatty acids and their derivatives can also serve as signalling molecules to regulate a variety of cellular activities (de Carvalho & Caramujo, 2018). Moreover, long-chain fatty acids are precursors to biologically active substances such as prostaglandins, thromboxane and leukotrienes, which are involved in a variety of physiological functions, including platelet aggregation, inflammation, etc (de Roos *et al*, 2009). The ratio of specific fatty acids has also been shown to act as a biomarker for the prediction of disease risks (Nishizaki *et al*, 2020; Xu *et al*, 2021a).

1.1 Classification of fatty acids

Fatty acids are comprised of carboxylic acids, and typically have a carbon atom count ranging from 4 to 28 in natural occurrences (Moss *et al*, 1995). Based on the carbon atom length of fatty acids they can be classified into the following: 1. Short-chain fatty acids (SCFAs) with 1-6 carbon atoms, produced by gut microorganisms during the fermentation of carbohydrates in the mammalian digestive tract (Silva *et al*, 2020). 2. Medium-chain fatty acids (MCFAs) with 6-12 carbon atoms (van Nuland *et al*, 2017). 3. Long-chain fatty

acids (LCFAs) with 14-18 carbon atoms, which are the most commonly consumed fatty acids in the diet (Mett, 2021). 4. Very long-chain fatty acids (VLCFAs) with more than 22 carbon atoms in the main chain (Kihara, 2012). In addition, dietary fatty acids can be further categorized into three groups based on the presence or absence of carbon-carbon double bonds (alkenyl group) and the number of double bonds: saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) (Panickar & Bhathena, 2010; Saini & Keum, 2018). SFAs are solid at room temperature and have no double bonds between carbon atoms in their chemical structure (Xu *et al*, 2021b). They are commonly found in animal-based foods such as meat, dairy, and butter. MUFAs contain one double bond in their structure and are usually liquid at room temperature (Hu *et al*, 2017). They are commonly found in olive oil, avocados, and nuts. PUFAs contain two or more double bonds in their structure and are also usually liquid at room temperature. They are commonly found in fatty fish, seeds, and vegetable oils (Kapoor *et al*, 2021). PUFAs can be further classified based on the position of their first double bond relative to the methyl end (also known as the omega, or "n," end) of the fatty acid molecule, when counting from the methyl end, PUFAs are referred to as n-3 PUFAs, n-6 PUFAs, and n-9 PUFAs (Mariamenatu & Abdu, 2021). The classification of PUFAs based on their "n" value reflects the importance of their specific molecular structure and determines their biological functions (Wiktorowska-Owczarek *et al.*, 2015). For example, n-3 PUFAs such as alpha-linolenic acid (ALA) and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and the n-6 PUFA arachidonic acid (AA) play crucial roles in brain function, cardiovascular health, and immune system regulation, while n-6 PUFAs such as linoleic acid (LA) are involved in skin health and the production of hormones (Abedi & Sahari, 2014). A balanced intake of different types of PUFAs is important for overall health (Calder, 2015).

1.2 The fatty acid discovery process

A century ago, fatty acid function was not comprehensively understood. Fatty acids were simply viewed as a component of dietary fats and a source of energy (Spector & Kim, 2015). A landmark discovery in 1929 showed that removing fat from the diets of animals resulted in a deficiency disease, which could be treated through the supplementation of linoleic acid (Burr & Burr, 1973). This finding demonstrated that fatty acids were

not just a source of energy, but were also of unique nutritional value. In the 1970s, researchers found that the incidence of acute myocardial infarction was low among Inuits in Greenland (Dyerberg *et al*, 1978), this was attributed to the low levels of cholesterol, triglycerides, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) in their plasma lipid and lipoprotein composition, which was strongly associated with the daily diet of Inuits (de Knijff *et al*, 1992). Subsequent analysis of fatty acids in blood of Inuits showed that they had significantly higher levels of n-3 PUFA, particularly EPA, and significantly lower levels of AA in their blood (Dyerberg *et al*, 1975). In recent years, epidemiological studies have shown that individuals with elevated levels of n-3 PUFA, indicated by a high n-3 index (defined as the percentage of EPA + DHA in total erythrocyte fatty acids) exceeding 8%, have a risk of sudden cardiovascular death that is less than 90% compared to those with an omega-3 index below 4%. (Mozaffarian & Wu, 2011; Von Schacky, 2010). Today, there is an increasing interest in understanding the physiological effects of PUFA for the human metabolism. Studies have shown that fatty acids play an important role in multiple biological processes. They serve as a source of energy and are essential components of cell membranes, influencing their fluidity and stability (de Carvalho & Caramujo, 2018). Additionally, fatty acids are involved in cell signaling pathways and play a role in gene expression regulation, helping to control the expression of genes responsible for various physiological processes (Papackova & Cahova, 2015). These findings highlight the diverse and important functions that fatty acids play in maintaining human health. Fatty acid oxidation is a process in which fatty acids are broken down to release energy (Esteves *et al*, 2021). This process takes place in mitochondria, the powerhouses of cells, and is an important source of energy. Fatty acid oxidation also produces a range of metabolites that can have either positive or negative effects on biological processes (Vieira *et al*, 2017). For example, excessive oxidation of lipids can lead to changes in the physicochemical properties of phospholipid membranes, causing severe cellular dysfunction (Fruhwirth *et al*, 2007). Understanding fatty acid oxidation is important for gaining insights into the role of fatty acids for human health and disease.

1.3 Synthesis of fatty acids

In mammals, some fatty acids cannot be produced within the body and must be obtained through diet (Kaur *et al*, 2014). These fatty acids, refered to as essential fatty acids (EFAs), include linoleic acid (LA, C18:2n6) and alpha-linolenic acid (ALA, C18:3n3),

which are precursors for n-6 PUFAs and n-3 PUFAs, respectively (Lee *et al*, 2016). The human body can use ALA as a raw material to synthesize EPA via desaturase and elongase to extend the carbon chain, and then convert EPA into DHA. These reactions are in competition with the synthesis of n-6 PUFAs, fatty acids derived from LA (Schunck *et al*, 2018). Thus, an increase in one product can lead to a decrease in the other.

In addition to dietary intake, the distribution of fatty acids in the human body is influenced by the fatty acid desaturases (FADS) gene cluster, which includes the genes FADS1, FADS2, and FADS3 (Lattka *et al*, 2011). The FADS gene cluster is a group of genes located on the human chromosome in the 11q12-11q13.1 region that plays a crucial role in fatty acid metabolism (Schaeffer *et al*, 2006). The gene for fatty acid desaturase 1 (FADS1) specifically encodes the rate-limiting enzyme Δ-5 desaturase which helps in the formation of certain fatty acids (Mathias *et al*, 2010).

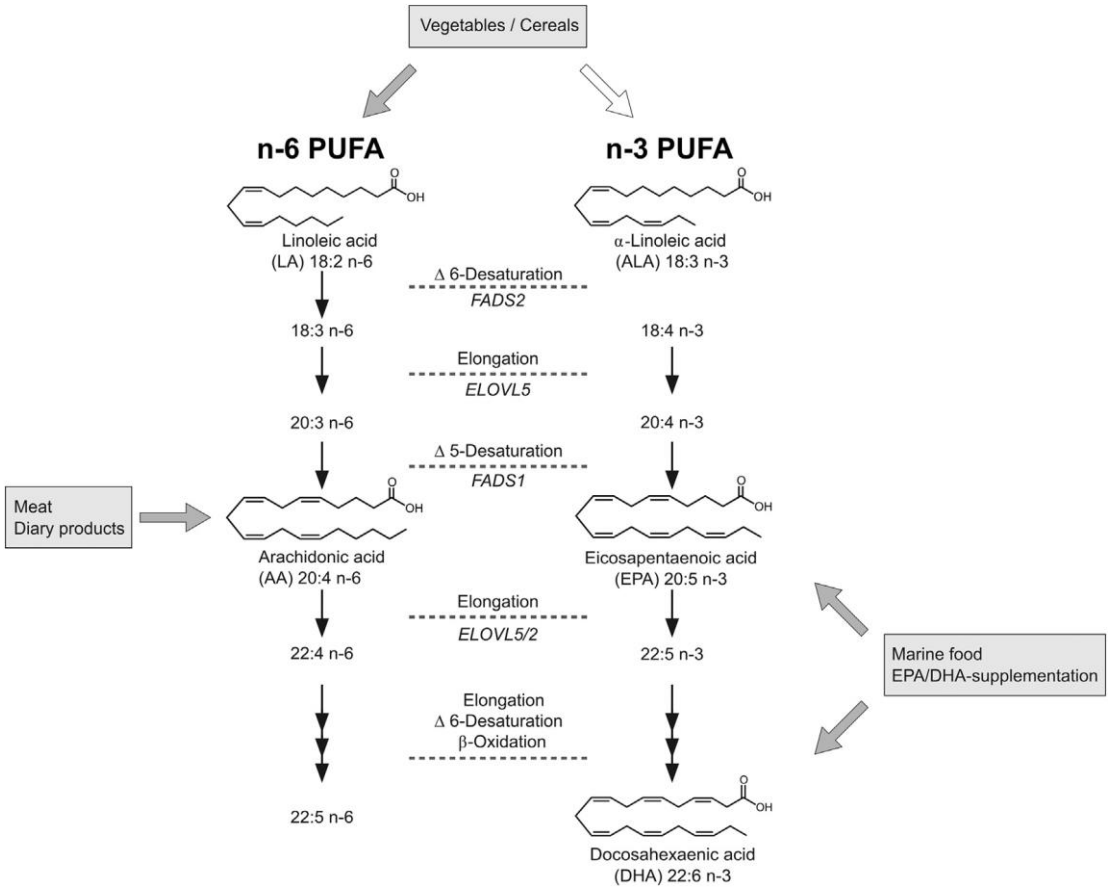


Figure 1. Elongation and desaturation pathways of n-3 PUFA and n-6 PUFA

(Figure 1, Schunck *et al.*, 2018)

1.4 Fatty acids and clinical disease

The ratio of n-6 PUFA to n-3 PUFA in human diet has shifted from the historically balanced 1:1 to current ratios ranging from 10:1 to 20:1 (Yang *et al*, 2022), which indicates that the current diet is leading to a deficiency of n-3 PUFA in humans. A lack of n-3 PUFAs can display negative effects on human health. Studies have shown that a deficiency of n-3 PUFAs can increase platelet aggregation and reduce the deformability of red blood cells, thus raising the risk of thrombosis (DiNicolantonio & J, 2019). Studies of patients with hyperlipidemia have indicated that n-3 PUFA can have an impact on lipids, and high-dose supplementation of n-3 PUFA can lead to a decrease in LDL cholesterol levels and plasma triglycerides (Zuliani *et al*, 2009). Clinical trials have shown that combining fish oil supplements with anti-rheumatic drugs can significantly improve joint pain in individuals with rheumatoid arthritis (Rajaei *et al*, 2015). Ulcerative colitis, psoriasis and melanoma have also been shown to benefit from n-3 PUFA (Charpentier *et al*, 2018; Millsop *et al*, 2014). Studies on animal models have shown that supplementation of n-3 PUFAs can reduce the number and size of tumors, and also increase the time it takes for tumors to form (Gu *et al*, 2015; Weylandt *et al*, 2011). Studies in newborn babies have shown that DHA is essential for the healthy development of the retina and brain (Kuratko *et al*, 2013).

