Application based personalized food choices and health sustainment: scientific background and investigation of biomarkers in human tissue specimens

Gesundheitserhaltende Ernährung: Wissenschaftlicher Hintergrund einer App zur personalisierten Lebensmittelauswahl und Identifizierung von Biomarkern für die Ernährungsweise in Humangewebe

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Abstract

Dietary fatty acids serve as objective biomarkers for the estimation of habitual diet mainly because biomarkers are free of memory bias or inaccuracies of food databases. The aim of the present work encompassed the implementation of a gas chromatographical method coupled with a mass spectrometrical and flame-ionization detector for analysis of fatty acid biomarkers in human biospecimens, their analytical determination and statistical evaluation in two different study populations and different biospecimens as well as the elaboration of adverse reactions to food ingredients with special focus on food allergies and food intolerances in the context of a possible implementation into an application for consumer health.

The first aim was the identification of potential influence of fatty acid biomarkers on desaturase and elongase indexes (Δ9DI, Δ6DI, Δ5DI and ELOVLI5), which are factors in type 2 diabetes risk, in breast adipose tissue from healthy women. Influence of further variables on respective indexes was also investigated. 40 samples were investigated and potential variables were either collected by questionnaire or determined. Principle component analysis was applied for fatty acid biomarkers ($PC_{diet}1$, $PC_{diet}2$ and $PC_{diet}3$ representative for the dietary intake of vegetable oils/nuts, fish and partially hydrogenated vegetable oils), endogenous estrogens (PC_E1) and oxysterols ($PC_{Oxy}1$). Multiple linear regression models were applied. Δ9DI and Δ6DI were influenced non-significantly and significantly negatively by $PC_{diet}2$ supporting a putative beneficial effect of vegetable oils and nuts on type 2 diabetes risk factors. ELOVLI5 and Δ5DI were influenced significantly and non-significantly positively by $PC_{diet}1$ supporting a putative beneficial effect of fish consumption on type 2 diabetes risk factors. On the other hand, $PC_{diet}1$ also significantly and non-significantly positively influenced Δ9DI and Δ6DI supporting a putative adverse effect of fish biomarkers on type 2 diabetes risk factors. The opposing influences of $PC_{diet}1$ suggesting an ambivalent role of dietary intake of fish on investigated indexes. Δ6DI was significantly positively influenced by $PC_{diet}3$ and number of pregnancies supporting a putative adverse effect of partially hydrogenated vegetable oils and pregnancies on type 2 diabetes risk factors. Lifestyle factors like smoking significantly and non-significantly in-

fluenced Δ9DI and Δ6DI putatively adversely. Δ5DI was influenced significantly positively by estrogen active drugs suggesting a putative beneficial effect on type 2 diabetes risk factors. It must be considered that a variation coefficient of up to 0.44 only explained 44% of variance of the respective indexes, suggesting other influencing factors might play a role.

The second aim was the implementation of a gas chromatographical method coupled with a mass spectrometrical and flame-ionization detector for analysis of fatty acid biomarkers in human biospecimens. The method was optimized for separation and detection of 40 fatty acids. Mean recovery for tridecanoic acid was $\bar{x}_{\text{tridecanoic acid}}$ = 90.51% and for nonadecanoic acid $\bar{x}_{\text{nonadecanoic acid}}$ = 96.21%. Thus, there was no significant loss of fatty acids with shorter and longer carbon chains over the extraction process to be expected. Limit of detections were calculated in adipose tissue samples and ranged from 0.007 to 0.077% of the proportion of the respective fatty acid to total fatty acids.

The third aim was the investigation if differentiation between breast glandular and adipose tissue had a relevant impact on the analysis of dietary fatty acid biomarkers or if contamination of breast glandular with breast adipose tissue and vice versa was neglectable for the analysis of dietary fatty acid biomarkers. No statistical significant differences were observed for all investigated fatty acid biomarkers (pentadecanoic-, heptadecanoic-, *trans* palmitoleic-, eicosapentaenoic-, docosahexaenoic-, linoleic and α-linolenic acid) between breast glandular and adipose tissue. Thus, differentiation between breast glandular and adipose tissue seems not to be necessary for the analysis of fatty acids serving as biomarkers for the intake of specific food groups. Potential influence of mixed breast tissue on fatty acid biomarkers analysis seems to be neglectable.

The fourth aim was the determination of fatty acid biomarkers in adipose tissue in another study population from healthy participants. 27 adipose tissue samples were analyzed. Milk and ruminant fat biomarkers exhibited proportions of 0.47% for pentadecanoic acid, 0.34% for heptadecanoic acid and 0.25% for *trans* palmitoleic acid. Fish fatty acid biomarkers revealed proportions of 0.034% for eicosapentaenoic acid and 0.061% for docosahexaenoic acid. The mean proportion of vegetable oils and nuts biomarkers were 9.58% for linoleic acid and 0.48% for α-linolenic acid in all adipose tissues. Principle component analysis was applied for the fatty acid biomarkers to provide objective markers of habitual diet for this study population. $PC_{diet}1$ was mainly characterized by pentadecanoic acid, heptadecanoic acid and *trans* palmitoleic acid and therefore served as a principle component for the dietary intake of milk and ruminant fat. $PC_{diet}2$ and $PC_{diet}3$ only exhibited pattern for $\omega 3$

and $\omega 6$ fatty acids but not for dietary intake of specific food groups and could therefore not used as objective marker. PC_{diet} 1, 2 and 3 explained 82.76% of variance.

The last aim of this thesis was the elaboration of adverse reactions to food ingredients with special focus on food allergies and food intolerances in the context of a possible implementation into an application for consumer health. Scientific information on adverse reactions to food ingredients and trigger substances was provided in this thesis and possible implementation strategies were evaluated. For food allergens, which have regulatory requirements in the context of labelling, a strategy was elaborated, where it is necessary to provide information on the list of ingredients, the nexus 'contain' and the respective food allergen as well as information on the name of the product. For food intolerances, which do not have regulatory requirements, limits were shown in the context of the application. If the elaborated food intolerances shall be implemented into the application, a professional dietary concept has to be developed for every food intolerance because of the complexity of the implementation.

Zusammenfassung

Die vorliegende Arbeit umfasste die Implementierung einer analytischen Methode zur Bestimmung von Fettsäurebiomarkern in unterschiedlichen Bioproben, die analytische Bestimmung und statistische Evaluation von Fettsäurebiomarkern in zwei Studienpopulationen und unterschiedlichen Bioproben sowie die Ausarbeitung und Bereitstellung wissenschaftlicher Information zu adversen Reaktionen von Lebensmittelzutaten mit besonderem Fokus auf Nahrungsmittelallergien sowie Nahrungsmittelunverträglichkeiten im Kontext einer strategischen Implementierung dieser adversen Reaktionen in eine Applikation im Sinne des Verbraucherschutzes.

Das erste Ziel war die Identifizierung von potentiellen Einflüssen durch Fettsäurebiomarker der Ernährung auf Desaturase- und Elongase-Indices (Δ9DI, Δ6DI, Δ5DI, ELOVLI5), welche Einflussfaktoren auf das Typ 2 Diabetes Risiko darstellen, in Brustfettgewebe von gesunden Frauen. Der potentielle Einfluss von weiteren Variablen auf Desaturase- und Elongase-Indices wurde ebenfalls untersucht. 40 Proben wurden untersucht und potentielle Variablen sowohl mithilfe eines Fragebogens erhoben als auch analytisch ermittelt. Hauptkomponentenanalysen wurden für Fettsäurebiomarker ($PC_{diet}1$, $PC_{diet}2$ und $PC_{diet}3$ repräsentativ für eine Ernährung reich an pflanzlichen Ölen/Nüssen, Fisch und gehärteten Ölen), endogene Estrogene (PC_E1) und Oxysterole ($PC_{Oxy}1$) angewendet. Potentielle Einflussfaktoren wurden mittels multiple linearer Regressionsanalyse ermittelt. Δ9DI und Δ6DI wurden nicht-signifikant und signifikant durch $PC_{diet}2$ beeinflusst. Dies unterstützt einen möglicherweise vorteilhaften Effekt von Fettsäurebiomarkern repräsentativ für die Aufnahme von pflanzlichen Ölen und Nüssen auf Typ 2 Diabetes Risikofaktoren. $PC_{diet}1$ hatte einen möglicherweise vorteilhaften signifikanten und nicht-signifikanten Einfluss auf ELOVLI5 und Δ5DI. Auf der anderen Seite hatte $PC_{diet}1$ einen möglicherweise adversen signifikanten Einfluss auf Δ9DI und Δ6DI, was auf eine ambivalente Rolle von Fettsäurebiomarkern des Fischkonsums hindeutet. Möglicherweise adverse signifikante Einflüsse auf Δ6DI hatten $PC_{diet}3$ sowie die Anzahl an Schwangerschaften. Rauchen hatte einen signifikanten und nicht-signifikanten möglicherweise adversen Einfluss auf Δ9DI und Δ6DI. Einen möglicherweise vorteilhaften Einfluss auf ELOVLI5 wurde mit der Einnahme von

estrogenaktiven Substanzen beobachtet. Berücksichtigt werden muss allerdings, dass mit einem Variationskoeffizienten von bis zu 0.44 nur 44% der Varianz der entsprechenden Indices erklärt werden konnte und somit weitere Einflussfaktoren eine Rolle spielen könnten.

Das zweite Ziel war die Implementierung einer gaschromatographischen Methode für die Trennung und Detektion von 40 Fettsäuren. Die Chromatographie war simultan an einen Massenspektrometer und einen Flammenionisationsdetektor gekoppelt. *Recovery* Versuche zeigten für die Internen Standards Tridecansäure und Nonadecansäure Wiederfindungsraten von $\bar{x}_{\text{Tridecansäure}} = 90.51\%$ und $\bar{x}_{\text{Nonadecansäure}} = 96.21\%$. Die Nachweisgrenzen der Fettsäuren wurden mit Fettgewebsproben bestimmt und reichten von 0.007 bis 0.077% Anteil der jeweiligen Fettsäuren an der Gesamtfettsäureverteilung.

Die Untersuchung der Fragestellung ob die Differenzierung zwischen Brustdrüsen- und Brustfettgewebe einen relevanten Einfluss auf die Analyse von Fettsäurebiomarkern repräsentativ für eine Ernährung reich an Milch, Fisch und pflanzlichen Ölen/Nüssen hat war das dritte Ziel der vorliegenden Arbeit. Es wurde kein statistisch signifikanter Unterschied zwischen den prozentualen Anteilen der Fettsäurebiomarkern (Pentadecan-, Heptadecan-, *trans* Palmitolein-, Eicosapentaen-, Docosahexaen-, Linol- und α-Linolensäure) in Brustdrüsen- und Brustfettgewebe entdeckt. Eine Differenzierung zwischen Brustdrüsen- und Brustfettgewebe scheint daher in Bezug auf die Analyse von Fettsäurebiomarker der Ernährung nicht notwendig zu sein. Der potentielle Einfluss von, mit Brustdrüsen- und Brustfettgewebe, gemischtem Gewebe scheint damit in Bezug auf die Analyse von Fettsäurebiomarkern vernachlässigbar zu sein.

Das vierte Ziel der vorliegenden Arbeit war die Bestimmung der Fettsäureverteilung mit besonderem Fokus auf Fettsäurebiomarker für die Ernährung in Fettgewebe in einer anderen Studienpopulation von gesunden Teilnehmerinnen und Teilnehmern. 27 Fettgewebsproben wurde untersucht. Der Anteil an Fettsäurebiomarker für den Konsum von Milch- und Wiederkäuerfette betrug 0.47% für Pentadecansäure, 0.34% für Heptadecansäure und 0.25% für *trans* Palmitoleinsäure. Fettsäurebiomarker repräsentativ für Fischkonsum hatten Anteile von 0.034% für Eicosapentaensäure und 0.061% für Docosahexaensäure. Für Fettsäurebiomarker repräsentativ für den Konsum von pflanzlichen Fetten und Nüssen wurden Anteile von 9.58% für Linolsäure und 0.48% für α-Linolensäure gefunden. Anschließend wurde eine Hauptkomponentenanalyse der Fettsäurebiomarker für die Ernährung durchgeführt. $PC_{diet}1$ wurde hauptsächlich durch Pentadecansäure, Heptadecansäure und *trans* Palmitoleinsäure charakterisiert. $PC_{diet}1$ wurde folglich als Hauptkomponente für

den Konsum von Milch- und Wiederkäuerfetten interpretiert. $PC_{diet}2$ und $PC_{diet}3$ wurden ausschließlich von $\omega 3$ und $\omega 6$ Fettsäuren charakterisiert. Eine eindeutige Zuordnung zu speziellen Lebensmittelgruppen war nicht möglich. Die Biomarker für den Konsum von Fisch und pflanzlichen Fetten sowie Nüssen können daher nicht durch $PC_{diet}2$ und $PC_{diet}3$ zusammengefasst werden. $PC_{diet}1$, 2 und 3 erklärten 82.76% der Varianz.

Das letzte Ziel der vorliegenden Arbeit war die Ausarbeitung von adversen Reaktionen gegenüber Lebensmittelinhaltsstoffen mit speziellem Fokus auf Nahrungsmittelallergien und Nahrungsmittelunverträglichkeiten im Kontext einer möglichen Implementierung in eine Applikation im Sinne des Verbraucherschutzes. Hierfür wurden wissenschaftliche Informationen zu adversen Reaktionen gegenüber Lebensmittelinhaltsstoffen und potentiellen *Triggersubstanzen* zusammengetragen und mögliche Implementierungsstrategien evaluiert. Für Nahrungsmittelallergene, welche spezielle regulatorische Voraussetzungen im Sinne von Beschriftung und Deklaration besitzen, wurde eine Strategie ausgearbeitet, welche die Notwendigkeit der Informationen von Zutatenliste, der Verknüpfung der Signalwörter 'enthält' und dem entsprechenden Allergen wie auch den Namen des Produktes beinhaltet. Zutaten und Inhaltsstoffe, die Nahrungsmittelunverträglichkeiten hervorrufen können, haben keine speziellen regulatorischen Voraussetzungen. Limitierungen einer möglichen Implementierung von Nahrungsmittelunverträglichkeiten in die Applikation wurden im Rahmen dieser Arbeit aufgezeigt. Aufgrund der Komplexizität der Implementierung einer jeder einzelnen Nahrungsmittelunverträglichkeit in die Applikation sollte für jede einzelne ausgewählte Unverträglichkeit ein individuelles Konzept entwickelt werden.

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abbreviations

ADT Adipose tissue

FA fatty acid

FFQ food frequency questionnaire

WAT white adipose tissue

BAT brown adipose tissue

VAT visceral adipose tissue

SAT subcutanenous adipose tissue

TAG triacylglycerides

SCFA short chain FAs

MCFA medium chain FAs

LCFA long chain FAs

SFA saturated FAs

UFA unsaturated FAs

MUFA monounsaturated FAs

PUFA polyunsaturated FAs

TFA *trans* FAs

fig figure

ch chapture

CoA coenzyme A

FAS FA synthase

ER endoplasmic reticulum

Δ**9D** stearoyl-CoA desaturase

FADS fatty acids desaturase

Contents

Δ5D Δ5 desaturase

Δ6D Δ6 desaturase

HUFA highly unsaturated FAs

ELOVL Elongases of very long chain FAs

GC gas chromatography

MS mass spectrometry

NADP Nicotinamide adenine dinucleotide phosphate

FID flame ionization detection

EI electron-impact ionization

MI molecular ion

FI fragmentation ions

FAME fatty acid methyl ester

t_R retention time

eq. equation

T2D type 2 diabetes

E2 17β-Estradiol

MP menopausal

E1 estrone

Chol Cholesterol

PCA Principal component analysis

PC Principal components

exVar explanatory variables

R^2 coefficients of regressions

7β-OHC 7-β-OH-Cholesterol

5,6β-epoxyC 5,6β-epoxy-Cholesterol

5,6α-epoxyC 5,6α-epoxy-Cholesterol

n_0 initial number of copies

ct cycle threshold

HPRT1 hypoxanthin-guanin-phosphoribosyltransferase

oil% percentages of oil

TIC total ion chromatograms

IS Internal Standard

QM quality measurements

CF correction factor

LOD limit of detection

CV coefficients of variation

DI desaturase index

ELOVLI ELOVL index

Δ**5DI** Δ5D-index

Δ**6DI** Δ6D-index

Δ**9DI** Δ9D-index

ELOVLI5 ELOVL5-index

ISOCROSS Isoflavones: Cross-species comparison of metabolism, estrogen sensitivity, epigenetics, and carcinogenesis

WASP Würzburg analysis of steroid profiles

EAD estrogen active drugs

BMI Body mass index

GLT glandular tissue

Contents

ROS reactive oxygen species

$CHCl_3$ Chloroform

EtOH Ethanol

H_2O Water

MTBE Methyl tert-butyl ether

MeOH Methanol

NaCl Sodium chloride

TMSH Trimethylsulfoniumhydroxide

4:0 Butyric acid

6:0 Caproic acid

8:0 Caprylic acid

10:0 Capric acid

11:0 Undecylic acid

12:0 Lauric acid

13:0 Tridecylic acid

14:0 Myristic acid

14:1ω**5** Myristoleic acid

15:0 Pentadecylic acid

15:1ω**5** 10Z-pentadecenoic acid

16:0 Palmitic acid

16:1ω**7 _trans_** *trans* Palmitoleic acid

16:1ω**7** Palmitoleicacid

17:0 Margaric acid

17:1ω**7** Margaroleic acid

22:6 ω3 Docosahexaenoic acid

postMP postmenopausal

HRT hormone replacement therapy

preMP premenopausal

EFSA European Food Safety Authority

SOP standard operation procedure

GTIN global trade item number

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1. Introduction

Dietary habits play an important role in the occurence of non-communicable diseases such as type 2 diabetes (T2D). Dietary measurement errors (e.g. caused through memory bias and unvalidated questionnaires) may cause problems in estimation of diet-disease associations but also in evaluating influence of diet on potential risk factors of known diseases. Since measurement errors are common in nutritional epidemiology studies, which use assessment tools on the basis of the memory of the participants, the use of validated dietary biomarkers or the combination of both, dietary biomarker and such assessment tools are highly recommended (Freedman et al. 2011). Dietary fatty acid (FA) serve as objective biomarkers for the habitual diet and analysis of FAs in Adipose tissue (ADT) is assumed to be the best choice for study of long term FA intake though habitual diet mainly because of its slow turnover (summarized in Hodson, Skeaff, and Fielding 2008). In addition to the impact of dietary habits to common diseases such as T2D, nutritional factors play also a role in food-associated diseases, where specific food ingredients may trigger adverse reactions.

2. Theoretical background

In the following section, theoretical background shall be provided for all related subjects concerning this manuscript. First, human biospecimens (human ADT and breast GLT, respectively) will be described in the context of their constitutions (ch. 2.1). Subsequently, FAs will be described in terms of their constitution, *de novo* synthesis, habitual diet and FA biomarkers, which reflect the dietary intake of specific food groups (ch. 2.2). Information on analysis of FAs will be provided thereafter (ch. 4.2.3). Background information on biomarkers for desaturases and elongases and known influencing factors of these biomarkers will be depicted in ch. 2.4. Furthermore some information about the development of an application for consumer health regarding food-associated diseases, where specific food ingredients may trigger adverse reactions, will be provided in ch. 2.5.

2.1. Human Biospecimens

2.1.1. Human adipose tissue

In Humans, ADT is distributed throughout the whole body. Dependent on its major type, ADT is called white adipose tissue (WAT) or brown adipose tissue (BAT). WAT is distinguished into two major depots, subcutanenous adipose tissue (SAT) and visceral adipose tissue (VAT). The distribution of SAT is concentrated in abdominal, gluteal and femoral areas but also in the area of the human female breast, whereas VAT is concentrated in the abdominal cavitiy around internal organs (Choe et al. 2016) (see figure (fig) 2.1).
WAT is considered to provide energy storage and serves as an adipokine secreting tissue (Choe et al. 2016). BAT is a specialized tissue that is involved in non-shivering thermogenesis through lipid oxidation. Because of its high oxidation rate, BAT consists of exceptionally high number of mitochondria. BAT is generally observed in neonates and is gradually replaced by WAT with aging (Richard and Picard 2011). ADT contains, besides adipocytes, connective tissue matrix, nerve tissue, stromavascular cells, and immune cells (Kershaw and Flier 2004). Due to the presence of large amounts of lipid droplets, adipocytes are
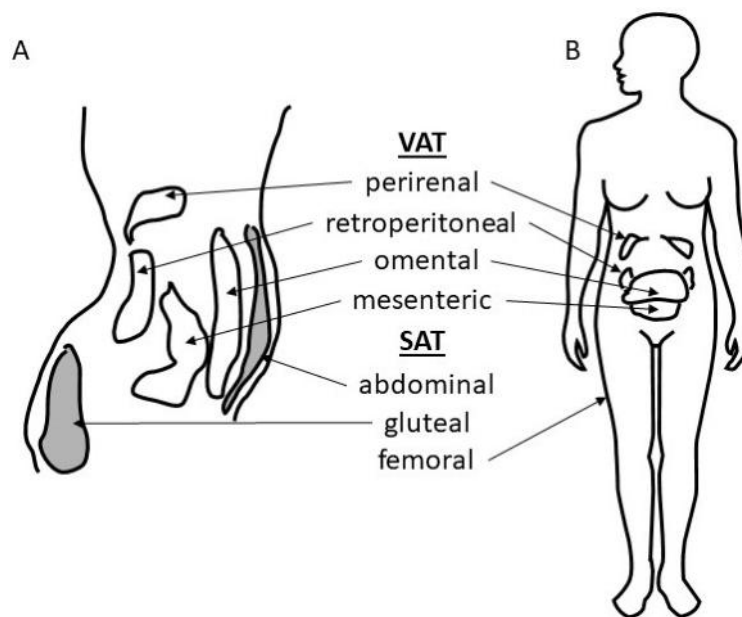
Figure 2.1.: Anatomy and location of VAT and SAT (modified according to Choe et al. 2016. (A) Cross-section of the human body with focus on the location of VAT and SAT. VAT, which is concentrated in the abdominal cavitiy, is further devided in perirenal, retroperitoneal, omental and mesenteric depots. SAT depots are located inter alia abdominal, gluteal as wee as femoral. VAT surrounds intra-abdominal organs, whereas SAT is located throughout the whole body underneath the skin. (B) Front-view of the human body with focus on the location of VAT and SAT. VAT visceral adipose tissue. SAT subcutaeous adipose tissue.

morphologically different from other cells (Gesta, Tseng, and Kahn 2007) and consists of mainly triacylglycerides (TAG) that can be used for energy.

2.1.2. Human breast glandular tissue

Female mammary glandular tissue (GLT) consists of parenchymatous tissue and stroma (Bocker, Hungermann, and Decker 2009). The parenchyma is characterized by a cluster of ducts ending in 15-25 lobular units (fig 2.2). The parenchyma is surrounded by stroma and fat tissue. The stroma is further subdevided in intralobular stroma, which is located between the ductules within the lobule, and interlobular stroma, which comprise the area between lobules (Bocker, Hungermann, and Decker 2009). Interindividual differences of the human female breast lead to a heterogeneous composition of GLT and ADT. The

percentage amount of lobules can vary from very little up to 50% of the whole gland (Bocker, Hungermann, and Decker 2009). The result of a heterogeneous composition of GLT and ADT was also observed from mammoplasty specimens after reduction mammoplasty from healthy women (Pemp, Geppert, et al. 2020).
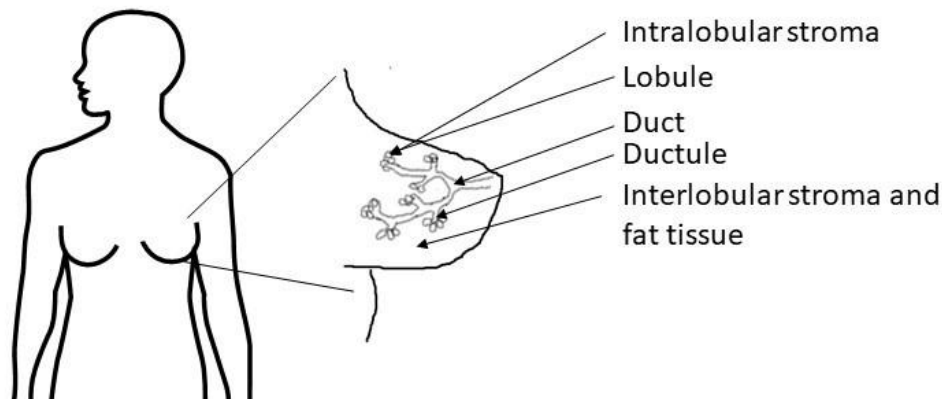
Figure 2.2.: Anatomy of the human female breast (modified according to Bocker, Hungermann, and Decker 2009). The intralobular stroma is located between the ductules within a lobule. The interlobular stroma comprises the area between lobules. Embedded in the fat tissue are 15 to 20 lobular units.

2.2. Fatty acids

As their storage form, FAs are bound on glycerol forming TAGs. Acyl lipid hydrolysis releases aliphatic carbon chains with a methyl group at one end of the molecule and a carboxyl group on the other end (fig. 2.3).
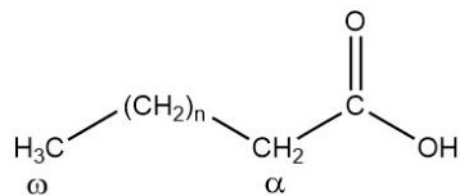
Figure 2.3.: FAs are composed of an aliphatic carbon chain with a methyl group at one end of the molecule (ω carbon atom) and a carboxyl group on the other end. The carbon atom next to the carboxylic carbon atom refers to the α carbon atom.

The carbon atom next to the carboxylic carbon atom refers to the α carbon atom whereas the methyl group at the end of the molecule indicates the ω carbon atom. FAs can be

divided into groups according to chain length, number, position and configuration of their double bonds (fig. 2.4).
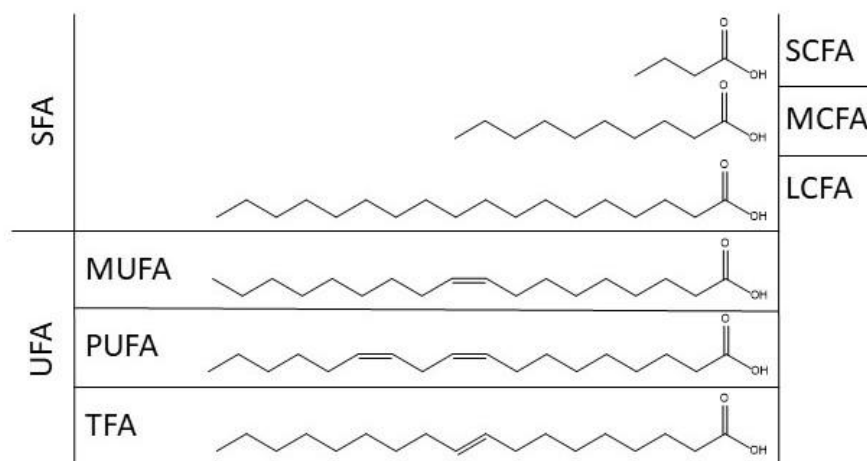
Figure 2.4.: Dependent on their chain length, FAs can be devided in SCFA (1 to 6 carbon atoms), MCFA (7 to 12 carbon atoms) and LCFA (>12 carbon atoms). SFAs only contain alkyl groups whereas UFAs contain one or more allyl groups in their acyl residue. UFAs are further subdevided in MUFAs (one double bond), PUFAs (two or more double bonds) and TFAs (one or more *trans* double bonds).

Dependent on their chain length, FAs can be divided into short chain FAs (SCFA), medium chain FAs (MCFA) and long chain FAs (LCFA) (summarized in Schonfeld and Wojtczak 2016). Straight-chain molecules with an even or an odd number of carbon atoms refer to saturated FAs (SFA). unsaturated FAs (UFA), which are the dominated part in ADT, contain one or more allyl groups in their acyl residues (fig. 2.4). Dependent on the number of double bonds in the molecule, FAs can further be divided into monounsaturated FAs (MUFA), polyunsaturated FAs (PUFA) and *trans* FAs (TFA). Unlike MUFAs and PUFAs, TFAs have one or more *trans* double bonds in their carbon chain. *Trans* means that the two carbon atoms adjacent to the double bond point into the opposite direction, whereas *cis* means that the two carbon atoms point into the same direction (Belitz, Grosch, and Schieberle 2009). The position of the double bond can vary along the carbon chain (fig. 2.4). The ω nomenclature is applied when counting starts from the ω carbon atom (refers to the methyl group at the end of the molecule) side. Thus, dependent on its location, UFAs can be classified in $\omega 3$, $\omega 5$, $\omega 6$, $\omega 7$ and $\omega 9$ FAs (fig. 2.5) (Belitz, Grosch, and Schieberle 2009). FAs are usually denoted in the literature by a "shorthand description". For the purpose of this thesis, FAs will be clustered with numbers in accordance to their chain length and amount of double bonds. Further they will be classified by their position of its first
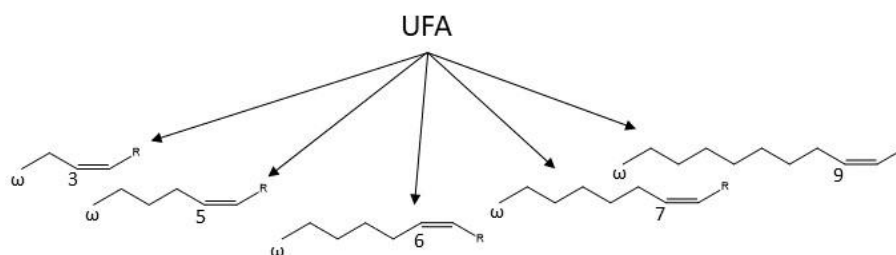
Figure 2.5.: Dependent on the position of the double bond, UFAs are classified by the ω nomenclature. ω3 FAs are characterized by the location of the double bond three carbon atoms away from the ω end. For ω5 FAs, the double bond is five carbon atoms away from the ω end. For all further ω FAs (ω6, ω7 and ω9), the ω nomenclature is applied the same way.

double bond in the molecule corresponding to the ω nomenclature. All double bonds are considered to be *cis*; whenever *trans* double bonds are present, an additional *trans* is shown, e.g. 18:1 ω9 refers to oleic acid, whereas 18:1 ω9 *trans* represents elaidic acid. Full names of every mentioned FA will be depicted in the abbreviations. FAs can either be received by dietary intake (see chapture (ch) 2.2.2) or *de novo* synthesized from acetyl-coenzyme A (CoA) and malonyl-CoA (ch 2.2.1).