The role of fatty acids in cardiovascular disease has been thoroughly studied. The American Heart Association recommends that patients with CVD should take n-3 polyunsaturated fatty acids to reduce the incidence of cardiovascular events (Siscovick *et al*, 2017), and studies indicate that the intake of n-3 PUFAs can improve the function of the endothelial cells and enhance the ability of the blood vessels to relax and expand (Zanetti *et al*, 2015).

Hypercholesterolemia is an important risk factor for cardiovascular disease (Tietge, 2014). Statins can effectively reduce the levels of total cholesterol and low-density lipoprotein cholesterol (Rosenson, 2006). Therefore, statins are comprehensive blood lipid regulators and are considered the first choice for the medical therapy of hypercholesterolemia in patients with cardiovascular diseases (Zhou & Liao, 2009). However, high triglycerides are also an important risk factor for cardiovascular disease (Ye *et al*, 2019). Elevated levels of triglycerides are a risk factor for the development of CVD, such as heart attack, stroke, and peripheral arterial disease. Hence, controlling triglyceride levels is crucial in reducing the risk of CVD (Peng *et al*, 2017). Medical statin therapy is not sufficient

to completely eliminate the risk of cardiovascular disease (CVD). Despite the proven efficacy in reducing LDL-cholesterol, the use of statins alone may not fully address other factors contributing to CVD, such as high triglycerides levels. It is well established that n-3 PUFA can lower triglycerides (Backes *et al*, 2016).

A Japanese lipid intervention study (JELIS) addressing EPA levels showed that supplementation with high-purity EPA significantly reduced the risk of CVD in statin-treated patients, especially in those with hyperlipidemia and low HDL-cholesterol levels (Matsuzaki *et al*, 2009). Recently, the REDUCE-IT clinical trial did also study the impact of EPA supplementation on cardiovascular disease. It involved a large number of participants and the results showed a significant reduction (25%) for the risk of major adverse cardiovascular events in participants with EPA supplementation (Bhatt *et al*, 2019). These trials provide evidence for the beneficial effects of EPA on cardiovascular health.

Aside from dietary intake, activity of desaturase enzymes also plays a role for the regulation of polyunsaturated fatty acids levels in the human body (Czumaj & Śledziński, 2020). Δ-5 desaturase is the rate-limiting enzyme in PUFA elongation and is encoded by the FADS1 gene (Huang *et al*, 2022). An association between altered Δ5 desaturase activity and risk of cardiovascular disease has been reported (Mayneris-Perxachs *et al*, 2014). The D5D index is calculated by the ratio of n-6 PUFA (n-3 PUFA are more susceptible to daily diet) product (AA) to precursor (DGLA) for evaluating the activity of desaturase (Tosi *et al*, 2014). Statins can also alter the synthesis of long-chain fatty acids to increase concentrations of AA in plasma: Tanaka et al. found that statins stimulate the mRNA expression of FADS1 and FADS2 in HepG2 cells (Tanaka *et al*, 2019). However, there is still limited knowledge about the changes in fatty acid content and desaturase activity in patients receiving statin therapy.

The study presented here aimed to investigate the relationship between fatty acid composition and presence of coronary artery disease (CAD) in patients undergoing cardiac catheterization. It also aimed to determine whether the levels of essential fatty acids and the D5D index, a measure of desaturase activity, differ between CAD and non-CAD patients, and examined the effect of statin therapy on fatty acid profiles.

2 Methods

2.1 Research subjects

The study population consisted of patients who were admitted for invasive coronary angiography to the University Hospital Brandenburg/Havel in Germany (Wang *et al*, 2022). These patients either had a known CAD with suspicion of disease progression or were referred for suspected CAD. Following the results of the invasive angiography, patients were categorized into the CAD group if they had a 50% diameter stenosis or more, which is considered as indicative of obstructive CAD. On the other hand, control patients were those without significant CAD.

Exclusion criteria for the study included a history of active cancer, inability to provide informed consent, and being under the age of 18 years. All subjects provided written informed consent, and the study was approved by the Ethics Committee of the Medical Association of the State of Brandenburg (AS69(bB)/2016) (Wang *et al.*, 2022).

2.2 Sample collection and clinical data acquisition

Blood was collected from the participants after they had fasted overnight and before undergoing a cardiac catheterization procedure. The blood samples were stored in EDTA tubes and kept at minus 80°C until being analyzed for fatty acids. Additionally, a standard lipid panel, including total cholesterol, LDL-C, HDL-C, and triglycerides, was immediately measured using validated clinical assays in the hospital's central laboratory after the blood sample was collected.

2.3 Sample preparation and determination by GC

Fifty microliters of whole blood per sample was utilized for gas chromatography (GC) analysis. Fatty acid extraction and methylation was performed using a standard protocol (Kang & Wang, 2005).

2.3.1 Fatty acid extraction and methylation

1) Reaction systems:

Reagents	Volume (μL)
PDA (1 mg/mL)	50 μL

Blood Sample	50 µL
Borontrifluoride in 14% methanol	500 µL
n-hexane	500 µL

2) After vortexing, samples were incubated:

Reaction temperatures	Time (min)
100 °C	60 min

3) Cooling down to room temperature, the mixture was added to 750 µL water, vortexed, and extracted for 4 min.
4) Then all samples were centrifuged for 5 min (RT, 3500 rpm).
5) From each sample, 100 µL of the upper n-hexane layer was transferred into a micro-insert (placed in a GC glass vial), tightly closed, and analyzed by GC.

2.3.2 Determination of FAs by GC

Gas Chromatography (GC) was performed using a 7890B GC System with an HP88 Column and the analysis was done using OpenLAB CDS ChemStation Edition.

1 µL of each sample was injected into the injector (splitless injection, 280 °C) analysis was performed with the following temperature gradient (Kang & Wang, 2005):

Temperature	Temperature/min
50 °C to 150 °C	20 °C/min
150 °C to 240 °C	6 °C/min
240 °C	10 min
Total run time	30 min
Nitrogen was used as carrier gas	constant flow 1 mL/min

The flame ionization detector (FID) analysis was performed at 250 °C with the following gas flows:

Gas	Flow
Hydrogen	20 mL/min
Air	400 mL/min
Nitrogen	make up 25 mL/min

Methylated FAs in the samples were identified by comparing the retention times with those of known methylated FAs of the Supelco® 37 FAME MIX standard and single FAME standards purchased from Cayman Chemicals (Ann Arbor, MI, USA). The methylated fatty acids in the samples were identified by comparing their retention times with those of known standards. FA values are presented as percentage (%) of total FA content. For the study, 16 FAs were included as follows:

Common name	Lipid number
myristic acid	C14:0
palmitic acid	C16:0
stearic acid	C18:0
arachidic acid	C20:0
behenic acid	C22:0
lignoceric acid	C24:0
palmitoleic acid	C16:1 n-7c
oleic acid	C18:1 n-9c
nervonic acid	C24:1 n-9
eicosapentaenoic acid (EPA)	C20:5 n-3
docosapentaenoic acid (DPA)	C22:5 n-3
docosahexaenoic acid (DHA)	C22:6 n-3
linoleic acid (LA)	C18: 2 n-6
dihomo-gamma-linolenic acid (DGLA)	C20:3 n-6
arachidonic acid (AA)	C20:4 n-6
adrenic acid (AdA)	C22:4 n-6

2.4 Statistics

The statistical analysis of the results was performed using unpaired Student's t-test and Chi-square test, as appropriate, with the help of Prism GraphPad 5 software. Significance

of the results was determined by evaluating the *p*-value. A *p*-value less than 0.05 was considered statistically significant. Results with a *p*-value between 0.01 and 0.05 were considered as having a weak statistical significance (denoted by *), whereas results with a *p*-value between 0.001 and 0.01 were considered as having a moderate statistical significance (denoted by **). Results with a *p*-value less than 0.001 were considered as having a strong statistical significance (denoted by ***).

3 Results

3.1 Patient clinical data and records

In this study, we enrolled a total of 273 patients undergoing cardiac catheterization (Wang *et al.*, 2022). In total, 81 of these patients were not diagnosed with cardiovascular disease, while 192 patients were diagnosed with cardiovascular disease. The general information of the patients is shown in Table 1, in which the CAD patients were older and heavier than patients without CAD. Notably, 59% of patients in the CAD group were treated with statins, while only 13% of patients in the non-CAD group had treatment with statins. Therefore, CAD patients had significantly lower levels of cholesterol, HDL and LDL than non-CAD patients. However, triglycerides and HbA1c were significant higher in CAD patients.

Table 1 Patient's Characteristics: Differences between the groups were tested with the unpaired Student's t-test and Chi-square test. Data are presented as mean ± standard error of the mean (Table 1 taken from Wang *et al.*, 2022).

Patient's Characteristics	**No CAD**	**CAD**	***p***
Male/Female	35/46	138/54	< 0.0001
Age (years)	57.42 ± 1.67	67.92 ± 0.94	< 0.0001
Weight (kg)	81.17 ± 1.90	86.42 ± 1.30	0.0241
BMI	27.77 ± 0.50	28.83 ± 0.38	0.0927
HbA1c (mmol/mol)	36.10 ± 0.44	44.43 ± 0.95	< 0.0001
Cholesterol (mmol/L)	5.28 ± 0.12	4.67 ± 0.10	< 0.0001
HDL (mmol/L)	1.50 ± 0.04	1.20 ± 0.03	< 0.0001
LDL (mmol/L)	3.56 ± 0.11	3.01 ± 0.09	< 0.0001
TGs (mmol/L)	1.34 ± 0.06	1.72 ± 0.08	< 0.0001
Diabetes mellitus	1 (1%)	71 (37%)	< 0.0001
Statin use	10 (13%)	102 (59%)	< 0.0001

3.2 Comparative analysis of total fatty acids in patients with CAD and controls

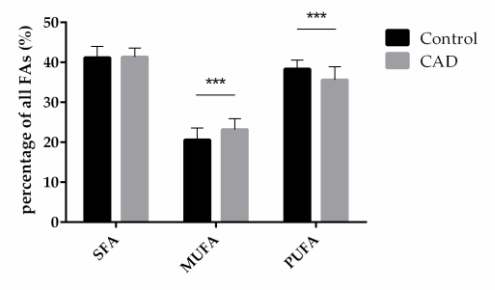
To analyse the differences of fatty acid profiles in patients with and without CAD, we extracted patient's blood total FA composition and analysed this by gas chromatography. Compared to non-CAD patients, CAD patients had significantly higher levels of MUFA ($p < 0.0001$), which include C16:1n7c, C18:1n9c, and C24:1n9. Polyunsaturated fatty acids

(PUFA, $p < 0.0001$) were significantly lower in CAD patients, which include C20:5 n-3, C22:5n-3, C22:6n-3, C18: 2n-6, 20:3n-6, 20:4n-6 and C22:4n-6. However, saturated fatty acids (SFA) had no significant difference between CAD and non-CAD patients, which include C14:0, C16:0, C18:0, C20:0, C22:0, C24:0 (Figure 2a).