2.2.1. *De novo* synthesis

Under physiological conditions, lipogenesis and lipolysis are set into a dynamic equilibrium to sustain fat mass in ADT (Song, Xiaoli, and Yang 2018). Under non-physiological conditions, e.g. in fasting or overeating periods, the maintained equilibrium is disrupted. In overeating periods, excessive energy is available for *de novo* synthesis of FAs. A surplus of glucose derived from dietary carbohydrates is transported into cells via specific glucose transporters. In cytoplasm it is converted to pyruvate via glycolysis. Pyruvate is further transported into mitochondria for entering into the *Krebs Cycle* resulting into its endproduct citrate. In the presence of excessive energy, citrate is exported into the cytoplasm and further degraded into acetly-CoA, which is then modified into malonyl-CoA (Song, Xiaoli, and Yang 2018). The conversion of acetyl-CoA into malonyl-CoA is the first and speed-limiting step of *de novo* FA synthesis in humans. After entering into the *de novo* pathway FAs are synthesized in the multi-enzyme protein complex FA synthase (FAS). From each round of elongation, the growing FA chain is carried between the active sites of FAS upon reaching a carbon chain length of 16 resulting into 16:0 (fig. 2.6 (A); Song, Xiaoli, and Yang 2018).
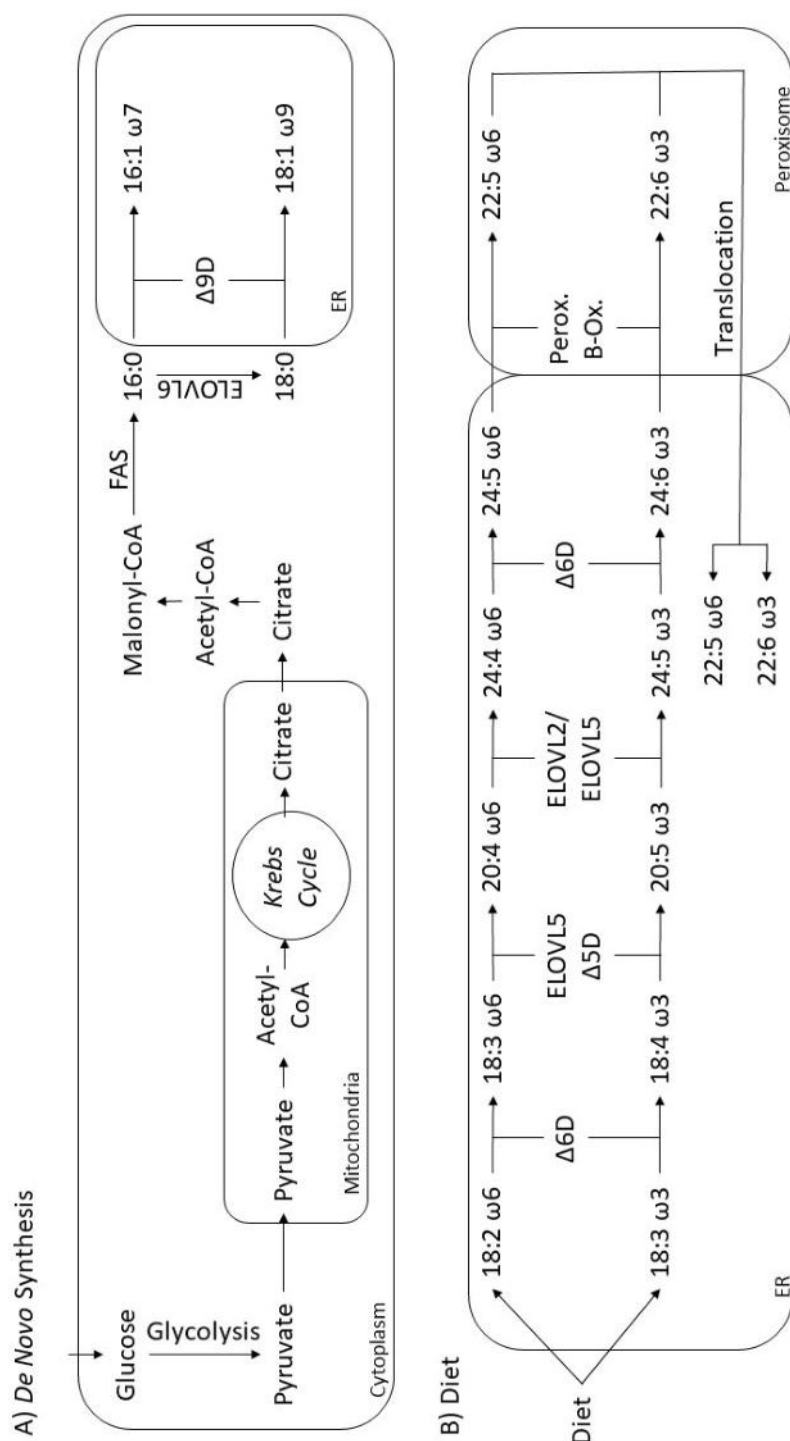
Figure 2.6.: FA biosynthesis in mammals (modified according to Guillou et al. 2010). (A) *De novo* synthesis from excessive energy surplus. Glucose is transported into cells and is further converted to pyruvate via glycolysis in cytoplasm. After transport into mitochondria, pyruvate is further degraded to acetyl-CoA, which enters into the *Krebs cycle* resulting into its endproduct citrate. Citrate is exported into cytoplasm, further degraded to acetly-CoA, which is modified to malonyl-CoA entering the *de novo* pathway via FAS resulting in 16:0. At the surface of ER, 16:0 is either desaturated to 16:1 ω7 via Δ9D or elongated to 18:0, which is then desaturated to 18:1 ω9. (B) Synthesis of PUFA from dietary 18:2 ω6 and 18:3 ω3. After dietary intake, 18:2 ω6 and 18:3 ω3 are both desaturated and elongated in ER via Δ6D, Δ5D and ELOVL2/5 resulting into 24:5 ω6 and 24:6 ω3, respectively. After translocation into peroxisomes, 24:5 ω6 and 24:6 ω3 are both β-oxidated into 22:5 ω6 and 22:6 ω3 and translocated back into ER for further usage.

The primary endproduct of *de novo* lipogenesis is 16:0. To generate its byproduct 18:0 Elongases of very long chain FAs (ELOVL) use malonyl-CoA and Nicotinamide adenine dinucleotide phosphate (NADP) to introduce two carbon atoms in its chain (fig. 2.6 (A); Song, Xiaoli, and Yang 2018). While ELOVL1, ELOVL3 and ELOVL6 elongate SFAs and MUFAs, ELOVL2 and ELOVL5 elongate PUFAs (Guillou et al. 2010). At the surface of the endoplasmic reticulum (ER) 16:0 enters the desaturation pathway to synthesize UFAs. Dependent on its enzyme form, FA desaturation introduce a *cis* double bond in a specific position of LCFA (M. T. Nakamura and Nara 2004). Among the desaturation enzymes, stearoyl-CoA desaturase (Δ9D) introduces a *cis* double bond exclusively at the carbon atom 9 and 10 resulting into its products of MUFAs (Enoch, Catala, and Strittmatter 1976). Biochemical studies confirming that the subtrate-binding tunnel has a hydrophobic interior with a sharp kink around carbon atom 9 and 10 of the bound acyl-CoA (H. Wang et al. 2015). Δ9D requires for the aerobic mechanism molecular oxygen, NADP-cytochrome *b5* reductase and the electron acceptor cytochrome *b5*. The conversion of 16:0 ends in its product 16:1 ω7 (fig. 2.6 (A)). It has been reported that Δ9D not only prefers 16:0 but also FAs with chain lengths of 17, 18 and 19 carbon atoms (Enoch, Catala, and Strittmatter 1976). Important desaturation products from *de novo* synthesis and dietary intake are represented by 16:1 ω7 and 18:1 ω9 (fig. 2.6 (A)).

Different to the synthesis of MUFAs, humans do not have the enzymatic capacity to synthesize PUFAs from acetyl-CoA (Tocher, Leaver, and Hodgson 1998). However, they are capable of synthesizing PUFAs from the essential precursors 18:2 ω6 and 18:3 ω3 (fig. 2.6 (B)). Thus, the precursors are dietary essential. Two PUFA desaturases have been identified in humans which are encoded by the fatty acids desaturase (FADS) gene cluster. Within this gene cluster, Δ5 desaturase (Δ5D) is encoded by FADS1 (Cho, M. Nakamura, and Clarke 1999) whereas Δ6 desaturase (Δ6D) is encoded by FADS2 (Cho, M. T. Nakamura, and Clarke 1999). Like Δ9D, those enzymes are ER membrane bound desaturases and require for the aerobic reaction the same equipment (Tocher, Leaver, and Hodgson 1998). Δ5D and Δ6D are both classified as front-end desaturases because they introduce a double bond between the pre-existing double bond and the carboxylic end of the FA (M. T. Nakamura and Nara 2004). Consequently Δ5D introduce a double bond at carbon atom 5 and 6 whereas Δ6D at carbon atom 6 and 7. The conversion of 18:2 ω6 and 18:3 ω3 to highly unsaturated FAs (HUFA) refers to the ω6 and ω3 pathway, respectively. The initial and rate-limiting step in HUFA biosynthesis is the desaturation of 18:2 ω6 and 18:3 ω3 by Δ6D, followed by ELOVL5 carbon chain elongation and Δ5 desaturation by Δ5D to form the first important intermediates 20:4 ω6 and 20:5 ω3, respectively (Sprecher et al. 1995). Those intermediates can further be elongated first by either ELOVL 5 or 2

and second by ELOVL 2 to synthesize 24:4 ω6 and 24:5 ω3, respectively (fig. 2.6 (B)). A last desaturation step is performed by Δ6D to form the intermediates 24:5 ω6 and 24:6 ω3. Subsequent translocation to peroxisomes and removal of two carbon atoms by a single cycle of β-oxidation leads to the endproducts 22:5 ω6 and 22:6 ω6, respectively (Guillou et al. 2010). Afterwards, the endproducts are translocated back to ER for further usage (fig. 2.6 (B)).

Since ω3 and ω6 FAs compete for the same metabolic pathway and produce metabolites with differing effects in health and disease, it may be assumed that the balance of the two classes of PUFAs play an important role in the pathogenesis of inflammatory diseases (Simopoulos 2002). Additionally, humans have limited capacity for conversion of essential FAs to HUFAs (Burdge 2004; Plourde and Cunnane 2007). In vivo studies in humans indicate, that the conversion rate of labelled 18:3 ω3 to 20:5 ω3 is <10 % and only traces of 22:6 ω3 are formed (Hussein et al. 2005; Plourde and Cunnane 2007). Despite the low effect of interconversion from essential FAs, HUFAs are also received from habitual diet.

2.2.2. Habitual diet

Since the german habitual nutrition is mainly impacted from the western diet, the highest amount of fat is received from fatty and processed meat and products thereof (Pfau, Oltersdorf, and Brombach 2008). Also important suppliers of fat are animal and vegetable derived oils and fats (e.g. butter and margarine). Fatty, processed meat and products thereof as well as animal and vegetable derived oils and fats encompasses more than 40% of total fat intake in men (Pfau, Oltersdorf, and Brombach 2008). Interestingly, in women, besides animal and vegetable derived oils and fats, milk and products thereof as well as cheese mainly contributes to total fat intake (approximately 40%). Sweets, baking goods and meals on the basis of meat further contribute to total fat intake. However, these just take on a subordinate role. Interestingly, neither the intake of nuts (e.g. walnuts) nor fish was mentioned for the significant contribution to total fat intake, which is typical for the western diet. Median intake of total fat are lower in women (68 g/day) than men (92 g/day).

2.2.2.1. Habitual intake of nuts

The average intake of nuts (e.g. cashew nuts, peanut butter, peanuts, walnuts) is identically low in both women and men (2 g/day). Interestingly, the 50th percentile is indicated as intake of 0 gram per day, whereas the 95th percentile eats 0.13 gram per day (Pfau, Oltersdorf, and Brombach 2008). Concludingly, only few participants eat nuts on a regularly basis.

2.2.2.2. Habitual diet of vegetable and animal derived oils and fats

On average, men consume more total fat than women (29 g/day vs. 20 g/day) per day. The highest amount of total fat intake per day is derived from animal oils and fats (summarized in mean intake of butter per day, men 16 g/d, women 10 g/day; Pfau, Oltersdorf, and Brombach 2008). However, also the intake of vegetable oils and fats (summarized in mean intake of margarine per day, men 11 g/d, women 7 g/d; Pfau, Oltersdorf, and Brombach 2008) significantly contributes to total fat intake. The specific intake of vegetable oils such as sunflower, palm, olive and rapeseed oil was not assessed in the fat classification of the "'Nationale Verzehrsstudie II"' (Pfau, Oltersdorf, and Brombach 2008). Therefore, total fat intake from the "'Nationale Verzehrsstudie II"' is only described more in detail for spread-like dietary intake of animal and vegetable derived fats and oils. However, recent assessment on mean palm oil intake per day was based on data from the german national food consumption survey (Pfau, Oltersdorf, and Brombach 2008) and estimated a mean intake of 5.43 g/d palm oil for adults (95th percentile 23.49 g/d; Hearty et al. 2021). Interestingly, the main contributing food categories to palm oil intake were biscuit, cakes, bread, cereal and margarine (Hearty et al. 2021).

2.2.2.3. Habitual diet of milk and products thereof

The average intake of milk and products thereof (such as yogurt, cheese, quark but also meals like cornflakes with milk) is nearly similar in both women and men (227 g/d vs. 248 g/d; Pfau, Oltersdorf, and Brombach 2008). The highest proportion of total intake per day is derived from milk and milk-mixed drinks (98 g/d vs. 131 g/d), followed by milk derived products such as yogurt (88 g/d vs. 75 g/d) as well as cheese and quark (41 g/d vs. 43 g/d). Milk based meals (e.g. cornflakes with milk) only contribute small amounts to total daily intake (17 g/d for both; Pfau, Oltersdorf, and Brombach 2008).

2.2.2.4. Habitual diet of fish

The average intake of fish, products thereof and meals based on fish is nearly identical in both women and men (23 g/d vs. 29 g/d; Pfau, Oltersdorf, and Brombach 2008). Fish and products thereof (especially fatty fish) are suppliers of HUFAs, and especially $\omega 3$ PUFAs. Interestingly, the 5th percentile is indicated as intake of 0 gram per day and the 50th percentile as 15 gram per day, whereas the 95th percentile consumes 103 grams per day fish, products thereof and meals based on fish (Pfau, Oltersdorf, and Brombach 2008). Concludingly, the german population is a low fish consumer society, which is also typical for the western diet.

2.2.3. Biomarker reflecting habitual diet

Besides the estimation of habitual dietary intake (as mentioned in ch. 2.2.2) and especially dietary FAs through assessment tools such as food frequency questionnaire (FFQ), 24-hour dietary recall and food record, it is also possible to analyze objective dietary biomarkers, which occure exclusivly in diet. Dietary measurement errors (e.g. caused through memory bias, sincerity of the study participants but also through unvalidated questionnaires) may cause problems in estimations of diet-disease associations but also in evaluating influence of diet on potential risk factors of known diseases. Since measurement errors are common in nutritional epidemiology studies, which use assessment tools on the basis of the memory of participants, the use of at least validated dietary biomarkers or the combination of objective biomarkers and such assessments tools are highly recommended (Freedman et al. 2011). Of all possible human biospecimens used for estimating dietary FA biomarkers, ADT is seen as that compartment, which reflects the previous two to three years of dietary FA intake (summrized in Hodson, Skeaff, and Fielding 2008). Thus, smaller intra-variability of dietary FA biomarkers of interest can be expected in comparison to estimated blood FA biomarkers. Therefore, over the past years, dietary FA biomarkers were assessed and validated against diverse assessment tools for milk and ruminant fat (ch. 2.2.3.1), vegetable oils and nuts (ch. 2.2.3.2), fish (ch. 2.2.3.3) as well as partially hydrogenated vegetable oils (ch. 2.2.3.4).

2.2.3.1. Biomarker reflecting intake of dairy and ruminant fat

Since milk and dairy products constitute an important part of western diets and are highly consumed in the german population (ch. 2.2.2 and 2.2.2.3), they are an important supplier of dietary fat. Dairy products comprise a complex and heterogeneous group (ch. 2.2.2.3). Therefore valide biomarkers, which describe objectively and comprehensively the heterogeneity of this group, are needed. Naturally, microbial fermentation in the bovine rumen can turn 16:0 and 18:0 through α-xidation into the odd-chain FAs 15:0 and 17:0. The fermentation process is considered unique to the flora of the rumen. Correlation studies between the dietary intake of milk and ruminant fat and 15:0 as well as 17:0 concluded, that these two odd-chain FAs are the most strongly correlated FAs with total dairy and dairy fat intakes (summmarized in Pranger, Joustra, et al. 2019). For 15:0 and 17:0, in general, strongest correlation were found in ADT. 18:1 ω7 *trans* and 16:1 ω7 *trans*, two *trans* FAs, are also synthesized via microbial fermentation. Only recently, 18:1 ω7 *trans* was found to be useful as biomarker of dairy fat intake (Pranger, Corpeleijn, et al. 2019). 16:1 ω7 *trans* was also found useful as a dietary FA biomarker for dairy intake, however

16:1 ω7 *trans* can be found not only in diet but also be synthesized by α-oxidation of 18:1 ω7 *trans* (Pranger, Joustra, et al. 2019). It seems therefore advisable to analyze all *trans* FAs occuring naturally in the flora of the rumen.

2.2.3.2. Biomarker reflecting intake of vegetable oils and nuts

Vegetable oils and nuts comprise a complex and heterogeneous group. Therefore valide biomarkers, which describe objectively and comprehensively the heterogeneity of this group, are needed. In general, the habitual intake of nuts is extremely low in the german population (ch. 2.2.2.2). It is therefore assumed, in the context of this manuscript, that the main source of dietary intake of nuts in the german population is walnuts. The main FAs in nuts are 18:1 ω9, 18:2 ω6 and 18:3 ω3. Walnuts are particularly characterized by high levels of 18:3 ω3. 18:3 ω3 was therefore found as a reasonable FA biomarker for the dietary intake of walnuts (summarized in Garcia-Aloy et al. 2019). Nuts are also a high source of 18:2 ω6 and were consistantly found in correlation studies (summarized in Garcia-Aloy et al. 2019), however other food sources contain also high amounts of 18:2 ω6 such as vegetable oils. Vegetable oils also contain considerable amounts of 18:3 ω3 which clearly means that neither 18:2 ω6 nor 18:3 ω3 can solely indicate the intake of nuts (summarized in Garcia-Aloy et al. 2019). Vegetable oils such as flaxseed oil, rapeseed oil and sunflower oil are characterized through 18:2 ω6 and 18:3 ω3 and are therefore measured as FA biomarkers in different compartments (summarized in Garcia-Aloy et al. 2019). Since both, vegetable oils and nuts, are characterized by high amounts of 18:2 ω6 and 18:3 ω3, is is practcable to use those FAs as biomarkers for both, vegetable oils and nuts intake.

2.2.3.3. Biomarker reflecting intake of fish

It is well-known that long-chain ω3 FAs serve as dietary biomarker to evaluate fish consumption. Fish fat is the main source of 20:5 ω3 and 22:6 ω3, however 20:5 ω3 and 22:6 ω3 contribute only small amounts to total ω3 FAs in human biospecimens (summarized in Baylin and Campos 2006). Since consumption of fish is extremely low in the german population (ch. 2.2.2.4), it is assumed, that 20:5 ω3 and 22:6 ω3 will only contribute to small amounts to total ω3 FAs in human biospecimens in the german population. In ADT, 20:5 ω3 and 22:6 ω3 correlated with dietary fish intake (Baylin, Kabagambe, et al. 2002).

2.2.3.4. Biomarker reflecting intake of partially hydrogenated vegetable oils

Industrial *trans* FAs in foods, in difference to naturally occuring *trans* FAs (ch. 2.2.3.1), originate mainly from the use of partially hydrogenated vegetable oils. During industrial hydrogenation, which is used to produce semi-liquid and solid fat for production of margarine etc., a complex mixture of *trans* FAs with its most dominant *trans* FA 18:1 ω9 *trans* arise (Efsa Panel on Dietetic Products 2010). In correlation studies, intakes of highly processed foods were strongly correlated with levels of plasma 18:1 ω9 *trans* in men and women (Chajès et al. 2011) suggesting, that 18:1 ω9 *trans* seems to be a reasonable FA biomarker for the intake of partially hydrogenated vegetable oils.

2.3. Analysis of FAs

Typically, FAs are analyzed as their respective fatty acid methyl ester (FAME) in GC analysis. The most common derivatisiation method is the use of trimethylsulfonium hydroxide (fig. 2.7)
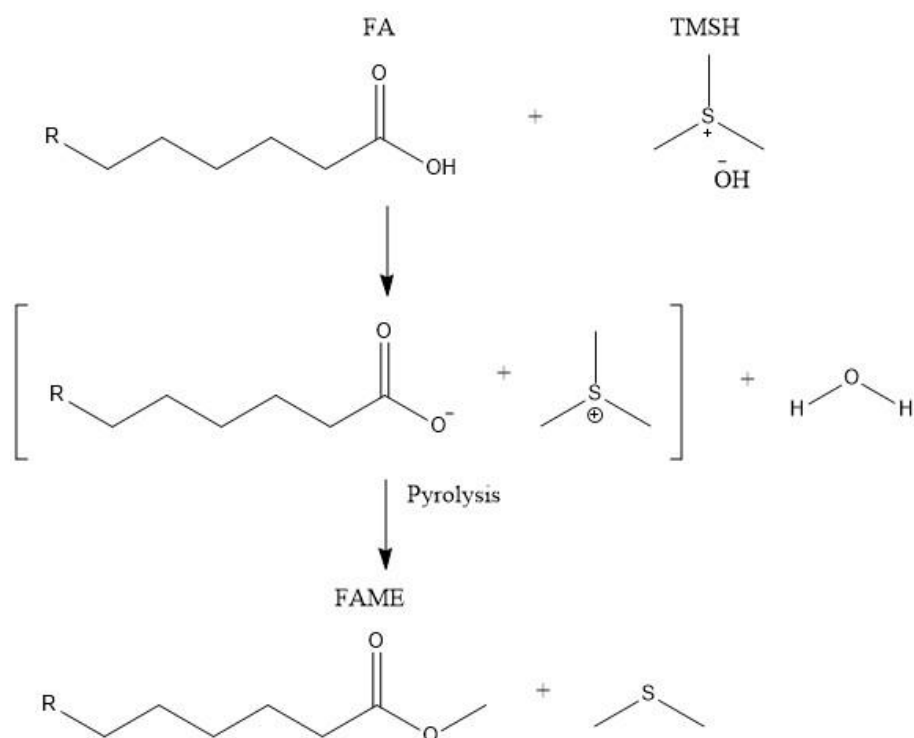
Figure 2.7.: Conversion of FAs into their respective FAMEs by base-catalyzed transesterification and followed pyrolysis with trimethylsulfonium hydroxide (modified acc. to Gries et al. 2021).

The transesterification improves sample volatility and therefore improvement in GC analysis due to their raised non-polarity. Furthermore derivatisation with trimethylsulfonium hydroxide does not need a secondary extraction step and has short incubation times (Gries et al. 2021).

2.3.1. Separation on gas chromatography columns

FAMEs are polarizable compounds consist of carbon and hydrogen atoms with an ester group and facultative *cis* and *trans* configured double bonds. Therefore only polar columns are suitable for separation of FAMEs in biospecimens. For analysis of FAMEs in biological matrices stationary phases typically contain high percentages of cyanopropyl functional groups. Due to the structural properties of FAMEs strong dispersive (van der Waals), very strong dipole-dipole, very strong dipole-induced dipole, and moderate basic interactions are expected (Merck KGaA 2019b; Merck KGaA 2019a). Using a capillary column coated with high percentages of cyanopropyl groups, the separation of FAMEs follows strict stereochemical patterns. FAMEs are separated according to their carbon chain length, their number of double bonds as well as the position of the double bonds and the geometric configuration. The longer the carbon chain the higher its interaction with the cyanopropyl groups and its resulting retention time (t_R) are. Due to lower interaction of *trans* isomers with the cyanopropyl-dipole, *trans* isomers elute before their corresponding *cis* isomers resulting in lower t_R. As the number of double bonds increases, the t_R increases because of stronger dipole-dipole interactions. Furthermore, the position of double bonds in the carbon chain has structural consequences and therefore differing impact on the t_R. The nearer the double bond to the ω end the lower the structural consequences in its chain and the higher its interaction with the cyanopropyl groups are. Consequently, t_R increases with decreasing distance between double bonds and the ω end of the carbon chain (Agilent Technologies, Inc. 2019). FAMEs are typically detected by flame ionization detection (FID), mass spectrometry (MS) or in combination of both.

2.3.2. Flame ionization detector

Up to now, the most frequently used method for FA analysis is gas chromatography (GC) coupled with FID. The detector response results from the combusting of FAME molecules in a hydrogen-air diffusion flame (Poole 2015). As an ionization-based mass-selective detector, production of ions are required. The formation of detectable ions results from

following chemical ionization as shown in equation (eq.) 2.1.

$$CH^\bullet + O^\bullet \rightarrow CHO^+ + e^-$$ (2.1)

The CHO^+ ion then reacts further to produce H_3O^+ ions and other hydrocarbon ions (eq. 2.2).

$$CHO^+ + H_2O \rightarrow H_3O^+ + CO$$ (2.2)

The ionization is excepted to be a first order reaction and therefore explaining the linear response of the FID (Poole 2015). Theoretically, the produced hydrocarbons are unstable and undergo a cascade of reactions producing its logical end point Methane (T. 1999). Consequently, each carbon atom in the FA molecule is capable of producing the same signal (Poole 2015). The presence of the ester group in the FAME molecule reduces the effective carbon atoms by 1.5 due to the prevention of the carbon atom from the $C = O$ and $C - O$ structure entering into the radical pathway (T. 1999). Thus, the overall FID response of a specific FA is proportional to the sum of above mentioned effective carbon atoms (Poole 2015). Consequently, an increase in carbon atoms leads to higher responses in FID and must therefore be considered in FA analysis. The lack of selectivity limits the usefulness of FID when applied to complex matrices, since only detector response and t_R are measured. Therefore misidentification of FAMEs in the presence of contaminants, artefacts and coeluting compounds are possible (Dodds et al. 2005).

2.3.3. Single quadrupole mass spectrometry

GC-FID does not allow structural profiling of FAs in biological matrices. Thus GC coupled to MS is a powerful alternative combining the separation power of GC with structural profiling obtained by MS detection. Typically, GC-MS with electron-impact ionization (EI) is used for FA analysis (Christie and Han 2010). In EI energetic electrons are accelerated by an electric field through the high vacuum chamber containing the gaseous sample. Consequently, such electrons will transfer their energy to the neutral analyte, obtaining molecular ion (MI) through the reaction (Packer et al. 2010):

$$M^{+\bullet}e^- \rightarrow M^+ + 2e^-$$ (2.3)

EI is generally considered as a severe ionisation process resulting in further fragmentation of the obtained MI. The fragmentation pathway of FAs follows specific rules for SFAs and UFAs (fig. 2.8).
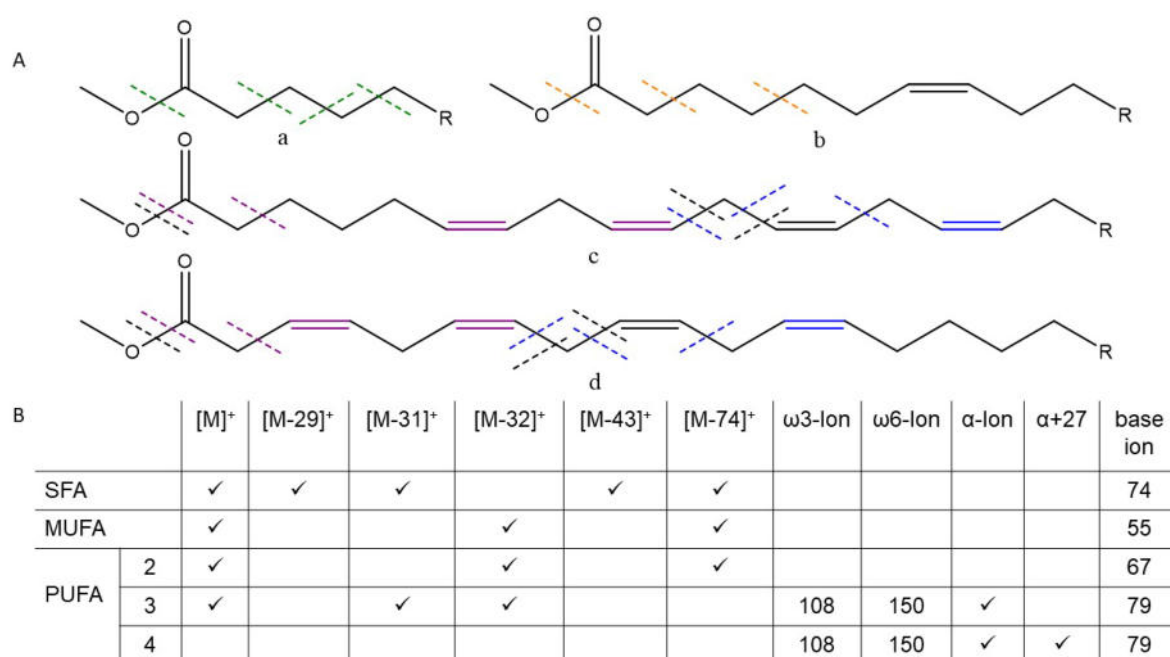
Figure 2.8.: Typical fragmentation pattern for FAMEs. (A) Fragmentation pattern for the identification of FAMEs with MS. a indicates SFA, b MUFA, c ω3 PUFA and d ω6 PUFA. *omega*3/6 PUFA only when R=$-CH3$. Green cleavage = fragmentation pattern for SFA; Orange cleavage = fragmentation pattern for MUFA; Purple cleavage = fragmentation pattern for PUFA with two double bonds; Black cleavage = fragmentation pattern for PUFA with three double bonds; Blue cleavage = fragmentation pattern for PUFA with four or more double bonds. (B) Selected fragmentation patterns for the identification of FAME. ✓indicated the ions, which were used for identification of specific FAMEs. PUFA 2, 3 and 4 indicated PUFAs with two (2), three (3) or four and more (4) double bonds.

Typically, MIs of SFA methyl esters are abundant $[M]^+$, as is an ion with $[M-31]^+$ confirming the existence of a methyl ester (Christie and Han 2010). The fragmentation ions (FI) $[M-43]^+$ (loss of carbon atom 2 to 4) and $[M-29]^+$ (loss of carbon atom 3 to 4) are also diagnostic for SFAs. As indicated by its chemical structure, SFA fragmentation leads to $m/z = 74$ via McLafferty rearrangement (Christie and Han 2010). Typically, $m/z = 74$ represents the most intense ion in the mass spectra of SFAs (fig. 2.8). Starting from $m/z = 74$, the homologous series of FIs ($m/z = 87$, 101, 115 etc.) are evidential for carbon chains of general formula $[CH_3OCO(CH_2)_n]^+$ without interruptions of other functional groups or alkyl branches. These are formed via cleavage of neutral aliphatic radicals from the ω part of the molecule. Similar to the SFA fragmentation pattern, the MIs $[M]^+$ of

MUFAs are clearly seen (fig. 2.8). A loss of $m/z = 32$ indicates the elimination of methanol ($[M - 32]^+$), whereas $[M - 74]^+$ indicates the FI after McLafferty rearrangement (fig. 2.8). Loss of a fragment containing the carboxyl-group by cleavage between carbon atom 5 and 6 with addition of a rearranged hydrogen atom forms $[M - 116]^+$ (Christie and Han 2010). Thus, contrarily to the fragmentation pattern of SFAs, FIs with the general formula of $[C_nH_{2n-1}]^+$ dominate the spectra with $m/z = 55$ as the most intense FI (fig. 2.8). The mass spectra of MUFAs do not allow any statement concerning the location of its double bond. Similarly to MUFAs, PUFAs with two double bonds have the same fragmentation pattern. However, FIs with the general formula $[C_nH_{2n-3}]^+$ dominate the spectra with $m/z = 67$ as its base ion (fig. 2.8; Christie and Han 2010). Similarly to UFAs with one or two double bonds, the MI and the loss of $[M - 31/32]^+$ are indicative for trienoic PUFAs. FIs with the general formula $[C_nH_{2n-5}]^+$ dominate the spectra with $m/z = 79$ as its base ion. Opposing to PUFAs with one or two double bonds, the location of the $\omega 3$ and $\omega 6$ defining terminal double bonds indicate specific fragmentation pattern for identification purposes. For $\omega 3$ FAs an ion at $m/z = 108$ is indicative for an $\omega 3$ terminal double bond with methylene interrupted unsaturation, whereas $m/z = 150$ is indicative for an $\omega 6$ end (fig. 2.8; Christie and Han 2010). Aside the ω FIs, α FIs formed by a similar cleavage at the ω end, resulting into a fragment containing the first two double bonds and the second methylene group. Depending on the location of its first double bonds, the m/z of α FIs vary across the molecule (tab. 2.1).