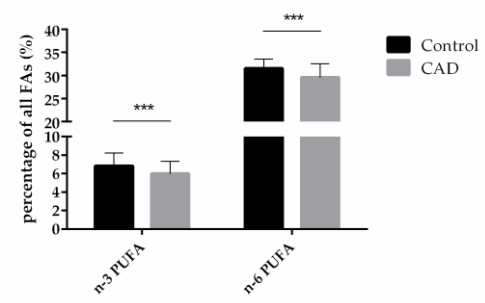
We found that n-3 PUFA and n-6 PUFA were both lower in CAD patients in comparison to non-CAD patients ($p < 0.0001$, $p < 0.0001$). In the analysis of individual n-3 PUFA content, EPA and DPA were significantly lower in CAD patients ($p < 0.0001$, $p < 0.0001$), DHA also showed a lower trend in CAD patients. For n-6 PUFA, LA, DGLA, and AdA were significantly lower in CAD patients ($p = 0.0101$; $p < 0.0001$; $p < 0.0001$), but there was no significant difference in AA (Figure 2b).

Studies have shown an association between D5D activity changes and CAD progression. In this study, we assessed the activity of D5D by calculating n-6 PUFA product (AA) to substrate (DGLA) ratios, also known as the D5D-Index. We found that D5D-Index was significantly higher in CAD patients than non-CAD patients ($p < 0.0001$), indicating that there was an increased activity of the D5D enzyme in CAD (Figure 2f).

To investigate whether there was a correlation between n-3 PUFA and TG in patients in this study, we performed a correlation analysis between the two parameters. The results showed that patients with high levels of n-3 PUFA had lower levels of TG, which indicates that there was an inverse relationship between n-3 PUFA and TG ($p < 0.0001$) (Figure 3a). We also analysed the correlation between HDL-cholesterol, LDL-cholesterol with n-3 PUFA (Figure 3b, c). The results showed a positive correlation between HDL-cholesterol and n-3 PUFA, especially for EPA, while a negative correlation was observed between LDL-cholesterol and n-3 PUFA, especially for DHA (Figure 3d, e).



(**a**)



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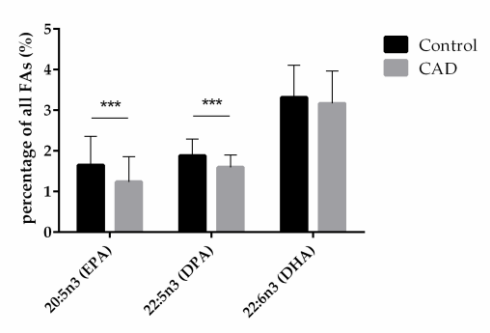

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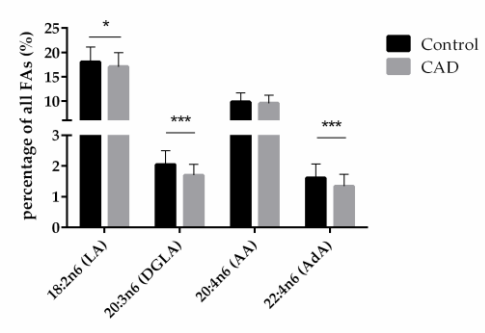

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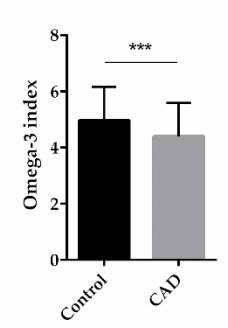

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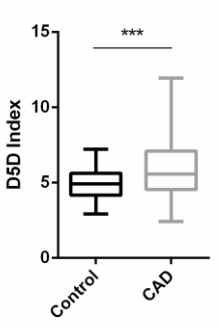
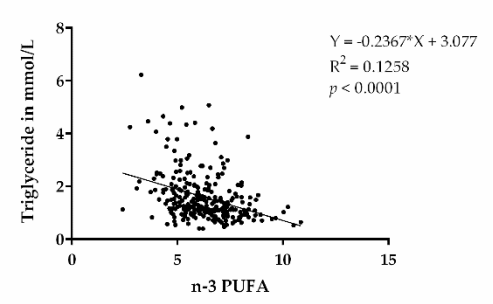


(**f**)

Figure 2. FA levels in blood from patients without and with CAD. (a) Relative content of SFAs, MUFAs, and PUFAs in control and CAD patients. (b) Relative content of n-3 and n-6 PUFAs in control and CAD groups. (c) Comparison of individual n-3 PUFAs in CAD and control patients. (d) Comparison of individual n-6 PUFAs in CAD and control patients. (e) n-3 index in control and CAD group. (f) D5D index as indicator of desaturase activity in CAD versus control patients. (n = 81 for the control group, and n = 192 for the CAD group; * indicates $0.01 < p < 0.05$, *** indicates $p < 0.001$) (Figure 1 taken from Wang *et al.*, 2022).



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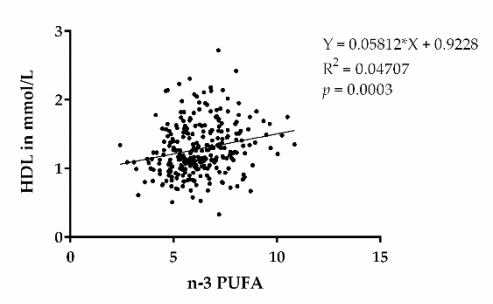

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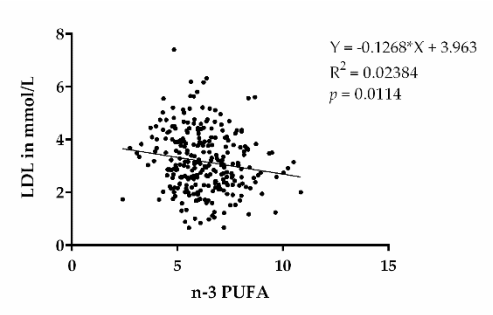

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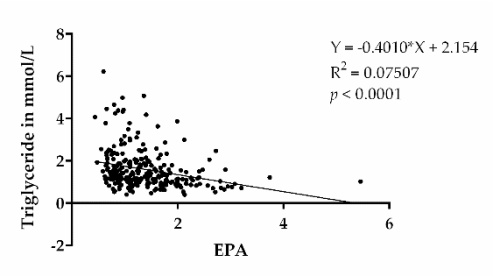

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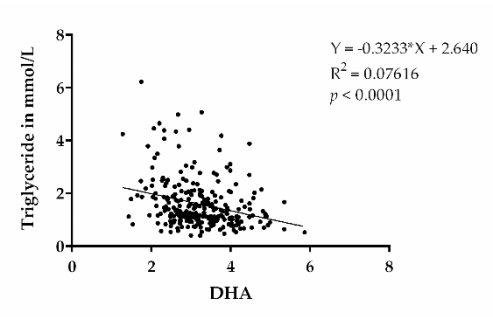
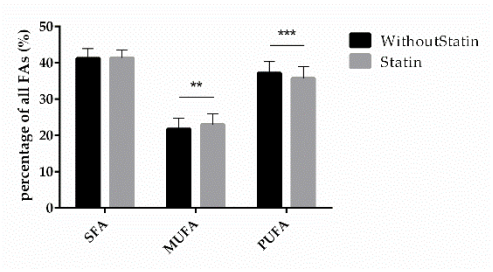


(**e**)

Figure 3. n-3 PUFAs and dyslipidemia. (a) Correlation between TGs and n-3 PUFA (EPA + DPA + DHA). (b) Correlation between HDL cholesterol and n-3 PUFA (EPA + DPA + DHA). (c) Correlation between LDL cholesterol and n-3 PUFA (EPA + DPA + DHA). (d) Correlation between TGs and EPA. (e) Correlation between TGs and DHA. n = 273 (Figure 2 taken from Wang *et al.*, 2022).

3.3 Comparing fatty acid levels in patients with and without statin therapy

We screened patients who had been treated with statins and analysed the fatty acid differences in comparison to those patients who had not been treated with statins. We found that patients treated with statins had significantly higher MUFA ($p = 0.0011$) and significantly lower PUFA ($p = 0.0006$) (Figure 4a). We also found that compared to non-statin treated patients, patients treated with statins had a significantly lower level of n-6 PUFA ($p < 0.0001$), especially for LA ($p < 0.0001$), DGLA ($p < 0.0001$), while there were no significant differences for AA and n-3 PUFA (Figure 4b, c). Importantly, we found that D5D-index was significantly higher in patients after statin treatment ($p < 0.0001$) (Figure 4d), which indicated that statins increase the activity of the D5D enzyme to alter the distribution of fatty acids in patients.



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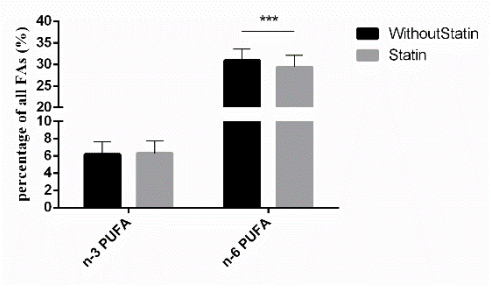

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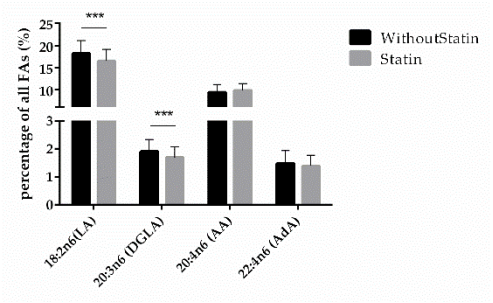

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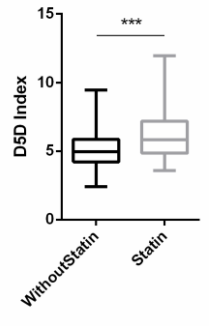

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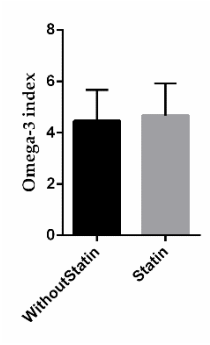


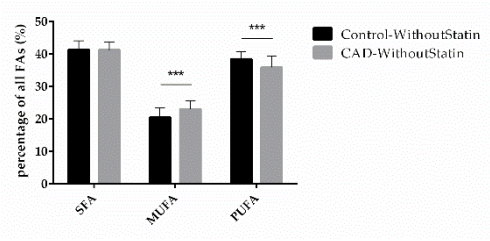
(**e**)

Figure 4. Correlation of FAs and statin treatment.(a) Relative content of SFAs, MUFAs, and PUFAs depending on statin treatment vs. no statin treatment. (b) Relative content of n-3 and n-6 PUFAs according to statin treatment. (c) Relative content of different n-6 PUFAs according to statin treatment. (d) D5D index and (e) omega-3 index according to statin medication. (n = 142 for the without-statin group, n = 112 for the statin group; ** indicates $0.001 < p < 0.01$, *** indicates $p < 0.001$) (Figure 3 taken from Wang *et al.*, 2022).