Table 2.1.: α ions from the respective fragmentation pattern of different PUFAs depending on the location of the double bonds.

first double bonds	$\Delta 4, 7$	$\Delta 5, 8$	$\Delta 6, 9$	$\Delta 7, 10$	$\Delta 8, 11$	$\Delta 9,12$	$\Delta 10, 13$	$\Delta 11, 14$
FI m/z	166	180	194	208	222	236	250	264

Additionally, in the fragmentation pattern of PUFAs with four and more double bonds, a FI with $m/z = \alpha + 27$ occurs (2.8; Christie and Han 2010). Furthermore a thermodynamically favoured rearrangement occurs with increasing number of double bonds to produce a stable tropylium ion with $m/z = 91$. Similarly to trienoic PUFAs, higher PUFAs generate FIs with the general formula $[C_nH_{2n-5}]^+$ and $m/z = 79$ as its base ion (fig. 2.8).

2.4. Desaturase and elongase indexes

The use of FA product-to-precursor biomarker ratios as a proxy of Δ9D, Δ6D and Δ5D activity is supported by recent studies. Δ9DI calculated from ADT FA composition (ratio of 16:1 ω7 to 16:0) significantly positively correlated with its ADT mRNA expression. Contrarily, the correlations for ADT Δ6DI (ratio of 18:3 ω6 to 18:2 ω6) and Δ5DI (ratio of 20:4 ω6 to 20:3 ω6) with their corresponding ADT mRNA expression were not significant (Sjogren et al. 2008). However, these indexes are well-established indirect measures of Δ6D and Δ5D in different human biospecimens in epidemiological studies (Weir et al. 2020, Jacobs et al. 2015, Schiller et al. 2014) and ADT Δ6DI and Δ5DI were associated with variants in or near desaturase encoding gene loci (Marklund et al. 2018). Indexes of elongases have been much less extensively studied than desaturase indexes. No established ELOVLI5 biomarker exists, yet and calculation of respective elongase index differs between studies (Cormier et al. 2014, Djousse et al. 2012). Since the main dietary ω6 FAs in the diet of western population includes only 18:2 ω6 and to a lesser extent 20:4 ω6, it may be assumed that only traces of 20:3 ω6 and 18:3 ω6 in ADT FAs have exogenous origin. Therefore the use of the ratio 20:3 ω6 to 18:3 ω6 for calculating the ELOVL5 activity is mostly used in analyses. Individual studies observed consistent positive associations of Δ9D and Δ6D and negative associations of Δ5D and ELOVL5 biomarkers with T2D in various biological specimens (Takkunen et al. 2016, Lankinen et al. 2015, Jacobs et al. 2015, Jo, An, and Y. Park 2013, Chow et al. 2013, Kroger et al. 2011, Harris et al. 2016, Yary et al. 2016, Mahendran et al. 2014; fig. 2.9).

Diet has been shown to affect biomarkers of Δ9D, Δ6D, Δ5D and ELOVL5, respectively, indicating putative beneficial and adverse effects of diet in T2D. Besides diet, there are other known influencing factors on desaturase and elongase biomarkers (ch. 2.4.1), respectively, which may indirectly affect the risk of T2D through influencing of Δ9D, Δ6D, Δ5D and ELOVLI5, respectively.

2.4.1. Factors influencing desaturase and elongase indexes

Only a few epidemiological studies systematically investigated the association of anthropometric, demographic, dietary and lifestyle factors so far. Some factors were identified influencing Δ9DI, Δ6DI and Δ5DI, such as BMI, smoking habits and alcohol consumption, in compartments different to ADT, whereas either unclear, no or conflicting associations were observed for diet and dietary FA biomarkers. Limited or no human data are available for age, parity, menopausal status, endogenous and exogenous estrogens as well as oxidative stress. Possible mechanism for every influencing factor will be depicted below.
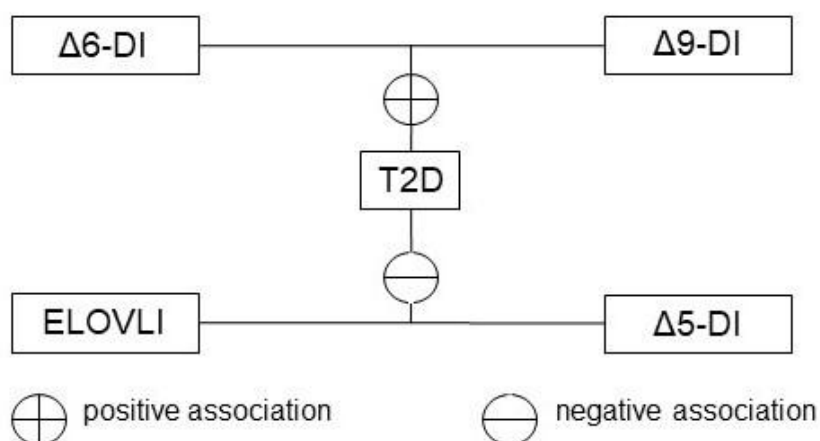
Figure 2.9.: In the literature described positive and negative association of Δ9D, Δ6D, Δ5D and ELOVL5 biomarkers with T2D risk. Δ9DI biomarker for the estimation of Δ9D activity; Δ6DI biomarker for the estimation of Δ6D activity; Δ5DI biomarker for the estimation of Δ5D activity; ELOVLI biomarker for the estimation of ELOVL5 activity; T2D type 2 diabetes.

Furthermore, no study ever investigated the influence of various factors on ELOVLI5, yet.

<u>BMI</u>

BMI as a marker of general obesity positively influenced Δ9DI and Δ6DI (Schiller et al. 2014; Del Pozo et al. 2020; Daneshmand et al. 2017; Warensjo, Ohrvall, and Vessby 2006; Vinknes, Elshorbagy, Nurk, et al. 2013; Alsharari, Riserus, et al. 2017) whereas Δ5DI was negatively influenced by BMI (Daneshmand et al. 2017; Warensjo, Ohrvall, and Vessby 2006; Alsharari, Riserus, et al. 2017; Del Pozo et al. 2020; Schiller et al. 2014) in compartments different to ADT.

<u>Smoking habits</u>

Elevated plasma cotinine levels, as a biomarker of smoking habits, were found inversely associated with serum Δ5DI and Δ6DI in patients with suspected coronary heart disease (Skeie et al. 2015). Furthermore, smoking habits have been found positively influencing Δ9DI and Δ6DI in human biospecimens different to ADT (Schiller et al. 2014, Saadatian-Elahi et al. 2009, Warensjo, Ohrvall, and Vessby 2006, Kishino et al. 2008). Recent studies found unclear influence of smoking on Δ5DI (Schiller et al. 2014, Kishino et al. 2008). Thus smoking may increase T2D risk through interaction with Δ9DI, Δ6DI and Δ5DI. It may be hypothesized that the oxidative condition caused by smoking depletes UFAs through

lipid peroxidation (Pryor 1997) and therefore could possibly stimulate the desaturation-elongation-pattern. However, the interaction is not fully understood.

Alcohol consumption
The relationship between T2D and alcohol consumption seems ambivalent since low and moderate alcohol consumption is associated with lower risk and heavy alcohol consumption with higher risk of T2D in the sense of a U-shaped relationship compared to non consumers (Baliunas et al. 2009, X. H. Li et al. 2016). Alcohol consumption has been found positively influencing Δ9DI and Δ6DI in human specimens different to ADT (Schiller et al. 2014, Saadatian-Elahi et al. 2009, Alsharari, Leander, et al. 2019, Vinknes, Elshorbagy, Nurk, et al. 2013, Laguzzi et al. 2018, Daneshmand et al. 2017). Positive or no associations were observed for alcohol consumption and Δ5DI, however the positive association were found non-linear (Laguzzi et al. 2018, Schiller et al. 2014). It is postulated, that the metabolites of alcohol induces the production of reactive oxygen species (ROS), which may induce catabolism of PUFAs. This, in turn, may stimulate the desaturation-elongation pathway (summarized in Pawlosky and Salem 2004). However the interaction is not fully understood.

Dietary FA biomarkers and dietary fat intake
Diverse sources of dietary fat and types of FAs has been thought to be possible implicated in the development of T2D (Wolfram et al. 2015, WHO 2000). Only recently, a meta-analyses of prospective cohort studies investigated the associations of FA biomarkers and risk of T2D (Chen et al. 2020). Only higher levels of 18:2 ω6 (but no other FA biomarker) were associated with a lower risk of T2D (relative risk = 0.03; 95%CI 0.53 - 0.77; Chen et al. 2020). Furthermore, the intake of vegetable fat (summary relative risk = 0.81; 95% CI 0.76 - 0.88) was associated with a decrease in T2D incidence (Neuenschwander et al. 2020). The intake of animal-based long chain ω3 PUFA were associated with an increase in T2D incidence (summary relative risk = 1.10; 95% CI 1.06 - 1.15). However, these observations were mostly nonlinear and the findings were biased by measurement errors via self-reporting FFQs (Neuenschwander et al. 2020). In a recent Mendelian randomisation study (Yuan and Larsson 2020), 18:2 ω6 and 18:3 ω3 were found causal-negative associated with the incidence of T2D (OR 0.96 95% CI 0.94 - 0.98 and OR 0.90 95% CI 0.90 - 0.96, respectively). 20:5 ω3 and 22:6 ω3 were found causal-positive associated (OR 1.08 95% CI 1.03 - 1.12 and OR 1.04 95% CI 1.02 - 1-07, respectively) with the incidence of T2D (Yuan and Larsson 2020). Both, dietary FA intake assessed by FFQ and dietary FA biomarker from total PUFA, ω6 PUFA, ω3 PUFA as well as individual FAs negatively influenced Δ9DI in human biospecimens different to ADT (Vinknes, Elshorbagy, Drevon, et al. 2013, Vinknes, Elshorbagy, Nurk,

et al. 2013, Perez-Heras et al. 2018, Manni et al. 2017). Only one study did not find an influencing impact of dietary FAs on Δ9DI (Schiller et al. 2014). However, with respect to measurement errors (ch. 2.2.3), dietary FAs were assessed by FFQ. Also no influence of dietary FAs was observed for Δ6DI and Δ5DI in that study. In a dietary intervention study Δ5DI and Δ6DI in serum from healthy men and women were significantly higher and lower in a rapeseed-oil diet, rich in 18:1 ω9, 18:2 ω6 and 18:3 ω3, compared to a high saturated fat diet (Warensjo, Riserus, et al. 2008). Also significantly higher Δ5DI and lower Δ6DI were observed in serum after a three month dietary intervention in healthy subjects with supplementation of 20:5 ω3 and 22:6 ω3 (Vessby, Gustafsson, Tengblad, and Berglund 2013). However in another randomized controlled nutrition study with 17 postmenopausal women, a low fat diet with supplementation of 20:5 ω3 and 22:6 ω3 did not alter either plasma Δ6DI or Δ5DI (Raatz et al. 2012). Studies in experimental animals found significantly decreased Δ9D, Δ6D, Δ5D and ELOVL5 mRNA expressions after supplementation of fish oil and diets high in 18:2 ω6 and 18:3 ω3 (Y. Wang et al. 2005, Kim, Choi, and Y. Park 2019, Igarashi et al. 2007). It is hypothesized that PUFAs are the main dietary components that downregulates in the sense of a feedback regulation DIs through interaction with two transcription factors, sterol regulatory element binding protein-1c and peroxisome proliferator-activated receptor α, based on the availability of subtrates and products (summarized in M. T. Nakamura and Nara 2004 and Vessby, Gustafsson, Tengblad, Boberg, et al. 2002).

Endogenous and exogenous estrogens

In a recent population-based cohort study and meta-analysis with 3117 postmenopausal (postMP) women 17β-Estradiol (E2) was associated with increased risk of T2D. Their findings were strengthened by a systematic meta-analysis corroborating that E2 is associated with T2D risk in postMP women (Muka et al. 2017). However, the causal context is not understood yet and contrarily large randomized controlled trials have consistently found lower T2D incidence in postMP women with estrogen treatment (Bonds et al. 2006, Kanaya et al. 2003, Margolis et al. 2004). Estrogens may affect lipid metabolism through downregulation of lipogenic enzymes (summarized in Gao and Dahlman-Wright 2013). Studies in experimental animals suggest negative influence of estrogens on desaturases and elongases (Al-Qahtani et al. 2017, Paquette et al. 2008, Bryzgalova et al. 2008, Gao, Bryzgalova, et al. 2006, Alessandri et al. 2011), however inconsistent results were obtained in human biospecimens. In postMP women receiving hormone replacement therapy (HRT), ADT Δ9D mRNA expression decreased after three months treatment compared to baseline (Lundholm et al. 2008). Contrarily, HRT in two healthy postMP populations significantly

increased levels of 20:4 $\omega6$ and 22:6 $\omega3$ suggesting an increased activity of elongases and desaturases (Giltay et al. 2004, Sumino et al. 2003). However, both studies did not adjust for dietary consumption and therefore confounding effects can not be excluded.

Age and menopausal status

There is an ongoing debate, whether menopause, and its subsequently decreasing estrogen concentration or aging or both are responsible for higher risk of T2D development (summarized in Mauvais-Jarvis et al. 2017 and Paschou and Papanas 2019). Lower premenopausal (preMP) E2 levels at the onset of menopausal transition were positively associated with an increased risk of T2D (S. K. Park et al. 2017). Furthermore, early beginning of menopausal transition was also associated with an increased risk of T2D development (summarized in Anagnostis et al. 2019). Inconsistent results were obtained for the influence of age and menopause on DIs. Positive correlations were found between age and erythrocytes Δ9DI and Δ6DI in postMP women (Fuhrman et al. 2006). In the same study population Δ5DI and Δ6DI were lower and higher in postMP women, respectively. However, only small and weak positive influence of age on erythrocytes Δ9DI and Δ6DI were observed (Schiller et al. 2014). Furthermore, no difference was observed for serum Δ5DI and Δ6DI in preMP and postMP women (Liu et al. 2000). ADT Δ6DI significantly decreased in healthy women by age independent of diet, nevertheless significance was lost after adjusting for menopause (Bolton-Smith, Woodward, and Tavendale 1997).

Parity and reproductive history

Higher parity has been found significantly associated with an increased risk of T2D (Guo et al. 2017, P. Li et al. 2016), however causality was not explored, yet. In a prospective cohort study with 136.652 postMP women a greater number of parity was associated with increased risk of T2D development, but these results were attenuated and became non-significant when adjusting for weight gain, suggesting that weight gain, not parity itself, is responsible for T2D development (Luo et al. 2019). However, during pregnancy, the supply of PUFA, especially 22:6 $\omega3$, is crucial for the development of the fetus, therefore maternal plasma 22:6 $\omega3$ increases (summarized in Demmelmair and Koletzko 2015). Consequently, human erythrocytes Δ9DI and Δ6DI increase until delivery and decrease postpartum (Kuipers et al. 2011). The increasing 22:6 $\omega3$ biosyntheses was confirmed in pregnant sprague-dawley rats based on increased Δ6D protein levels during pregnancy (Chalil et al. 2018). However, up to date, it is unknown, if Δ9DI and Δ6DI postpartum are altered in comparison or return back to their pregestational stage.

<u>Oxidative stress</u>

Accumulating evidence suggest, that oxidative stress resulting from hypergycaemic states could be involved in the development of T2D. Hyperglycemia increases levels of oxidative stress induced DNA-damage biomarkers, lipid-peroxidation and protein oxidation products (summarized in Oguntibeju 2019). Male Wistar rats showed decreased activities by an iron rich diet compared to control (Valenzuela et al. 2018). However, no association was observed for γ-glutamyltransferase, another biomarker for oxidative stress, in human serum of health subjects (Wu, Baylin, and Colacino 2018) suggesting putative different associations between oxidative stress biomarkers and desaturases/elongases. Further possible oxidative stress biomarkers are oxysterol metabolites, which results from autoxidational processes under the influence of ROS. Thereby arise predominantly the metabolites 7-β-OH-Cholesterol (7β-OHC), 5,6α-epoxy-Cholesterol (5,6α-epoxyC) and 5,6β-epoxy-Cholesterol (5,6β-epoxyC), respectively (summarized in Niki et al. 2005). However, no study ever investigated the influence of oxysterol metabolites in desaturase and elongase indexes.

2.5. Background information application consumer health

The chair of food chemistry, institute for pharmacy and food chemistry, Julius-Maximilians university of wuerzburg together with Scannel GmbH (sponsored by Bayern Innovativ GmbH) wants to develop a smartphone application with the aim of consumer protection. In the case of this application, a consumer shall be defined as a person, who has adverse reactions to food or special food ingredients. Therefore this application aims to monitor, based on individual profile settings, food and food ingredients on a risk-based approach and alerts users, based on their profile settings, about potential risks outgoing from the respective food or food ingredient. The function of the application shall be based on the global trade item number (GTIN). The user scans the GTIN of the respective food item and information will be provided for the scanned food item (such as list of ingredients). In case of a positive hit (due to the respective profile setting of the user) a pop-up window will occure with information the the respective food ingredient on the basis of the profile settings. The aim of this pop-up window shall only be focused to provide information about potential risks outgoing from the scanned food because of accordance between food ingredient(s) and the respective profile setting of the user was found. This application shall not replace consultation of medicinal staff and it shall not make diagnosis. It only provides information of potential risks outgoing from food stuff and their respective food ingredients due to the profile settings of the respective users.

3. Objectives

Dietary fatty acid biomarkers play an important role in estimation of diet-disease associations but also in evaluating influence of diet on potential risk factors of known diseases. An association between dietary fatty acid biomarkers and desaturase as well as elongase indexes (putative beneficial and risk factors for type 2 diabetes risk) is currently the subject of controversy. Because of that, the first aim of this manuscript will be the identification of potential influence of dietary fatty acid biomarkers on desaturase as well as elongase indexes in breast adipose tissue from healthy women. Influence of further variables on desaturase as well as elongase indexes in breast adipose tissue will also be investigated. For that, breast adipose tissue from healthy women were obtained from adult women undergoing reduction mammoplasty. Further information, which could have an influence on desaturase and elongase indexes, were collected. At the chair of food chemistry potential explanatory variables, which could have an putative influence on desaturase and elongase indexes, were investigated. Potential explanatory variables encompassed, besides dietary fatty acid biomarkers, endogeneous estrogens, oxysterols and proportion of fat in breast adipose tissue. To identify and evaluate the associations between potential explanatory variables and respective indexes, multiple linear regression models using stepwise forward selection will be applied.

The dietary fatty acid biomarkers from the study population of adult women undergoing reduction mammoplasty were investigated by a former PhD student using gas chromatography coupled with flame-ionization detection. The lack of selectivity limits the usefulness of the flame-ionization detection, when applied to complex matrices, since only detector response and retention times of the respective analytes are measured. Therefore, the second aim of this manuscript will be the implementation of a novel gas chromatographical method which is simultaneously coupled with a mass spectrometrical and flame-ionization detector.

Adipose tissue is assumed to be the best choice for studying of long term fatty acid intake through habitual diet mainly because of its slow turnover. However, due to inter-

individual differences of the human female breast, potential difficulties to obtain breast glandular tissue lead to non-existence of fatty acid biomarker studies in that biospecimen so far. Consequently potential influence of mixed breast tissue (either adipose tissue contaminated with glandular tissue and vice versa) on fatty acid biomarkers is unknown. Therefore information on the differentiation between breast glandular and adipose tissue in the context of the analysis of fatty acids serving as biomarkers for the intake of specific food groups is lacking. Thus, the third aim of this manuscript will be the determination of the relative fatty acid profile in breast glandular tissue obtained from the same adult women undergoing reduction mammoplasty for the first time using the implemented gas chromatographical method. Furthermore, the relative concentrations of fatty acids serving as biomarkers for food intake will be compared with adipose tissue breast samples from the same women.

In the context of this manuscript, the implemented gas chromatographical method which is coupled with a mass spectrometrical and flame-ionization detector will further be applied on another study population from healthy participants undergoing surgery for cosmetic reasons. Thus, the fourth aim of this manuscript will be the determination of the fatty acid composition with special focus on the fatty acid biomarkers of this study population, followed by statistical evaluation to provide objective markers of diet and dietary intake of fatty acids for this study population.

Nutritional factors play also a role in food-associated diseases, where specific food ingredients may trigger adverse reactions. In the context of this manuscript, scientific information on adverse reactions to food ingredients shall be provided for possible implementation into an application for consumer health. Thus, the last aim of this manuscript will be the elaboration of adverse reactions to food ingredients with special focus on food allergies and food intolerances. Information provided will be sound-scientific and discussed in the context of practicability.

4. Materials and methods

4.1. Equipment, chemicals and solutions

4.1.1. Chromatography and laboratory equipment

Analytical balance	Mettler AM 100 (Mettler Toledo GmbH, Gießen, Germany)
	Mettler AT21 Comparator (Mettler Toledo GmbH, Gießen, Germany)
Bio-freezers	HDE40086FV, serial number 1117710801191114 (Thermo Fisher Scientific, Asheville, United States)
	991705032, serial number 3261619 (Dometic Germany GmbH, Germany)
Freezer	AEG öko-ARCTIS electronic (Elektrolux Hausgeräte GmbH Markenvertrieb AEG, Nürnberg, Germany)
Centrifuge	Universal 16 EBA 12 (Hettich lab technology, Tuttlingen, Germany)
Evaporator	Rotational-Vacuum-Concentrator RVC 2-25 CD with cold trap CT 04-50 SR (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and coupled membrane-vacuum pumps MZ 2C (Vacuubrand GmbH + Co. KG, Wertheim, Germany)
Pipettes	Microman®
	10-100 µL (M100; Part. No. F148504, Gilson, Middleton, Wisconsin, USA)
	50-250 µL (M250; Part. No. F148504, Gilson, Middleton, Wisconsin, USA)
	100-1000 µL (M1000; Part. No. F148506, Gilson, Middleton, Wisconsin, USA)

	Multipette® plus (Eppendorf SE, Hamburg, Germany)
GC-MS/FID	Thermo Scientific™ TRACE™ 1300 Gaschromatograph
	Thermo Scientific™ ISQ™ series single quadrupol GC-MS system
	Thermo Scientific™ AI/AS 1310 Autosampler ISQ Series
	(Thermo Fisher Scientific, Waltham, Massachusetts, USA)
Column	Supelco SP-2560 fused silica, 100 m x 0.25 mm; film thickness 0.20 μm, column material poly-biscyanopropylsiloxane (Sigma Aldrich, Munich, Germany)
Vortex	Vortex-Genie® 2 (Model: G560E, Part. No. 2-146860, Scientific industries, New York, USA)
Beaker	various sizes (VWR international GmbH, Darmstadt, Germany)
Nitrogen tanks	Serial Number: NPB2019430234X, CHART INC. 1300 Airport Drive, Ball Ground, GA 30107 USA

4.1.2. Laboratory consumables

Cryos	1.8 mL, with screw cap (Part. No.: 72.379, Sarstedt, Nürnbrecht, Germany)
Pipette tips	1000 μL (Part. No. CP1000 F148114 Gilson, Middleton, Wisconsin, USA)
	250 μL (Part. No. CP250 F148114, Gilson, Middleton, Wisconsin, USA)
	100 μL (Part No. CP100 F148414, Gilson, Middleton, Wisconsin, USA)
	Combitips® advanced (2.5, 5.0, 10.0, 50.0 mL incl. adapter (Eppendorf SE, Hamburg, Germany)
Glass culture tubes	GL 18, 100 x 16 mm with screw cap (Part. No. RG09, A. Hartenstein, Laborbedarf GmbH, Würzburg, Germany)

Vials	1.5 mL brown bottle (Part. No. 451101220, CZT Klaus Trott Chromatographie, Kriftel, Germany)
	1.5 mL brown bottle (Part. No. 451101012_E, CZT Klaus Trott Chromatographie, Kriftel, Germany)
	Screw PP with seals, 8-425, NK/TEF, red, 1.0 mm (Part. No. 3011015S1, Klaus Trott Chromatographie, Kriftel, Gemany)
	Screw PP with seals, 8-425, Sil/PTFE blue-white, 1.5 mm (Part. No. 3011S3015 Klaus Trott Chromatographie, Kriftel, Germany)
	Screw PP with seals, 9-425, Sil/PTFE white-red, 0.9 mm (Part. No. 3111C3010, Klaus Trott Chromatographie, Kriftel, Germany)
	micro-inserts, conical base, 5 x 30 mm (Part. No. 501105031, Klaus Trott Chromatographie, Kriftel, Germany)
	feathers (Part. No. 501167011, Klaus Trott Chromatographie, Kriftel, Germany
Beakers	diverse volumes (VWR International, Darmstadt, Germany)
Septa	Septa BTO coated 11mm (Thermo Scientific US)
Liner	LinerGOLD, GC Liners, Straight Liner with Wool) 4.0 mm x 6.3 mm x 78.5 mm (Thermo Fisher, US)
Syringe	10 µl SYR FN 60 mm T Ga 25, Cons Tip, Batch Q05-L1005 (Thermo Scientific, US)
Spatula	double spatula 18/8, stainless steel, 2 mm thickness (Neolab Laborbedarf Vertriebs GmbH, Heidelberg, Germany)
Volumetric flash	diverse volumes (Witeg Labortechnik GmbH, Wertheim, Germany and Duran Group, Wertheim, Germany)
Pasteur pipettes	glass, 2 ml, 230 mm (VWR International, Darmstadt, Germany)
Swingsettes	Biopsy processing/embedding cassettes with lid. white (2588 Bernard-Pilon, Beloeil QC J3G 4S5, Canada)
measuring cylinder	diverse volumes (VWR International, Darmstadt, Germany)

4.1.3. Chemicals

CHCl₃	contains EtOH as stabiliser, >99.0-99.4% (Part. No.: 24216, Riedel-de Haën, Seelze, Germany)
EtOH	99.9% spoiled with PE (Part. No.: 1877, University Wuerzburg, Germany)
distilled *H₂O*	distilled water from internal water system (University Wuerzburg, Germany)
MTBE	PESTINORM® MTBE for pesticide residue analysis (Part. No.: 85932.290, VWR International, Leuven, BE)
MeOH	HPLC grade, ≥ 99.9% (Part. No.: 12490.4700, Bernd Kraft, Duisburg, Germany)
NaCl	Part. No.: 2532, University Wuerzburg, Germany
TMSH	LiChropur for GC-analysis, 0.25 M in MeOH (Part. No.: 92732, Sigma Aldrich, Munich, Germany)
liquid nitrogen	from internal system (University Wuerzburg, Germany)
37 FAME Mix	certified reference material in dichloromethane (varied concentrations), Supelco (Sigma Aldrich, Munich, Germany)
16:1 ω7 *trans* (FAME)	Product no. 20-1602-09, Methyl-Palmitelaidate (9E) (Larodan AB, Karolinska Institutet Science Park, 17165 Solna, Sweden)
18:1 ω7 *trans* (FAME)	Product no. 20-1813-9, Methyl Transvaccenate (11E) (Larodan AB, Karolinska Institutet Science Park, 17165 Solna, Sweden)
18:1 ω7 (FAME)	Product no. 20-1812-9, Methyl Vaccenate (11Z) (Larodan AB, Karolinska Institutet Science Park, 17165 Solna, Sweden)
13:0 (FA)	Tridecanoic acid (T0502-10G) (Sigma Aldrich, Munich, Germany)
19:0 (FA)	Nonadecanoic acid (N5252-1G) (Sigma Aldrich, Munich, Germany)

4.1.4. Solutions

$CHCl_3$-MeOH-solution
1050 mL of $CHCl_3$ (measuring cylinder) + 450 mL of MeOH (measuring cylinder)

$CHCl_3$-MeOH-IS-solution
4.76g 13:0 + 0.119g 19:0 // 200 mL volumetric flash dissolved in $CHCl_3$-MeOH-solution

0.9% NaCl solution
1.80g NaCl // 200mL volumetric flash dissolved in distilled water

TMSH-MTBE-solution (5:8, v/v)
495µL TMSH (Microman® Pipette) + 795µL MTBE (Microman® Pipette); fresh preparation before ever sample preparation.

3-component-FAME-Mix
800µg 16:1 ω7 *trans* + 800µg 18:1 ω7 *trans* + 800µg 18:1 ω7 dissolved in 1000µL dichloromethane (Microman® Pipette)

Authentic standard reference mix
125µL of 3-component-FAME-Mix (Microman® Pipette) + 500µL of 37 FAME Mix // 2mL volumetric flash dissolved in MTBE

4.1.5. Software

Statistical softwares
R version 3.6.3 (2020-02-29), Copyright (C) 2020 The R Foundation for Statistical Computing
OriginPro 2021 (64-bit) SR2 9.8.5.212, Copyright (C) 1991-2021 OriginLab Corporation

Chromatographical softwares
Thermo Xcalibur QualBrowser 4.0.27.10, Copyright (C) 1998-2015 Thermo Fisher Scientific Inc.

Further programs
PerkinElmer ChemDraw Version 19.0.1.28, Copyright (C) 1998-2019 PerkinElmer Informatics, Inc.

4.2. Methods

In the following section, methods used in the context of this manuscript are described. In this manuscript, two different study populations were analyzed and evaluated. Whenever necessary, the respective study will be named.

4.2.1. Acquisition of human tissue

In this manuscript, ADT from different body compartments from the WASP and breast ADT from healthy women from Isoflavones: Cross-species comparison of metabolism, estrogen sensitivity, epigenetics, and carcinogenesis (ISOCROSS) study population were used for analysis.

4.2.1.1. Study WASP

ADT from different parts of the human body were obtained from 27 adult women (n=26) and men (n=1) undergoing surgeries between 2019 and 2021. Participating women gave their written informed consent before inclusion in the study. Information on sex, age, type and location of surgery, height, weight, smoking history, alcohol consumption, diet, physical activity, intake of food supplements, intake of pharmaceuticals (excluding estrogen active drugs (EAD)), parity, breastfeeding, menopausal status as well as intake of EAD were assessed with self-administered questionnaires. Body mass index (BMI) was calculated as body weight (kg) divided by squared height (m^2). The study followed the declaration of Helsinki and has been approved by the Ethics Committee of the Faculty of Medicine at the University of Wuerzburg.

4.2.1.2. Study ISOCROSS

The acquisition of breast tissue specimens is described elsewhere (Pemp, Kleider, et al. 2019). Briefly, in the context of this manuscript, breast tissue specimens (ADT and GLT) were obtained from 40 adult women undergoing reduction mammoplasty between 2010 and 2015. Participating women gave their written informed consent before inclusion in the study. Smoking history (smoker, non-smoker), alcohol consumption as well as intake of EAD and information on age, height, weight and parity were assessed with a self-administered questionnaire. BMI was calculated as body weight (kg) divided by squared height (m^2). The study followed the declaration of Helsinki and has been approved by the Ethics Committee of the Faculty of Medicine at the University of Wuerzburg.

4.2.2. Preparation of human tissue

After receipt of respective biospecimen samples from the study of WASP or ISOCROSS the samples were prepared according to the following subchapters 4.2.2.1, 4.2.2.2 and 4.2.2.3.