3.4 Fatty acid profile comparison between control and coronary artery disease patients not on statin therapy

The previous results showed that statins can affect the distribution of fatty acids, so we planned to exclude the statin influence and therefore, we re-analyze the fatty acid

differences between patients with and without CAD not on statin treatment. The results show that, compared to patients without CAD, CAD patients had significantly higher level of MUFA ($p < 0.0001$) and significantly lower level of PUFA ($p < 0.0001$) (Figure 5a). The n-3 PUFA ($p < 0.0001$) and n-6 PUFA ($p = 0.0029$) both showed significantly lower levels in CAD patients (Figure 5b). In the analysis of individual n-3 PUFA content, EPA, DPA and DHA were significantly lower in CAD patients ($p < 0.0001$, $p < 0.0001$, $p = 0.0017$) (Figure 5c). For n-6 PUFA, DGLA, AA, and AdA were also significantly lower in CAD patients ($p < 0.0001$; $p = 0.0071$; $p < 0.0001$). However, there was no significant difference in LA (Figure 5d). Notably, the D5D index was not significantly different between CAD and non-CAD patients after excluding the statin factor (Figure 5e). These results further illuminate the impact of statins on the activity of the delta-5 desaturase and the increased conversion of LA to AA.



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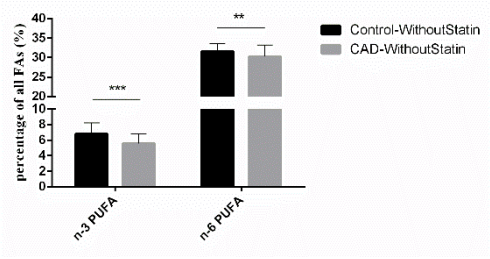

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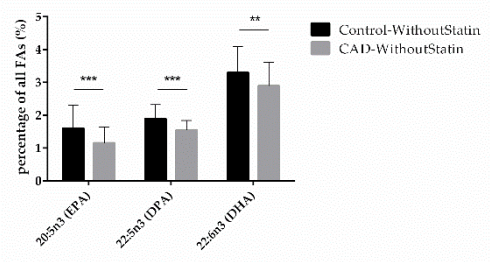

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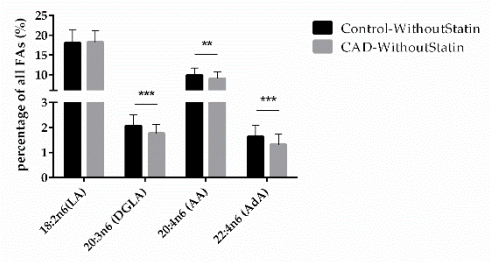

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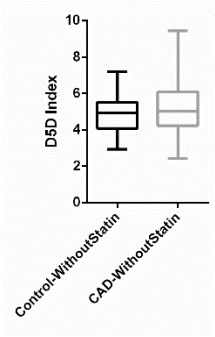

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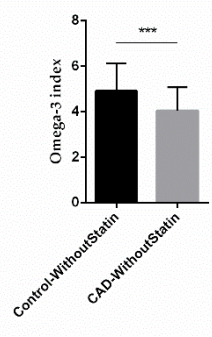

(**f**)

Figure 5. Correlation of FAs in control and CAD patient subgroups without statin treatment. (a) Relative content of SFAs, MUFAs, and PUFAs in control and CAD patients without statin treatment. (b) Relative content of n-3 and n-6 PUFAs in control and CAD patients without statin treatment. (c) Relative content of different n-3 PUFAs and (d) n-6 PUFAs in control and CAD patients without statin treatment. (e) D5D index and (f) omega-3 index in control and CAD patients without statin treatment. (n = 70 for the control-without-statin group, n = 72 for the CAD-without-statin group; ** indicates $0.001 < p < 0.01$, *** indicates $p < 0.001$) (Figure 4 taken from Wang *et al.*, 2022).

4 Discussion

The role and the distribution of fatty acids in CAD is a subject of significant debate. Our study demonstrated that individuals with CAD exhibit significantly lower levels of both n-3 PUFA and n-6 PUFA. Additionally, we found a negative correlation between n-3 PUFA and triglyceride as well as LDL levels, indicating that a deficiency of n-3 PUFA is closely linked to the development of CAD. Notably, the D5D-index was significantly higher in patients treated with statins. This suggests that statins probably affect the alteration of the rate-limiting enzyme D5D in fatty acid metabolism, which led to an active fatty acid metabolic pathway and thus accelerated the synthesis of pro-inflammatory fatty acid AA.

4.1 Relationship between n-3 PUFA and CAD

In this study, we found that the levels of both n-3 PUFAs and n-6 PUFAs in CAD patients were significantly lower compared to those without CAD. Analysis of individual n-3 PUFAs showed that levels of EPA and DPA were significantly lower in CAD patients, while DHA showed a decreased trend. In a study on the relationship of plasma fatty acids, oxidized lipids, and risk of acute myocardial infarction (AMI) in a Singaporean Chinese population, the results showed that long-chain n-3 FAs were inversely associated with AMI risk (Sun *et al*, 2016). This is consistent with our results. Further studies have found that a higher intake of n-3 PUFAs is associated with a reduced risk of cardiovascular disease, including heart attack and stroke (Cheng *et al*, 2015). There are several mechanisms by which n-3 PUFAs could improve cardiovascular disease. Endothelial dysfunction is a key factor in the development of hypertension (Gallo *et al*, 2021). N-3 PUFAs have been shown to improve endothelial function by increasing production of nitric oxide, a molecule that has positive effects on blood vessels relaxation and improves blood flow (Yamagata, 2020). This improved blood flow can help to lower blood pressure. EPA has been demonstrated to have anti-inflammatory effects through its ability to reduce the levels of pro-inflammatory cytokines and chemokines (Crupi & Cuzzocrea, 2022). Cytokines and chemokines are signaling molecules produced by immune cells that play a role in promoting inflammation (Shachar & Karin, 2013), and chronic inflammation is a contributing factor to the development of cardiovascular disease (Lopez-Candales *et al*, 2017). In 2017, a large, randomized, double-blind, placebo-controlled study, the REDUCE-IT

trial (Reduction of Cardiovascular Events with Icosapent Ethyl–Interventional Trial), investigated the effect of icosapent ethyl, which is a highly purified form of eicosapentaenoic acid (EPA), on cardiovascular outcomes in individuals with elevated triglyceride levels and cardiovascular disease or multiple risk factors for CVD (Bhatt *et al.*, 2019). The results showed that icosapent ethyl reduced the risk of major adverse cardiovascular events, including cardiovascular death, nonfatal myocardial infarction, and stroke, compared to placebo (Bhatt *et al.*, 2019). Additionally, icosapent ethyl reduced the risk of hospitalization for unstable angina and the need for coronary revascularization procedures (Peterson *et al*, 2021). These findings provide strong evidence for benefits of EPA supplementation in individuals with elevated triglyceride levels and established CVD or multiple risk factors for CVD. Furthermore, Nelson et al. demonstrated that EPA has the ability to lower triglyceride levels without affecting LDL-C levels, potentially providing positive impact on the formation of atherosclerotic plaques (Nelson *et al*, 2017).

DPA, one of the n-3 PUFAs, has been shown to have potential benefits for cardiovascular health, although research in this area is limited compared to other n-3 PUFAs such as EPA and DHA (Akiba *et al*, 2000). Studies have shown that compared to EPA, DPA has a stronger ability to inhibit platelet aggregation and promote endothelial cell migration (Akiba *et al.*, 2000; Kanayasu-Toyoda *et al*, 1996). Platelet aggregation is a key step in the formation of blood clots, which can contribute to the development of atherosclerosis (Wang & Tang, 2020). By inhibiting platelet aggregation, DPA may help to reduce the risk of cardiovascular disease. Endothelial cell migration is important for the repair and maintenance of blood vessels (Michaelis, 2014), and promoting this process can help to improve overall vascular health. Another mechanism by which DPA may affect cardiovascular health is through its effects on lipid metabolism. DPA has been shown to have a positive impact on blood lipid levels, including reducing triglycerides and increasing levels of high-density lipoprotein (HDL) cholesterol, which is known as "good" cholesterol (Drouin *et al*, 2019). This can help to improve the overall lipid profile and reduce the risk of cardiovascular disease. In addition, DPA may also have anti-inflammatory effects, which can down regulate the production and release of pro-inflammatory cytokines and chemokines (Balta *et al*, 2022; Zhao *et al*, 2021). Our finding of low levels of DPA in CAD patients might thus have pathophysiological relevance.

4.2 Effect of n-3 PUFA on triglycerides

It has been discovered that besides lowering cholesterol levels, elevated levels of triglycerides have a strong correlation with the residual risk of cardiovascular disease (Leatherman *et al*, 2022). Triglycerides are fats, which are found in the circulation and are involved in the development of cardiovascular disease (CVD) (Budoff, 2016). Elevated levels of triglycerides have been associated with an increased risk for CVD and other risk factors such as obesity, insulin resistance, and type 2 diabetes (Ye *et al.*, 2019). Triglycerides can contribute to the buildup of plaques in the arteries (Talayero & Sacks, 2011) and thus increase the risk of developing CVD. Managing elevated triglyceride levels is important for reducing the risk of CVD. Our study shows an inverse relationship between both n-3 PUFAs, specifically EPA and DHA, and TG, indicating that elevated levels of n-3 PUFAs may contribute to the regulation of TG levels in patients. N-3 polyunsaturated fatty acids (PUFAs), such as EPA and DHA, have been shown to lower triglyceride levels in the blood. This is considered to occur through a number of mechanisms: N-3 PUFAs have been shown to improve insulin sensitivity, which can help to regulate triglyceride levels and prevent their buildup in the blood (Lepretti *et al*, 2018). Furthermore, n-3 PUFAs have antioxidant properties that can reduce oxidative stress and inflammation, both of which have been linked to elevated triglyceride levels (Oppedisano *et al*, 2020). Moreover, n-3 PUFAs can increase the β-oxidation of fatty acids, which can reduce triglycerides stored in the body (Harris & Bulchandani, 2006) and decrease the synthesis of fat in the liver, which is a major contributor to elevated triglyceride levels (Sato *et al*, 2010).