4.2.2.1. Human tissue from the study WASP

ADT were obtained during plastic surgery by the surgeon itself. Immediately after removal, the ADT samples were put into swingsettes, flash-frozen in liquid nitrogen and stored in liquid nitrogen tanks at -80°C upon collection by laboratory workers. After collection, the ADT samples were further stored at -80°C in the bio freezer until analysis.

4.2.2.2. Human breast tissue from the study ISOCROSS

The preparation of breast tissue specimens is described elsewhere (Pemp, Kleider, et al. 2019). Briefly, the tissue specimens were put in ice cold Krebs-Buffer immediately after receipt. Within 10 to 15 minutes after removal, ADT and glandular tissue (GLT) with less than 15% adhering ADT were separated macroscopically, portioned, flash-frozen in liquid nitrogen and stored at -80°C in the biofreezer.

4.2.2.3. Homogenization of human tissue from WASP and ISOCROSS

Prior analysis, GLT and ADT samples were finely ground in a special contraption by hammering in liquid nitrogen by following the according standard operation procedure (SOP). Approximately 50 mg of GLT or ADT were hammered for analysis. The finely grounded powder was then weighed in a cryo (ch. 4.1.2) and stored at -80°C in the biofreezer (ch. 4.1.1) until analysis. During the whole procedure, the equipment and the tissues were kept in liquid nitrogen to avoid thawing.

4.2.3. FA analysis

In the following subchapters 4.2.3.1, 4.2.3.2 and 4.2.3.3, the implemented FA analysis method will be depicted. The implementation of method follows subsequently (ch. 4.2.3.5). To maintain constant measurements in FA analysis, quality assurance of the implemented FA method will be depicted next (ch. 4.2.3.6). Calcuation of desaturase and elongase indexes will close this chapter (ch. 4.2.3.7).

4.2.3.1. Extraction procedure

The extraction method of FAs was adapted and modified from an existing extraction procedure (Folch, Lees, and Sloane Stanley 1957). Briefly, approximately 50 mg tissue was weighed in and extraction of FAs was performed with 5 mL of a chloroform-methanol-solution and 200 μL of a chloroform-methanol-internal-standard-solution using Multipette® in glass culture tubes. The extraction solution was vortexed thereafter and allowed to extract for one hour. After extraction, 500 μL methanol and 500 μL 0.9% NaCl solution using Multipette® were added to the mixture, vortexed and centrifuged for 5 min at 25 g. The aqueous solution (upper phase) was removed with a glass pasteur pipette without penetrating the non-extractable residues at the interphase and the procedure was repeated three times. After the last washing step, the chloroform phase (lower phase) was collected with a glass pasteur pipette in a glass culture tube and evaporated at 50 mbar for 60 min and 25 mbar for 2h thereafter into dryness.

4.2.3.2. Derivatization

The residues of ADT samples from WASP and ISOCROSS were redissolved in 1000 μL MTBE using Microman® Pipettes in a glass culture tube, quantitatively transferred into a vial by using a glass pasteur pipette and diluted as followed:

$$dry\ residue + 1000\ \mu L\ MtBE\ (1) \tag{4.1}$$

$$(1)\ //\ 80\ \mu L + 520\ \mu L\ MtBE\ (2) \tag{4.2}$$

FAs were converted into their corresponding FAMEs by derivatization of diluted residue with TMSH:MTBE solution (5:8, v/v).

$$(2)\ //\ 20\ \mu L + 130\ \mu L\ TMSH : MtBE\ (5:8,\ v/v)\ (3) \tag{4.3}$$

1 μL of dilution (3) was used for FAME analysis.

The residue of GLT samples from ISOCROSS were redissolved in 200 μL MtBE using Microman® Pipettes in a glass culturetube, quantitatively transferred into a vial by using a glass pasteur pipette and diluted as followed:

$$dry\ residue + 200\ \mu L\ MtBE\ (1) \tag{4.4}$$

$$(1)\ //\ 30\ \mu L + 70\ \mu L\ MtBE\ (2) \tag{4.5}$$

FAs were converted into their corresponding FAMEs by derivatization of diluted residue with TMSH:MTBE solution (5:8, v/v).

$$(2) \mathbin{//} 20\ \mu L + 130\ \mu L\ TMSH : MtBE\ (5:8,\ v/v)\ (3) \tag{4.6}$$

1 µL of dilution (3) was used for FAME analysis. A flow diagram of the whole set up is depicted in Appendix A.1.

4.2.3.3. GC-MS/FID

FAMEs were separated and detected by a GC-MS/FID on a Supelco SP-2560 100m x 0.25 mm fused silica capillary column with 0.20 µm film thickness using splitless injection. The injector temperature was 250°C and carrier gas helium. The column oven was programmed at 120°C, ramped at 4°C/min to 240°C and was then kept at 240°C for 15 min. The temperature of the FID was 250°C. Detection in the FID was carried out at a hydrogen flow rate of 35 ml/min and an air flow rate of 250 ml/min. Mass spectrometric analysis of FAMEs was carried out with electron impact ionization and detection with total ion chromatograms (TIC) at m/z range of 50-450. Further relevant method parameters are depicted in Appendix A.2. For integration of FAME peak area the XCalibur software (version 4.0.27.10) was used. Relevant integration parameters for consistent integration are depicted in Appendix A.3.

4.2.3.4. Authentic standard reference mix

A standard reference mix (ch. 4.1.4) for peak identification of FAMEs and quality assurance was established. Relevant chromatographical and mass spectrometrical key parameters are depicted in tab. 5.1. Relevant key fragmentation pattern of FAMEs are explained in Chapter 2.3.3.

4.2.3.5. Implementation of method

The scope of implementation for FA analyses included comparison of FA compositions and individual FA percentages from the method described in ch. 4.2.3 and from an existing FA analysis method from the working group Lehmann (ch. 4.2.5.3) as well as calculation of the recovery and limit of detection (LOD)s of important FA biomarkers and FAs which are detected in the lowest range of FA composition.

FA composition

For final FA composition, the areas of individual FAMEs were firstly converted into their

respective FAs by multiplication with their respective ratio of the molar mass of the FA and FAME.

$$\text{FA } (Area) = \text{FAME } (Area) * \frac{M(\text{FA})}{M(\text{FAME})} \tag{4.7}$$

Secondly, the total area for all FAs was calculated by the sum of all identified FA areas.

$$total\text{FA } (Area) = \sum \text{FA}_1 + \text{FA}_2 + \text{FA}_X \tag{4.8}$$

The proportion of individual FAs on its composition was thirdly calculated by dividing the individual FA by the sum of all identified FA areas multiplicated by 100%.

$$individual \text{ FA } (\%) = \frac{individual \text{ FA } (Area)}{total \text{ FAs } (Area)} * 100\% \tag{4.9}$$

Comparison of methods

Recently, FA compositions of breast ADT from the ISOCROSS population (ch. 4.2.1.2) were determined in the working group Lehmann with GC-FID (Mahdiani 2017, ch. 4.2.5.3). To compare the FA distribution between breast ADT and GLT from the ISOCROSS study population regarding dietary FA biomarkers, a comparison between the method described in this manuscript (4.2.3) and the method used to determine FA compositions in breast ADT (ch. 4.2.5.3) had to be done before. Comparison of methods was carried out by one independent weighed-in ADT from three donors (n=3; sample no. 21, 24 and 26; following the procedure shown in chapter 4.2.3) and re-injection of the same three donors from Mahdiani 2017 analyzed with the method described in chapter 4.2.3.3. Paired t-test for statistical evaluation between the two methods was carried out for every identified FA.

Recovery

Extraction efficiency was examined for two independent weighed-in ADT from three donors (n=6) by adding the $CHCl_3$-MeOH-IS-solution (ch. 4.1.4) at the beginning (according to the procedure shown in chapter 4.2.3) and after the extraction procedure (ch. 4.2.3.1). Recoverys were calculated as followed:

$$Recovery(\%) = \frac{Area \text{ IS}_{before}}{Area \text{ IS}_{after}} * 100\% \tag{4.10}$$

A flow diagram of recovery experiments are depicted in Annex A.15.

Limit of detection

LODs were determined for 21:0, 22:0, 23:0, 24:0, 15:1 ω5, 24:1 ω9, 18:2 ω6 *transtrans*, 22:6 ω3, 18:3 ω6, 20:3 ω6, 20:3 ω3, 20:5 ω3 and 22:2 ω6 in ADT samples of WASP. LODs for FAs, which were not detected at all (21:0, 22:0, 23:0, 24:0, 15:1 ω5, 24:1 ω9, 18:2 ω6 *transtrans*) or in a subset (22:6 ω3) of ADT samples of WASP, were determined as followed:

The noise was integrated at the expected timeframe of the respective FAME ($Noise_{FAME}$). Subsequently, $Noise_{FAME}$ had to be calculated into respective $Noise_{FA}$:

$$Noise_{FA}\,(Area) = Noise_{FAME}\,(Area) * \frac{M\,(\text{FA})}{M\,(\text{FAME})} * 100\% \tag{4.11}$$

Secondly, LOD was expected to be reached at $Noise_{FA}$ multiplicated by three.

$$LOD_{FA}\,(Area) = Noise_{FA}\,(Area) * 3 \tag{4.12}$$

To calculate the potential proportion of respective FA on FA composition, the following formula was applied:

$$LOD_{FA}\,(\%) = \frac{LOD_{FA}\,(Area)}{LOD_{FA}\,(Area) + total\,FA\,(Area)} * 100\% \tag{4.13}$$

LODs for FAs, which were detected to small amounts in ADT (18:3 ω6, 20:3 ω6, 20:3 ω3, 20:5 ω3, 22:2 ω6), were determined as followed:

All detected signals of ADT samples from the 10th percentile and below of the respective FAs were chosen for LOD calculation. Firstly, $Noise_{FA}$ was calculated by deviding $Signal_{FA}$ by three.

$$Noise_{FA}\,(Area) = \frac{Signal_{FA}\,(Area)}{3} \tag{4.14}$$

Secondly, to calculate the potential proportion of respective FA at LOD on FA composition, the following formula was applied:

$$LOD_{FA}\,(\%) = \frac{Noise_{FA}\,(Area)}{Noise_{FA}\,(Area) + total\,FA\,(Area) - Signal_{FA}\,(Area)} * 100\% \tag{4.15}$$

4.2.3.6. Quality assurance

To maintain constant measurements in FA analyses, quality measurements (QM) with the authentic standard references mix (ch. 4.2.3.4) and $CHCl_3$-MeOH-IS-solution mix were established.

QM with authentic standard references

Besides the usage of t_R and fragmentation patterns of respective FAMEs for identification in FA analyses, QM for detector sensitivity reason of the FID and MS were established. Monitoring with the authentic standard references mix were carried out before and after every analyses. Briefly, t_R of 16:0 and 18:2 ω6, the peak areas of 16:0 and 18:2 ω6 in FID and MS, the ratio of the peak areas of respective FAMEs in FID and MS as well as their correction factor (CF), calculated as the ratio of real and theoretical proportion of respective FAMEs were documented. Monitoring of QM with the authentic standard references mix are depicted in the enclosed data CD-ROM.

QM with IS

Two IS allow a quality check of each sample since their ratio is expected to be constant when adding at the beginning of every preparation. Therefore, the ratio of the areas of 13:0 to 19:0 was calculated for every tissue preparation. Monitoring of QM with IS are depicted in the enclosed data CD-ROM.

4.2.3.7. Calculation of desaturase and elongase indexes

Desaturase and elongase indexes (DI and ELOVLI, respectively) were calculated as product-to-precursor ratios of individual FAs in ADT as followed:

$$\Delta5\text{DI} = \frac{Proportion\ of\ 20{:}4\ \omega6\ (\%)}{Proportion\ of\ 20{:}3\ \omega6\ (\%)} \tag{4.16}$$

$$\Delta6\text{DI} = \frac{Proportion\ of\ 18{:}3\ \omega6\ (\%)}{Proportion\ of\ 18{:}2\ \omega6\ (\%)} \tag{4.17}$$

$$\Delta9\text{DI} = \frac{Proportion\ of\ 16{:}1\ \omega7\ (\%)}{Proportion\ of\ 16{:}0\ (\%)} \tag{4.18}$$

$$\text{ELOVLI5} = \frac{Proportion\ of\ 20{:}3\ \omega6\ (\%)}{Proportion\ of\ 18{:}3\ \omega6\ (\%)} \tag{4.19}$$

4.2.4. Statistics

All statistical tests were carried out using OriginPro 2021 or R statistical software (ch. 4.1.5). Before statistical testing, all data were checked graphically for normal distribution and variance homogeneity.

4.2.4.1. Comparison of two samples

For comparison of two samples a decision tree is used (Fig. 4.1).
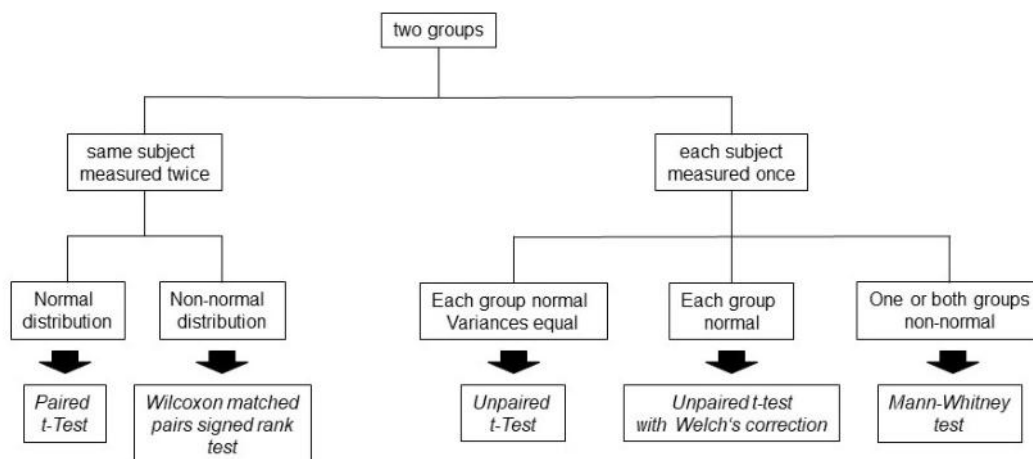
Figure 4.1.: Decision tree for two-sample comparison. All data is graphically checked on normal distribution and variance homogeneity. If the same subject was measured twice, the left path was chosen. If each subject was measured one, the right path was chosen.

Statistical significance was assumed if the P-value was <0.05. Analysis of two samples were performed with OriginPro 2021 (ch. 4.1.5). For more comprehensive statistical analysis (e.g. correlation analysis) R statistical software was used. For multi comparison of two samples P values were adjusted using the method of Holm using R statistical software.

```
adjpwerte <- p.adjust(pwerte$pWert, method="holm",28)
zus <- cbind(pwerte, adjpwerte)
write.table(zus,"testkatjaadjustiert.csv", quote=F, sep = ";", dec=",")
```

4.2.4.2. Correlation analyses

Correlation analysis was conducted using R statistical software (ch. 4.1.5). Complete R-scripts from correlation analysis are depicted in the enclosed data CD-ROM.

corGesamt <- rcorr(as.matrix(CorGes[,c(1:122)]), type=c("spearman"))

corGesamt.r <- data.frame(corGesamt$r)

corGesamt.P <- data.frame(corGesamt$P)

write.table(corGesamt.r,'corGesamtR.csv', sep=";", dec=",")

write.table(corGesamt.P,'corGesamtp.csv', sep=";", dec=",")

Significant correlation between numerical variables were assumed if the P-value was
< 0.05.

4.2.4.3. Principal component analysis

PCA was also performed by using R statistical software (ch. 4.1.5). Complete R-scripts
from PCA are depicted in the enclosed data CD-ROM.