4.3 Effect of statins on the distribution of n-6 PUFAs in CAD patients

In our study the majority of CAD patients were receiving statin therapy. This led us to speculate that statins may have impacted the distribution of n-6 PUFAs in these patients. Our results showed that patients treated with statins had significantly lower levels of LA and DGLA compared to those not treated with statins, while AA levels are slightly elevated. Statins work by inhibiting the activity of the enzyme HMG-CoA reductase, which is responsible for the production of cholesterol in the liver (Sirtori, 2014). This reduction in cholesterol production leads to an increase in the number of LDL receptors on the liver cells, which helps to clear LDL cholesterol from the blood (Sirtori, 2014). However, HMG-CoA reductase also plays a role in the biosynthesis of other molecules, including n-6

PUFAs. HMG-CoA reductase inhibitors contributed to a significant increase in the conversion of exogenous linoleic acid (C18:2 n-6) into its long-chain polyunsaturated fatty acid derivatives (Risé *et al*, 1997). Nakamura et al. evaluated the effect of HMG-CoA reductase inhibitors, also known as statins, on plasma polyunsaturated fatty acid concentrations in patients with hyperlipidemia, and the results demonstrated that the levels of AA in patients treated with HMG-CoA reductase inhibitors were significantly higher compared to those levels prior treatment initiation (Nakamura *et al*, 1998), which is in line with our findings. The exact mechanism behind these changes is not fully understood, but it is believed that statins may affect the activity of fatty acid desaturases, enzymes involved in the synthesis of PUFAs. In a study aiming to investigate the effect of simvastatin on the metabolic pathways of various fatty acid concentrations in human monocytic and hepatocytic cell lines, it was found that simvastatin altered the metabolism of n-6 PUFAs in human monocytic and hepatocytic cell lines, resulting in a significant increase in LA conversion, and a significant increase in Δ-5 desaturase and Δ-6 desaturase activities (Risé *et al*, 2005). Our results also showed an increase in D5D-index levels in patients treated with statins, suggesting an association between increased Δ-5 desaturase activity and statins. The effect of statins on Δ-5 desaturase activity leads to increased levels of AA in the body. However, this increase in AA can lead to increased inflammation and a thus higher risk of developing CVD, as AA is a precursor for pro-inflammatory eicosanoids (Nelson & Raskin, 2019). Furthermore, research has demonstrated that arachidonic acid is positively correlated with the risk of AMI (Sun *et al.*, 2016). A balanced diet that includes sufficient amounts of n-3 PUFAs such as EPA and DHA might be able to alleviate this effect. This needs to be analyzed in future studies.

4.4 Strengths and weaknesses of the study

This study has several limitations that should be noted (Wang *et al.*, 2022). Firstly, we were unable to analyze gene expression or genotype variation of genes involved in PUFA elongation and desaturation, including FADS1. Secondly, the lack of nutrition data from patients is a limitation and future studies should address this issue. Thirdly, the formation of n-3 PUFA-derived lipid mediators could not be studied as part of this work. Based on data in the literature and previous data from our group, these have important effects on inflammation homeostasis, and should be studied in the context of CVD and statin use in the future.

Despite these limitations, the data presented here provide new insights by showing differences in essential fatty acid levels between patients with and without CAD. These results support the need for further investigation of the effect of genetic, nutritional and pharmacological factors on fatty acid synthesis in CAD and the potential modification of AA synthesis by FADS1 genotypes.

Additionally, the results suggest the need to examine the dose- and specific statin-dependent effects on desaturase activity and the role of FADS1 genotypes.

Lastly, this study shows lower EPA levels in CAD patients, which provides further support for the concept of cardiovascular protection due to increased EPA levels.

5 Conclusions

In the present study, we used gas chromatography to analyze the differences in the distribution of various PUFAs in patients with and without CAD and found decreased levels of n-3 as well as n-6 PUFAs in patients with CAD. Furthermore, the results showed that statins enhance the conversion of LA and DGLA to arachidonic acid AA, and increase Δ-5 desaturase activity. The use of statin medication was associated with an increase in levels of arachidonic acid, which is a pro-inflammatory fatty acid associated with cardiovascular risk in CAD patients. These results might support the concept that supplementing statin treatment with n-3 PUFAs, such as EPA and DHA could enhance the beneficial effects of statins and mitigate the increase in AA levels. This combination approach may provide a more effective treatment for CAD patients, compared to the use of statins alone.

Further studies are necessary to fully understand the impact of statins and n-3 PUFAs on CAD and to establish clear guidelines for their use in CAD treatment.

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7 Statutory Declaration

“I, Wang, Chaoxaun, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic “Investigation on Polyunsaturated Fatty Acid Levels of Patients with and without Coronary Artery Disease and on the Impact of Statins on Fatty Acid Metabolism” [German title: Die Konzentration mehrfach ungesättigter Fettsäuren im Blut bei Patienten mit und ohne koronare Herzkrankheit und die Statinwirkung auf den Fettsäurestoffwechsel], independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (except for Figure. 1, I created all figures and tables presented in this work by myself) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; http://www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.”

Date Signature

8 Declaration of your own contribution to the publications

Chaoxuan Wang contributed the following to the below listed publications:

Wang C, Enssle J, Pietzner A, Schmöcker C, Weiland L, Ritter O, Jaensch M, Elbelt U, Pagonas N, Weylandt KH. Essential Polyunsaturated Fatty Acids in Blood from Patients with and without Catheter-Proven Coronary Artery Disease. Int J Mol Sci. 2022 Jan 11;23(2):766. doi: 10.3390/ijms23020766. PMID: 35054948; PMCID: PMC8775772.

Contribution:

Throughout my thesis research, I played a significant role in the design and execution of the study. Working closely with my supervisor and co-supervisor, I helped to develop the study plan, which involved conducting a blood sample fatty acid test. I was responsible for overseeing all aspects of the test, from the extraction of sample fatty acids to the fatty acid derivatization, GC assay, and integration of fatty acids.

Additionally, I was responsible for drafting the manuscript text and creating the tables and figures for this study, all figures and tables were created by myself. During the process, I collaborated with my supervisors and colleagues, incorporating their feedback and suggestions to ensure the accuracy and clarity of the data. My contributions in creating these tables and figures were crucial to the successful communication of the results.

_____

Signature, date and stamp of first supervising university professor / lecturer

_____

Signature of doctoral candidate

9 Printing copy of the publication

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Essential Polyunsaturated Fatty Acids in Blood from Patients with and without Catheter-Proven Coronary Artery Disease

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Abstract: Coronary artery disease (CAD) is the leading cause of death worldwide. Statins reduce morbidity and mortality of CAD. Intake of n-3 polyunsaturated fatty acid (n-3 PUFAs), particularly eicosapentaenoic acid (EPA), is associated with reduced morbidity and mortality in patients with CAD. Previous data indicate that a higher conversion of precursor fatty acids (FAs) to arachidonic acid (AA) is associated with increased CAD prevalence. Our study explored the FA composition in blood to assess n-3 PUFA levels from patients with and without CAD. We analyzed blood samples from 273 patients undergoing cardiac catheterization. Patients were stratified according to clinically relevant CAD (n = 192) and those without (n = 81). FA analysis in full blood was performed by gas chromatography. Indicating increased formation of AA from precursors, the ratio of dihomo-gamma-linolenic acid (DGLA) to AA, the delta-5 desaturase index (D5D index) was higher in CAD patients. CAD patients had significantly lower levels of omega-6 polyunsaturated FAs (n-6 PUFA) and n-3 PUFA, particularly EPA, in the blood. Thus, our study supports a role of increased EPA levels for cardioprotection.

Keywords: coronary artery disease; triglycerides; polyunsaturated fatty acids; n-3 PUFA; statins; arachidonic acid

1. Introduction

Coronary artery disease (CAD) is the leading cause of death in both developed and developing countries [1,2]. Lifestyle and environmental and genetic factors are risk factors for the development of CAD [3], with atherosclerosis being the underlying pathological mechanism.

Among other factors, triglycerides (TGs) play an important role in the development of atherosclerosis [4]. In addition, clinical trials showed that the recurrence rate of coronary events was significantly reduced if the TG level was lowered to less than 150 mg/dL in CAD patients [5].

N-3 polyunsaturated FAs (n-3 PUFA), found predominantly in fish oils, have been implicated in the prevention of CVD. Studies have shown that n-3 PUFAs have a va-

riety of cardiovascular-protective effects, including lowering blood pressure, improving cardiac function, reducing leucocyte-derived cytokine formation, and acting as an anti-inflammatory, as well as an anti-oxidative [6]. The American Heart Association recommends that patients with documented CVD take n-3 PUFAs at a dose of approximately 1 g/day in combination with the essential FAs (EFAs) eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) in the form of a fatty fish or fish oil supplement [7,8]. Similar recommendations are given by the European Society of Cardiology [9]. However, results from clinical trials are not consistent and the recently published STRENGTH trial failed to demonstrate a clinical benefit in high-risk patients treated with EPA/DHA [10], while the REDUCE-IT trial found a clear benefit in CAD patients with elevated TGs when treated with EPA [11].

Statins can significantly reduce the incidence of acute CAD in both patients with a history of CAD and patients with cardiovascular risk factors [12]. Statins inhibit the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol [13]. The lipid-lowering effect of statins has been the dominant approach to reduce and prevent CVD. As studies have confirmed that statins can lower serum cholesterol and significantly reduce CVD morbidity and mortality [14], their use is a basic principle for primary and secondary prevention of CVD. With regard to combining statins with n-3 PUFA, the JELIS study found that adding 1800 mg EPA per day to a statin medication in a group of hypercholesterolemic patients significantly decreased cardiovascular endpoints [15]. In a study exploring pitavastatin in combination with 1800 mg EPA per day for CVD treatment, it was found that patients in the combined pitavastatin/EPA treatment group had significantly higher plaque regression rates compared to pitavastatin alone [16]. In another randomized control trial (RCT) of statin use in CAD patients, compared to statins alone, patients supplemented with 3400 mg EPA and DHA per day had less fibrous coronary plaque progression [17]. Thus, the combination of statins and EPA apparently slows disease progression in CVD patients and improves outcomes, as recently confirmed by the REDUCE-IT trial [11].

Interestingly, statins can affect FA synthesis by changing Δ5-desaturase and Δ6-desaturase activities [18]. In vitro studies demonstrated that co-incubation of statins with different cell lines, such as HepG2 and THP-1 cells, significantly increased the mRNA content and protein expression of Δ5-desaturase [19,20]. This indicates that statins not only inhibit cholesterol biosynthesis, but also increase the efficiency of FA biosynthesis.

In this study, we aimed to characterize the FA composition in blood from patients undergoing cardiac catheterization for diagnosis of CAD in order to assess whether there are differences regarding essential FA, and, in particular, n-3 PUFA contents in patients with established CAD as compared to those without CAD.

2. Results

2.1. Patient Characteristics

A total of 273 patients undergoing cardiac catheterization were included in this analysis. Of those, 81 did not have signs of relevant coronary artery disease, while in 192 patients there were significant signs of CAD. Statin use was very different in both groups, with 13% in the CAD-free and 59% in the CAD group receiving statins. The patients' general characteristics are shown in Table 1. Notably, patients in the CAD group were significantly older and heavier than those in the control group, and they had significantly lower levels of cholesterol, and HDL and LDL cholesterol, while HbA1c levels were significantly higher in the CAD group.

Table 1. Differences between the groups were tested with the unpaired Student's *t*-test and Chi-square test. Data are presented as mean ± standard error of the mean.

Patient's Characteristics	No CAD	CAD	
Male/Female	35/46	138/54	<0.0001
Age (years)	57.42 ± 1.67	67.92 ± 0.94	<0.0001
Weight (kg)	81.17 ± 1.90	86.42 ± 1.30	0.0241
BMI	27.77 ± 0.50	28.83 ± 0.38	0.0927
HbA1c (mmol/mol)	36.10 ± 0.44	44.43 ± 0.95	<0.0001
Cholesterol (mmol/L)	5.28 ± 0.12	4.67 ± 0.10	<0.0001
HDL (mmol/L)	1.50 ± 0.04	1.20 ± 0.03	<0.0001
LDL (mmol/L)	3.56 ± 0.11	3.01 ± 0.09	<0.0001
TGs (mmol/L)	1.34 ± 0.06	1.72 ± 0.08	<0.0001
Diabetes mellitus	1 (1%)	71 (37%)	<0.0001
Statin use	10 (13%)	102 (59%)	<0.0001

2.2. Total FAs in CAD vs. Control Patients

To explore the FA profiles in CAD patients to that in patients without clinically relevant CAD, total FA composition in blood from patients in both groups was analyzed and compared between the two groups. Compared with control patients, CAD patients had significantly higher levels of monounsaturated FA (MUFA, $p < 0.0001$)—comprised of palmitoleic acid (C16:1 n-7c), oleic acid (C18:1 n-9c), and nervonic acid (C24:1 n-9)—and significantly lower levels of polyunsaturated fatty acids (PUFA, $p < 0.0001$)—comprised of eicosapentaenoic acid (EPA, C20:5 n-3), docosapentaenoic acid (DPA, C22:5 n-3), docosahexaenoic acid (DHA, C22:6 n-3), linoleic acid (LA, C18: 2 n-6), dihomo-gamma-linolenic acid (DGLA, 20:3 n-6), arachidonic acid (AA, 20:4 n-6), and adrenic acid (AdA, C22:4 n-6)—but there was no significant difference in the content of saturated fatty acids (SFA)—comprised of myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0), and lignoceric acid (C24:0)—(Figure 1a).

Compared with control patients, CAD patients had a significantly lower content of n-3 PUFAs and n-6 PUFAs ($p < 0.0001$; $p < 0.0001$) (Figure 1b). On the level of individual FAs, CAD patients had a significantly lower content of the n-3 PUFAs EPA and DPA ($p < 0.0001$; $p < 0.0001$), and DHA was also decreased in CAD patients vs. non-CAD patients, albeit non-significantly (Figure 1c). Regarding n-6 PUFAs, CAD patients had significantly lower content of LA, DGLA, and AdA ($p = 0.0101$; $p < 0.0001$; $p < 0.0001$), whereas AA did not significantly differ between the groups (Figure 1d).

The omega-3 (n-3) index is defined as the percentage of the two n-3 FAs, EPA and DHA, in total FAs. We analyzed the n-3 index in our samples presented here and found that the index was 5.0% in the control and 4.4% in the CAD patients ($p = 0.0005$) (Figure 1e).

Previous studies have indicated that increased *FADS1* gene activity could contribute to the development of CAD, as outlined in the Introduction. In order to assess *FADS1* activity on the level of the fatty acid product to substrate ratio in our study, we performed an analysis of the so-called delta-5-desaturase (D5D) index, calculated as ratio of arachidonic acid (AA, 20:4 n-6) to dihomo-gamma-linolenic acid (DGLA, 20:3 n-6). Compared with control patients, CAD patients had a significantly higher D5D index ($p < 0.0001$) (Figure 1f), indicating higher *FADS1* gene activity in CAD patients.

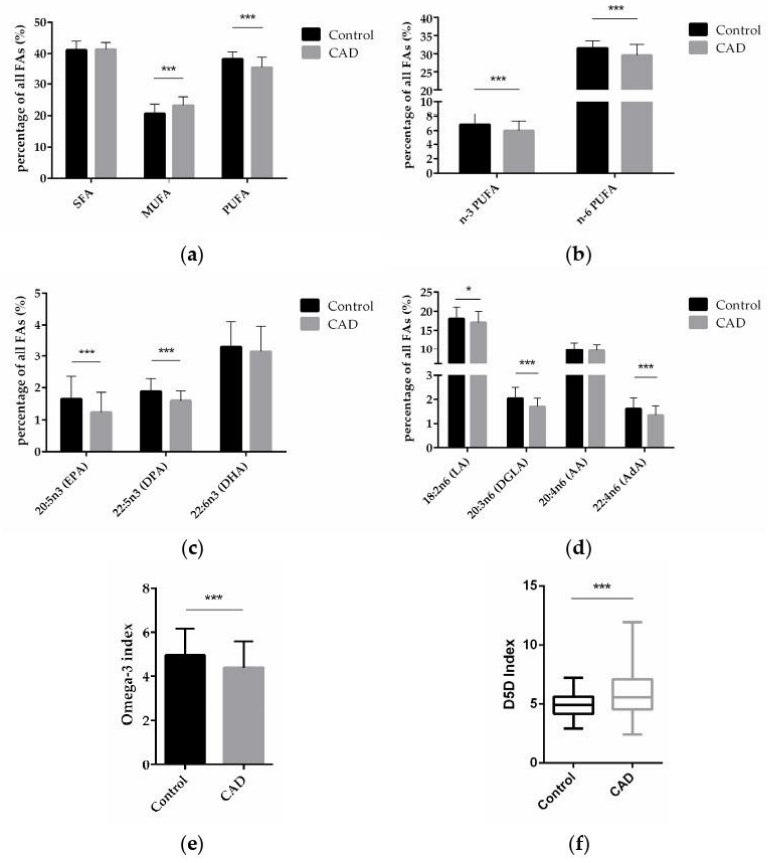

Figure 1. FA levels in blood from patients without and with CAD. (**a**) Relative content of SFAs, MUFAs, and PUFAs in control and CAD patients. (**b**) Relative content of n-3 and n-6 PUFAs in control and CAD groups. (**c**) Comparison of individual n-3 PUFAs in CAD and control patients. (**d**) Comparison of individual n-6 PUFAs in CAD and control patients. (**e**) n-3 index in control and CAD group. (**f**) D5D index as indicator of desaturase activity in CAD versus control patients. ($n = 81$ for the control group, and $n = 192$ for the CAD group; * indicates $0.01 < p < 0.05$, *** indicates $p < 0.001$).

N-3 PUFAs were shown to decrease TGs and are approved as medical treatment for increased TG levels. In order to assess whether there were lower TG levels associated with higher n-3 PUFA levels in our cohort, we performed a correlation analysis of these parameters. TGs were inversely associated with n-3 PUFA levels ($p < 0.0001$) (Figure 2a). This indicates that high content of n-3 PUFAs, indeed, also contributes to decreased TG levels in our patient cohort. We also performed correlations between HDL and LDL with n-3 PUFA, as shown in Figure 2b,c, and found correlations for higher HDL cholesterol, as well as lower LDL cholesterol, with the n-3 PUFA levels. In addition, we also observed that EPA and DHA, individually, were inversely correlated with TG ($p < 0.0001$; $p < 0.0001$) (Figure 2d,e).

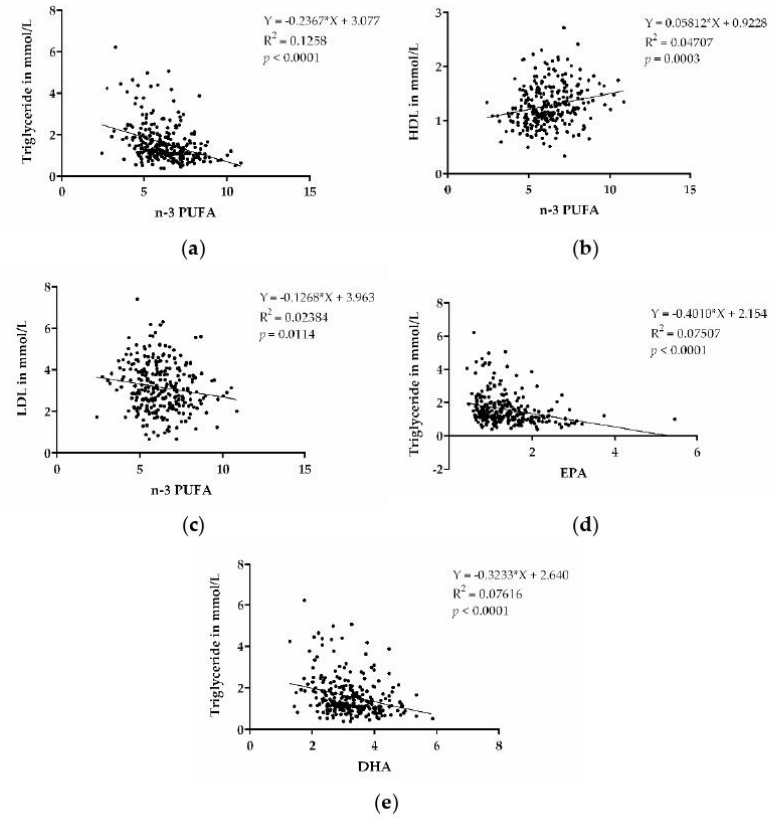

Figure 2. n-3 PUFAs and dyslipidemia. (**a**) Correlation between TGs and n-3 PUFA (EPA + DPA + DHA). (**b**) Correlation between HDL cholesterol and n-3 PUFA (EPA + DPA + DHA). (**c**) Correlation between LDL cholesterol and n-3 PUFA (EPA + DPA + DHA). (**d**) Correlation between TGs and EPA. (**e**) Correlation between TGs and DHA. (n = 273).

2.3. FAs and Statin Treatment

To investigate the effect of statins on FAs, we compared the FA differences between statin-treated and non-treated patients. The MUFA amount was significantly higher ($p = 0.0011$) and PUFAs were significantly lower ($p = 0.0006$) in patients on statin treatment (Figure 3a). Interestingly, it was the amount of n-6 PUFAs that was significantly lower ($p < 0.0001$) in statin-treated patients, while for n-3 PUFAs there was no significant difference according to statin treatment (Figure 3b). This was due to LA and DGLA being significantly lower ($p < 0.0001$; $p < 0.0001$), while AA and AdA were not significantly different (Figure 3c). Discernible in these data, the D5D index ($p < 0.0001$) was significantly higher in patients on statin medication (Figure 3d), while the omega-3 index remained unchanged (Figure 3e).

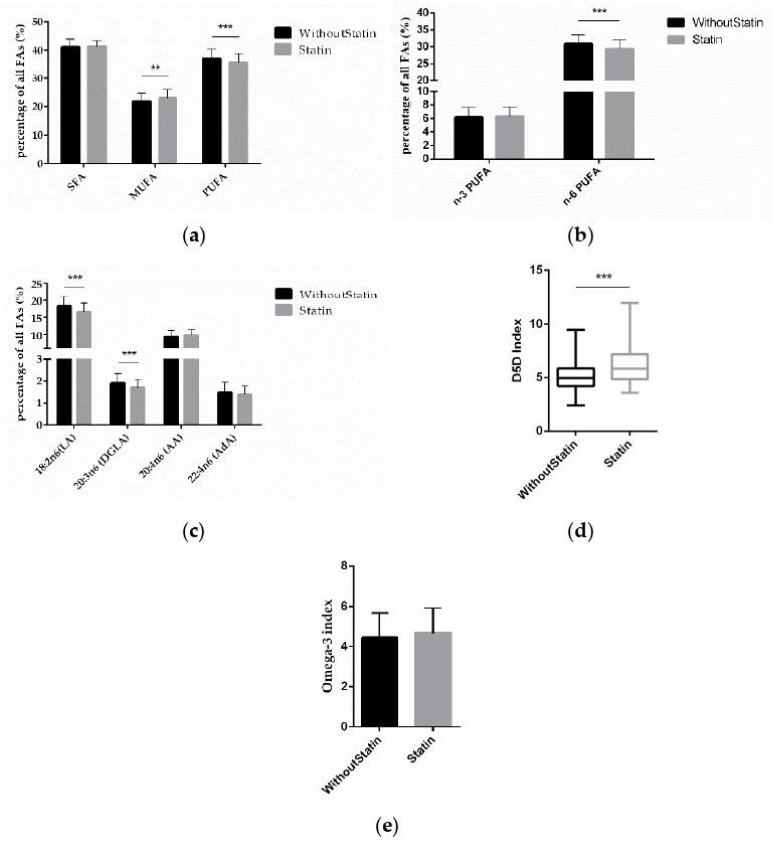

Figure 3. Correlation of FAs and statin treatment. (**a**) Relative content of SFAs, MUFAs, and PUFAs depending on statin treatment vs. no statin treatment. (**b**) Relative content of n-3 and n-6 PUFAs according to statin treatment. (**c**) Relative content of different n-6 PUFAs according to statin treatment. (**d**) D5D index and (**e**) omega-3 index according to statin medication. (n = 142 for the without-statin group, n = 112 for the statin group; ** indicates $0.001 < p < 0.01$, *** indicates $p < 0.001$).

2.4. FA Comparison in Control and CAD Patients without Statin Treatment

To determine the effect of essential FAs on CAD, we compared the differences of the FAs in the control and CAD patients, focusing on those without statin medication in both groups. We found that MUFA was significantly higher ($p < 0.0001$) and PUFA ($p < 0.0001$) was significantly lower in CAD patients without statin treatment (Figure 4a). Further analysis found that levels of n-3 PUFA and n-6 PUFA ($p < 0.0001$; $p = 0.0029$) were significantly lower in CAD patients without statin treatment (Figure 4b). The results of individual FA analysis for n-3 PUFA showed that EPA, DPA, and DHA ($p < 0.0001$; $p < 0.0001$; $p = 0.0017$) were significantly lower in CAD patients without statin treatment (Figure 4c). Regarding n-6 PUFA, DGLA, AA, and AdA ($p < 0.0001$; $p = 0.0071$; $p < 0.0001$) were also significantly lower (Figure 4d). Notably, also in these statin-free groups, a higher D5D index was found in CAD patients, although this was not significant ($p = 0.0654$) (Figure 4e), while the omega-3 index ($p < 0.0001$) was significantly lower in CAD patients (Figure 4f). After excluding the effect of statins on FAs, our results thus indicate that low levels of n-3 PUFA and n-6 PUFA, as well as higher D5D activity, might be present predominantly in CAD patients.

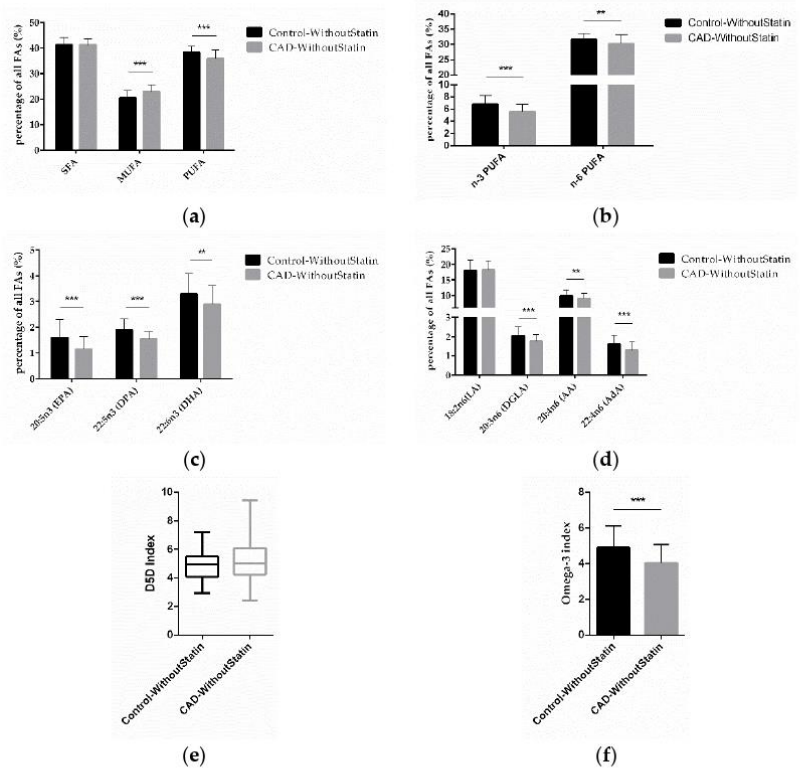

Figure 4. Correlation of FAs in control and CAD patient subgroups without statin treatment. (**a**) Relative content of SFAs, MUFAs, and PUFAs in control and CAD patients without statin treatment. (**b**) Relative content of n-3 and n-6 PUFAs in control and CAD patients without statin treatment. (**c**) Relative content of different n-3 PUFAs and (**d**) n-6 PUFAs in control and CAD patients without statin treatment. (**e**) D5D index and (**f**) omega-3 index in control and CAD patients without statin treatment. (n = 70 for the control-without-statin group, n = 72 for the CAD-without-statin group; ** indicates $0.001 < p < 0.01$, *** indicates $p < 0.001$).

3. Discussion

In this study, we found significant differences in FAs in CAD patients as compared to those without. N-3 and n-6 PUFA content was significantly lower in CAD patients. Since n-3 PUFAs have been implicated to have cardioprotective effects, their lower content might be important in the development and progression of CAD. We further analyzed the content of different kinds of n-3 PUFA and found that EPA and DPA were significantly lower in our CAD patient cohort compared to the control cohort, with no significant difference for DHA. This is in accordance with the strong evidence for the beneficial cardiovascular effects of EPA [11,15], but less so for EPA plus DHA. For DPA, there are data from a meta-analysis based on prospective cohort studies indicating that DPA levels were negatively correlated with stroke death [21]. In a second meta-analysis based on ten prospective cohort studies with 20,460 patients, a negative association between stroke risk and circulating DPA levels, but not with EPA, had been reported [22]. In addition, we found that DHA was significantly lower in CAD patients without statin treatment. However, this difference was not observed in statin-treated patients.

Mechanistically, intestinal cells take up dietary TGs and bind to apolipoprotein (apo) B-48 to form chylomicrons, which are transported through the pre-mesenteric lymphatic vessels and then through the thoracic duct into the blood circulation, where they are rapidly hydrolyzed by lipoprotein lipase [2] along the surface of the capillary lumen, leading to an elevation of free FAs and chylomicron residues [23]. High levels of TGs increase the risk of CVD. Studies have indicated that n-3 PUFA can increase lipoprotein lipase (LPL) activity and alter the kinetics of apoB100-containing lipoproteins [24]. A further study published by Allaire et al., found that EPA and DHA independently increased the rate of VLDL-apoB100 catabolism compared to the control [25]. In addition, n-3 PUFA is a ligand of the nuclear receptor family of transcription factors PPAR, which induces hepatic β-oxidation to reduce endogenous lipid production by activating PPARα [26,27]. In our study, we found that TG levels showed an inverse trend with the n-3 PUFA, in accordance with these mechanisms and the established effect of n-3 PUFA decreasing TG levels in the blood.

The importance of the effect of n-3 PUFA on TG-lowering and risk reduction of events in CAD is strongly supported by data from the REDUCE-IT trial [11,28]. Our previous study showed increased anti-inflammatory, anti-thrombotic, and antioxidant lipid metabolites in the blood of patients with n-3 PUFA supplementation [29]. This mechanism of action may also contribute to TG-lowering, as well as anti-atherosclerotic effects that have been postulated for n-3 PUFA in the context of CVD.

EPA serves as an n-3 PUFA, playing an important role in cardiovascular diseases [30]. High purity EPA showed beneficial effects and regression of atherosclerotic plaques [31]. Growing evidence supports the use of EPA, particularly, as an anti-atherosclerotic agent [32]. Apparently, it is EPA that reduces the residual risk after statin therapy by directly affecting atherosclerotic plaques [33]. It has been postulated that EPA—besides its well-established TG-lowering effect—also acts through EPA-derived anti-inflammatory lipid mediators [34] (Figure 5).

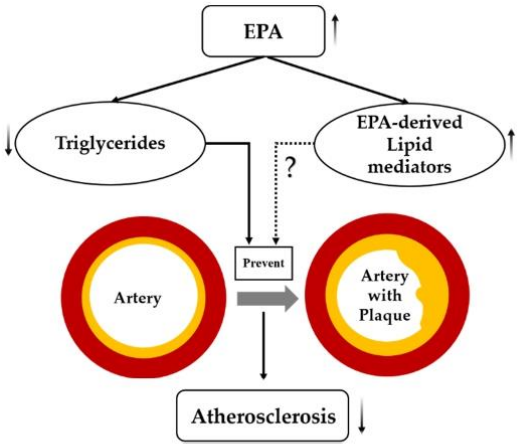

Figure 5. Postulated mechanism(s) by which EPA suppresses atherosclerosis. Dashed lines indicate possible effects of EPA that need further investigation, solid lines indicate effects of EPA that are well established in the scientific literature, arrows indicate increased or decreased.

The role of n-6 PUFAs in CVD is still inconclusive, but our study found significantly lower content in the total amount of n-6 PUFA in CAD patients. The analysis of individual n-6 PUFA content showed that the content of LA, DGLA, and AdA was significantly lower, whereas AA content was similar in CAD patients as compared to controls. Although AA can be metabolized via the cyclooxygenase and lipoxygenase pathways to produce pro-inflammatory mediators, such as prostaglandins and leukotrienes [34], which may be

related to the development of CAD, our study did not show any difference in AA content between CAD and control patients.

The clinical impact of n-6 PUFA on CVD risk and mortality remains controversial [35]. Yang et al., found that n-6 PUFA concentrations were negatively associated with CVD risk, and especially high LA concentrations were significantly associated with low CVD risk and CVD mortality [36].

FADS1 is a critical enzyme for the production of long-chain PUFA, and PUFA are a precursor for many important metabolites [37]. In our study, the D5D index describing *FADS1* gene activity was higher in CAD patients compared to control patients. Such results have also been described earlier and have been linked to the effect of specific FADS1 genotypes in combination with the impact of a western diet [38–40]. This could indicate that, with an n-6 PUFA-high western diet as a prerequisite, higher FADS1/D5D activity leads to the production of more AA, from which pro-inflammatory lipid mediators (LMs) are synthesized [34], which in turn promote CAD/CVD. As our previous studies indicate that PUFA levels, as well as lipid-lowering treatments, such as lipid apheresis or statins, can change PUFA and lipid mediators in blood and tissue [41–44], these effects could be modified by diet and/or medications. In contrast, Yang et al., suggested that the inhibition of D5D activity accelerates the development of atherosclerosis [36].

Statins are clearly shown to lower cardiovascular risk, which is mainly due to their potent LDL cholesterol-lowering effects, and guidelines recommend statins for aggressive LDL cholesterol-lowering in patients with CVD [9,45]. Within this study we also analyzed the effect of statin treatment on FA composition in the blood. Interestingly, n-6 PUFAs, but not n-3 PUFAs, were found to be significantly lower in patients receiving statin treatment, while a previous study had described a lowering effect on both classes of FAs [46], and others had found an increase of long-chain n-6 PUFA with simvastatin [47] and lowering of FAs with an increased AA/EPA ratio [48]. Our results show a significant decrease in DGLA in patients on statin treatment, which is consistent with observations that statins increase the activity of Δ6 and Δ5-desaturase enzymes [18,49]. We also found that patients receiving statin medication had a significantly higher D5D index compared to non-statin treated patients. Given the high proportion of statin-treated patients in the CAD group (Table 1), this statin effect could explain the increased D5D ratio in the CAD patients analyzed here. However, when we analyzed the D5D index in the subgroup of patients without statin treatment, we also found a trend towards a higher D5D index in CAD patients. This is in accordance with the fact that *FADS1* (and *FADS2*) gene polymorphisms have been associated with CVD in several non-experimental studies [36,50–53].

In this context, it is a central limitation of this study that we were not able to analyze gene expression—or genotype variation—of genes involved in the elongation and desaturation of PUFAs. In particular, we did not gain any information regarding FADS1 genotype and expression levels.

Another limitation of this study is the lack of nutrition data from the patients. This, as well as analyses regarding the formation of n-3 PUFA-derived lipid mediators, remain to be addressed in future studies.

This is, thus, a pilot study establishing a difference in essential fatty acid levels between patients with and without CAD. We hope that our data will add insight and rationale for future studies to further analyze the mechanisms and clinical impacts of our findings.

The results from this study raise the question of dose- and specific statin-dependent effects on desaturase activity, as well as the role of, for example, specific FADS1 genotypes on the observed effects. We propose to study these aspects specifically in future studies. Our study therefore supports the necessity to further investigate the effect of genetic and pharmacological factors on FA synthesis in CAD and to explore how FADS1 genotypes modify the synthesis of AA from DGLA.

Most importantly, however, our study shows lower EPA levels in CAD patients, confirming the emphasis on EPA as a potentially central compound mediating the biological

effects of omega-3 polyunsaturated fatty acids, and provides additional support to the concept of cardioprotection due to increased EPA levels in humans.

4. Materials and Methods

4.1. Study Population

Patients admitted for invasive coronary angiography were recruited at the University Hospital Brandenburg/Havel, Germany. Patients had either known CAD with suspicion for progress of the disease, or were referred for suspected CAD. Based on the findings of the invasive angiography, patients were then divided in the CAD group by using at least a 50% diameter stenosis as a cut off for the presence of (obstructive) CAD. Control patients were categorized as not having relevant CAD.

Exclusion criteria were active cancer disease, the inability to give informed consent, and an age of <18 years. Written informed consent was obtained from all subjects. The study was approved by the ethics committee of the medical association of the state of Brandenburg (AS69(bB)/2016).

4.2. Sample Collection

Blood samples were collected after overnight fasting and before the cardiac catheterization procedure. EDTA tubes were stored in −80 °C until FA analysis. A routine lipid panel (total cholesterol, LDL-C, HDL-C, and TGs) was measured by standard validated clinical assays immediately after sampling in the central laboratory of the hospital.

4.3. Sample Preparation

50 µL of full blood per sample was used for the gas chromatography (GC) preparation. Methylation and extraction of FAs were carried out on the basis of an established protocol [54]. Briefly, frozen samples were thawed at room temperature. All samples were then mixed with 50 µL pentadecanoic acid (PDA, 1 mg/mL, Merck Schuchardt OHG, Hohenbrunn, Germany) as internal standard, 500 µl borontrifluoride (BF_3, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) in 14% methanol (Merck KGaA, Darmstadt, Germany), and 500 µL *n*-hexane (Merck KGaA, Darmstadt, Germany) in glass vials and tightly closed. After vortexing, samples were incubated for 60 min in a preheated block at 100 °C. After cooling down to room temperature, the mixture was added to 750 µL water, vortexed, and extracted for 4 min. Then all samples were centrifuged for 5 min (RT, 3500 rpm). From each sample, 100 µL of the upper n-hexane layer was transferred into a micro-insert (placed in a GC glass vial), tightly closed, and analyzed by GC.

4.4. Determination of FAs Using GC

GC was performed on a 7890B GC System (Agilent Technologies, Santa Clara, CA, USA) with an HP88 Column (112/8867, 60 m × 0.25 mm × 0.2 µm, Agilent Technologies, Santa Clara, CA, USA), with the following temperature gradient: 50 °C to 150 °C with 20 °C/min, 150 °C to 240 °C with 6 °C/min, and 240 °C for 10 min (total run time 30 min). Nitrogen was used as carrier gas (constant flow 1 mL/min). 1 µL of each sample was injected into the injector (splitless injection, 280 °C). The flame ionization detector (FID) analysis was performed at 250 °C with the following gas flows: hydrogen 20 mL/min, air 400 mL/min, and make up 25 mL/min. Methylated FAs in the samples were identified by comparing the retention times with those of known methylated FAs of the Supelco® 37 FAME MIX standard (CRM47885, Sigma Aldrich, Laramie, WY, USA). Analysis and integration of the peaks were carried out with OpenLAB CDS ChemStation Edition (Agilent Technologies, Santa Clara, CA, USA). FA values are presented as percentage (%) of total FA content. For the study, 16 FAs were included as follows: myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0), lignoceric acid (C24:0), palmitoleic acid (C16:1 n-7c), oleic acid (C18:1 n-9c), nervonic acid (C24:1 n-9), eicosapentaenoic acid (EPA, C20:5 n-3), docosapentaenoic acid (DPA, C22:5 n-3), docosahexaenoic acid (DHA, C22:6 n-3), linoleic acid (LA, C18: 2 n-6), dihomo-gamma-

linolenic acid (DGLA, 20:3 n-6), arachidonic acid (AA, 20:4 n-6), and adrenic acid (AdA, C22:4 n-6). Statistical analysis of the results was carried out by unpaired Student's t-test and Chi-square test, where appropriate, using Prism GraphPad 5. Statistical significance was assumed when $p < 0.05$ (* $0.01 \leq p < 0.05$; ** $0.001 \leq p < 0.01$; *** $p < 0.001$).

4.5. N-3 Index and D5D Index

The omega-3 (n-3) index was defined as the percentage of the two n-3 FAs (FAs), EPA and DHA, of total FAs in analogy to the n-3 index level defined previously [55]. A low n-3 index has been suggested as a risk factor for CVD and its recommended range is between 4% and 8% [56].

Genes involved in the processing (elongation and desaturation) of long-chain (lc) PUFAs from short-chain (sc) precursors have been implicated in conferring diet-dependent risks of CVD [34,53,57]. The rate-limiting steps in the synthesis of lc PUFAs from sc PUFAs are catalyzed by the two FA desaturases, delta-5 desaturase (D5D) and delta-6 desaturase (D6D) [58], and encoded by FA desaturase 1 (*FADS1*) and FA desaturase 2 (*FADS2*), respectively. *FADS* gene cluster polymorphisms are, in addition to the nutritional regulation of FA supply and composition, a very important regulator of lc PUFA synthesis [59,60]. From the ratio of the FADS1 product AA to its substrate dihomo-gamma-linolenic acid (DGLA), the so-called delta-5 desaturase index (D5D index) can be calculated [61,62]. Considering this parameter, it is thus possible to draw conclusions about the efficiency of *FADS1* activity/gene expression.

5. Conclusions

We found lower levels of essential n-3 and n-6 PUFA in patients with catheter-proven CAD. Furthermore, there was an indication of increased desaturase activity leading to a higher D5D ratio in patients with CAD. In particular, EPA levels were significantly lower in CAD patients, supporting the concept of EPA-mediated cardioprotection.

Author Contributions: C.W. did FA GC analyses and prepared data and the manuscript; J.E. performed analyses of data; A.P. established GC measurements; C.S., N.P., O.R. and U.E. discussed and interpreted results; N.P., L.W., O.R. and M.J. provided samples; K.H.W. developed research questions, discussed and wrote the manuscript, and prepared and analyzed data. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the ethics committee of the medical association of the state of Brandenburg (AS69(bB)/2016).

Informed Consent Statement: Written informed consent was obtained from all subjects.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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10 Curriculum vitae

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection.

11 Publication list

1. **Wang C**, Enssle J, Pietzner A, Schmöcker C, Weiland L, Ritter O, Jaensch M, Elbelt U, Pagonas N, Weylandt KH. Essential Polyunsaturated Fatty Acids in Blood from Patients with and without Catheter-Proven Coronary Artery Disease. Int J Mol Sci. 2022 Jan 11;23(2):766. doi: 10.3390/ijms23020766. PMID: 35054948; PMCID: PMC8775772. (IF: 5.923)
2. Rohwer N, Jelleschitz J, Höhn A, Weber D, Kühl AA, **Wang C**, Ohno RI, Kampschulte N, Pietzner A, Schebb NH, Weylandt KH, Grune T. Prevention of colitis-induced liver oxidative stress and inflammation in a transgenic mouse model with increased omega-3 polyunsaturated fatty acids. Redox Biol. 2023 Aug;64:102803. doi: 10.1016/j.redox.2023.102803. Epub 2023 Jun 26. PMID: 37392516; PMCID: PMC10336695. (IF: 11.4)
3. Zouboulis CC, Hossini AM, Hou X, **Wang C**, Weylandt KH, Pietzner A. Effects of Moringa oleifera Seed Oil on Cultured Human Sebocytes In Vitro and Comparison with Other Oil Types. Int J Mol Sci. 2023 Jun 19;24(12):10332. doi: 10.3390/ijms241210332. PMID: 37373478; PMCID: PMC10299200. (IF: 6.208)

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