PCAChol <- data.frame(PCAChol[,"F183"], PCAChol[,"F205"], PCAChol[,"F226"], PCA-
Chol[,"F182"], PCAChol [,"F1819t"])

colnames(PCAChol) <- c("F183", "F205", "F226", "F182", "F1819t")

index2 <- apply(PCAChol,1,function(x)!any(is.na(x)))

PCAChol2 <- PCAChol[index2,]

pcaChol <- prcomp(PCAChol2, scale.=TRUE, retx = TRUE)

pdf("Screeplotpcan3FS.pdf", width=10, height=10)

screeplot(pcaChol, npcs = 5, type="lines")

dev.off()

pdf("PlotSummaryPCAn3FS.pdf", width=10, height=10)

plot(prcomp(PCAChol2))

summary(prcomp(PCAChol2, scale = TRUE))

dev.off()

biplot(prcomp(PCAChol2, scale = TRUE))

round(pcaChol$rotation[,1:5],2)

PCAChol$PC1[index2] <- pcaChol$x[,1]

PCAChol$PC2[index2] <- pcaChol$x[,2]

PCAChol$PC3[index2] <- pcaChol$x[,3]

write.table(PCAChol,"PCAnFS.csv", quote=F, sep = ";", dec=",")

The appropriate number of Principal components (PC) were identified by using the Kaiser-

Criterion (eigenvalues >1) and the scree plot, which graphs the eigenvalues against the PCs. The "elbow point" of this plot was used as cut-off criterion. For interpretation of PCs 2D- and 3D-factorloading plots were used.

4.2.4.4. Categorical Data

Relationship between categorical and numerical variables was evaluated by comparison of medians using unpaired Wilcoxon tests by using R statistical software (ch. 4.1.5). Complete R-scripts from unpaired Wilcoxon tests are depicted in the enclosed data CD-ROM.

```
Menoperi <- Komplett[which(Komplett$MP=='peri'),]
Menopost <- Komplett[which(Komplett$MP=='post'),]
Menopre <- Komplett[which(Komplett$MP=='pre'),]
wilcox.test(Menoperi$Age, Menopre$Age, paired = FALSE)
median(Menoperi$Age)
median(Menopre$Age)
```

Significant correlation between categorical and numerical variables were assumed if the P-value was < 0.05.

4.2.4.5. Multiple linear regression models

Multiple linear regression models were carried out by using R statistical software (ch. 4.1.5). Complete R-scripts from multiple linear regression models are depicted in the enclosed data CD-ROM.

```
attach(subsetModelle3)
step(lm(ELOVL5 ~1), scope=list
(lower= ~1,upper= ~ age+ MP+ SS+ Oel+ smoke+ alc+ BMI+ Fisch+ Pfl+ Hydr+ E+ ERD+
Oxy+ Chol ), direction="forward")
detach(subsetModelle3)
attach(subsetModelle3)
Modell <- lm(ELOVL5 ~ BMI + Fisch + alc)
summary(Modell)
detach(subsetModelle3)
png("ModellKonf.png")
qqnormkonf(Modell$residuals)
qqline(scale(Modell$residuals), lty = 3, col = 'grey50')
```

dev.off()

png("Modell.png")

par(mfrow=c(2,2))

plot(Modell)

dev.off()

cooks.distance(Modell)

To evaluate the associations between potential explanatory variables (*exVar*) and dependent variables, an automatic procedure, at which one *exVar* explaining the dependent variable best is chosen. Applying the Akaike information criterion, all possible *exVar*s from the model are added one after another to the one *exVar* explaining the dependent variable best until no further improvement of the model by adding *exVar*s is made. The significance of association between one *exVar* and the dependent variable as well as its size of impact is expressed by P values and coefficients of regressions (R^2), respectively. Statistical significance was reached if the P value was <0.05. To identify possible outliers, residual vs. leverage plots were used. If observations with Cook's distances >1 appreared, they were removed from the data set and the model was calculated anew. This process was repeated until no further observation had to be removed. Data distribution was evaluated by quantile-quantile plots with simulated confidence intervals. Analysis of the homoskedasticity assumption was evaluated by scale-location plots and fitted vs. residuals plots were used to detect several types of violations in the linear regression assumption (wether linearity and/or homoskedasticity holds and/or possible outliers). For adequate estimation of R^2, standard errors and confidence intervals at least two observations per *exVar* (Austin and Steyerberg 2015) were aimed.

4.2.5. Further data obtained in the working group Lehmann

4.2.5.1. endogenous estrogens in breast ADT from the ISOCROSS study

E1 and E2 in human breast ADT were quantified by GC-MS/MS (Varian 450-GC, 300-MS; Bruker Daltonics, Bremen, Germany) using their respective deuterated derivatives. A detailed description of the method can be found elsewhere (Pemp, Kleider, et al. 2019).

4.2.5.2. Oxysterols in breast ADT from the ISOCROSS study

7β-OHC, 5,6β-epoxyC and 5,6α-epoxyC were determined and quantified by GC-MS/MS (Varian 450-GC, 300-MS; Bruker Daltonics, Bremen, Germany) using their respective

deuterated derivatives. A detailed description of the method can be found elsewhere (Wunder et al. 2022).

4.2.5.3. FA composition in breast ADT from the ISOCROSS study

The FA composition in human breast ADT were determined by GC-FID (Agilent® 5890 GC Series II; Agilent® Technologies, Böblingen, Germany). Analysis of FA composition in ADT, according to the dissertation of Mahdiani 2017, was performed after extraction (according to Folch, Lees, and Sloane Stanley 1957) and derivatisation into their respective FAME with GC-FID (Agilent® 5890 GC Series II; Agilent® Technologies, Böblingen, Germany). Briefly, approximately 700 mg of ADT was extracted with 20 mL $CHCl_3$/MeOH (2:1, v/v) solution, containing 1 mg 11:0 (Sigma Aldrich, St. Louis, Missouri) as IS for one hour. Washing steps were performed by adding 2 mL MeOH and 2 mL of 0.9% NaCl solution to the mixture, vortexing and centrifuging for 5 min at 25 g. The aqueous solution was removed and the precedure was repeated three times. After the last washing step, the organic phase was collected with a glass pasteur pipette and was evaporated thereafter. The residue was redissolved and derivatized with TMSH (0.25 M) in MTBE at room temperature for 30 seconds. FAME were seperated on a Supelco® SP-2560 100 m x 0.25 mm fused silica capillary column with 0.20 µm film thickness (Sigma-Aldrich, Bellefonte, Pennsylvania) using splitless injection. The injector temperature was 260℃, and column oven started at 140℃, ramped at 3℃/min to 230℃, was kept at 230℃ for 5 min, and then ramped at 1℃/min to 240℃, finally holding this temperature for 20 min. Carrier gas helium was kept at a constant flow rate of 1 mL/min. The temperature of the FID was 260℃. Detection was carried out at a hydrogen flow rate of 40 mL/min, an air flow rate of 450 mL/min and a nitrogen flow of 45 mL/min. To identify the FAME in ADT, the t_R of FAMEs were compared with an authentic FAME standard mix (Supelco®, Sigma Aldrich, Bellefonte, Pennsylvania). For integration of FAME peak area, the Agilent ChemStatio software (G1701DA) was used. For final FA composition, the areas of individual FAMEs were firstly converted into their respective FAs by multiplication with their respective ratio of the molar mass of the FA and FAME. Secondly the total area for all FAs was calculated by the sum of all identified FA areas and the proportion of individual FAs on its composition was thirdly calculated by dividing the individual FA area by the sum of all identified FA areas multiplicated by 100%.

4.2.5.4. Gene expressions in breast ADT from the ISOCROSS study

Relative gene expression levels of Δ5D and Δ6D were determined by René Hauptstein in breast ADT with commercial TaqMan® gene expression assays. The initial number of

copies (n_0) for each transcript was calculated based on $1*10^{12}$ copies at cycle threshold (ct) and an efficiency of 100% :

$$n_0 = \frac{number\ of\ copies}{reaction\ efficiency^{ct}} = \frac{1*10^{12}}{2^{ct}} \tag{4.20}$$

Subsequently, n_0 of the respective desaturases were set into ratio with n_0 of the housekeeping-gene hypoxanthin-guanin-phosphoribosyltransferase (HPRT1):

$$relative\ gene\ expression = \frac{target\ gene_{n_0}}{HPRT_{n_0}} \tag{4.21}$$

Raw data of the respective gene expressions are depicted in the enclosed CD.

4.2.5.5. Proportion of fat in breast ADT from the ISOCROSS study

The proportion of compounds soluble in organic solvent expressed as percentages of oil (oil%) from ISOCROSS breast ADT was determined gravimetrically after extraction. A detailed description of the procedure can be found elsewhere (Pemp, Kleider, et al. 2019).

5. Results and discussion

5.1. Statistical evaluation of *exVars* influencing desaturase and elongase indexes in human breast ADT

Recent epidemiological studies indicated positive associations of Δ9DI as well as Δ6DI and negative associations of Δ5DI and ELOVLI5 with T2D (ch. 2.4). Factors influencing DIs and ELOVLI could therefore contribute indirectly to the risk of T2D through interaction with DIs and ELOVLI (ch. 2.4.1). However, data from studies in humans on how DIs and ELOVLI are modified in human ADT are scarce (ch. 2.4.1). Hence, the aim was to identify influencing factors on Δ9DI, Δ6DI and Δ5DI as well as ELOVLI5. Therefore, the influence of known and possibly new factors (ch. 2.4.1) on these indexes were statistically investigated in 40 breast ADT samples from the study population ISOCROSS (4.2.1.2) using multiple linear regression models using stepwise forward selection. Prior regression models, to identify possible correlations, and therefore co-linearities (which might hinder selection and/or influence of variables within the models), among possible numerical *exVar*s, correlation analysis was performed (ch. 5.1.1). For this purpose, Spearman's rank correlation analysis was used. Identified correlations (and therefore co-linearities) between numerical *exVar*s were solved by using PCA, to explain the variation observed within the data by constructing linear combinations of correlating variables. The resulting PCs were used as *exVar*s for multiple linear regression models (ch. 5.1.2). For statistical evaluation between numerical and categorical *exVar*s unpaired Wilcoxon-tests were performed. In order to investigate the relationships between possible *exVar*s and Δ9DI, Δ6DI and Δ5DI as well as ELOVLI5, multiple linear regression models using stepwise forward selection (ch. 5.1.3) was applied. All relevant numerical and categorical data from the 40 study participants of the ISOCROSS study are depicted in app A.4.

5.1.1. Correlation analysis of possible *exVar*s

Co-linearities between *exVar*s might hinder each other selection and/or influence of each other P-values within the multiple linear regression models. To prevent co-linearities, first,

it was checked if possible co-linearities exist between numerical *exVar*s and Δ9DI, Δ6DI and Δ5DI as well as ELOVLI5. For that correlation analysis (ch. 4.2.4.2) was performed. For identified co-linearities, PCAs were performed subsequently (ch. 5.1.2). Numerical *exVar*s included in correlation analysis were all continuous study characteristics (age, parity, oil%, BMI) as well as lifestyle characteristics (alcohol consumption) and all included analytical parameters (FAs (ch. 4.2.5.3), oxidative stress (ch. 4.2.5.2) and endogenous estrogens (ch. 4.2.5.1)). Due to known influence in desaturase and elongase homeostasis in compartments different to ADT (ch. 2.4.1), 18:2 ω6, 18:3 ω3, 20:5 ω3 and 22:6 ω3 were selected for correlation analysis. Furthermore 18:1 ω9 *trans*, representative for *trans* FAs, were chosen. The correlation coefficients were displayed in a coloured and clustered heatmap (fig. 5.1).

In correlation analysis with 40 samples 32 associations with a P-value <0.05 were observed (Annex A.5). Four main clusters were identified (fig. 5.1) including endogenous estrogens (ch. 4.2.5.1), oxysterols (ch. 4.2.5.2), FAs (ch. 4.2.5.3) and DIs as well as ELOVLI in ADT.

Correlation within FA biomarkers group

Several intervention and cross-sectional studies have provided proof of the use of FA composition in ADT as biomarker of dietary FA intake. In general, the most valid biomarkers represent FAs that can not be endogenously synthesized (ch. 2.2.3). Since measurement errors involved in the dietary assessment are common and lead to an underestimation of the association between dietary FAs and the potential outcome (Freedman et al. 2011), dietary FA biomarkers (ch. 2.2.3) were used for analyses. The selected FA biomarkers in this study (18:2 ω6, 18:3 ω3, 20:5 ω3, 22:6 ω3 and 18:1 ω9 *trans*, respectively) are mostly exogenous origin, however small amounts of 20:5 ω3 and 22:6 ω3 may be synthesized endogenously (ch. 2.2.1). Within the FA biomarkers group, two correlation clusters (the proportions of 18:2 ω6 and 18:3 ω3 as well as 20:5 ω3 and 22:6 ω3 were highly correlated with each other, fig. 5.1; app. A.5) were identified and allowed the clustering of three groups within the FA biomarkers group. Since vegetable oils and nuts are an extensive food sources in the western population (ch. 2.2.2) and 18:2 ω6 as well as 18:3 ω3 may serve as valid biomarkers of these sources (ch. 2.2.3), the correlation between 18:2 ω6 and 18:3 ω3 seems plausible. The correlation between 20:5 ω3 and 22:6 ω3 seems plausible, too, since fish intake correlates significantly in ADT FA analyses with 20:5 ω3 and 22:6 ω3 and both FAs are being used as biomarkers in several intervention and cross-sectional studies for years (ch. 2.2.3). No correlations were observed between 18:1 ω9 *trans* and the other investigated FAs (fig. 5.1). However, as the main unnatural *trans* FA isomer

occuring through partial hydrogenation of vegetable oils (ch. 2.2.3), no correlation with natural occuring FAs is plausible. Therefore, it was assumed, that co-linearities within the FA biomarker groups vegetable oils and nuts (18:2 ω6 and 18:3 ω3) and fish (20:5 ω3 and 22:6 ω3) exist. In order to prevent co-linearities for the group of FA biomarkers, PCA was performed subsequently for the FA biomarker groups fish (20:5 ω3 and 22:6 ω3), vegetable oils and nuts (18:2 ω6 and 18:3 ω3) as well as partially hydrogenated vegetable oils (18:1 ω9 *trans*). 18:2 ω6, 20:5 ω3 and 22:6 ω3 were all positively correlated with age (fig. 5.1; app. A.5). Significant positive correlations were also observed for 20:5 ω3 with Δ9DI and Δ5DI, respectively, as well as for 22:6 ω3 and Δ9DI. 18:2 ω6 was significantly negatively correlated with Δ9DI, Δ6DI and ELOVLI5, respectively, whereas 18:3 ω3 was only negatively correlated with Δ6DI (fig. 5.1; app. A.5). Further significant positive and negative correlations were observed for 20:5 ω3 with parity and 18:1 ω9 *trans* with BMI and alcohol consumption, respectively (fig. 5.1; app. A.5).

Correlation within oxidative stress biomarker group
Possible oxidative stress biomarkers are oxysterol metabolites, which result from autoxidational processes under the influence of ROS. Thereby arise predominantly the metabolites 7β-OHC, 5,6α-epoxyC and 5,6β-epoxyC, respectively (ch. 2.4). Therefore, 7β-OHC, 5,6α-epoxyC and 5,6β-epoxyC were used as representative biomarkers for oxidative stress. Tissue levels of investigated oxysterols were higly correlated with each others (fig. 5.1; app. A.5). Therefore, it was assumed, that co-linearities within the oxidative stress biomarker group exist. In order to prevent co-linearities for the group of oxysterol biomarkers, PCA was performed subsequently for the oxysterol biomarker group. 5,6α-epoxyC was significantly positivly correlated with 18:2 ω6. Also significant positive correlation was observed for 5,6β-epoxyC and 7β-OHC with 18:1 ω9 *trans* in ADT (fig. 5.1; app. A.5).

Correlation within endogenous estrogens
E1 and E2 were highly correlated with each other in ADT (fig. 5.1; app. A.5). The described correlation in ADT is plausible, due to precursor status of E1 for synthesis of E2 and vice versa. Therefore, it was assumed, that co-linearities between E1 and E2 exist in multiple linear regression models. In order to prevent co-linearities for endogenous estrogens, PCA was performed subsequently for E1 and E2. E2 was significantly negatively correlated with Δ9DI and ELOVLI5, respectively (fig. 5.1; app. A.5).
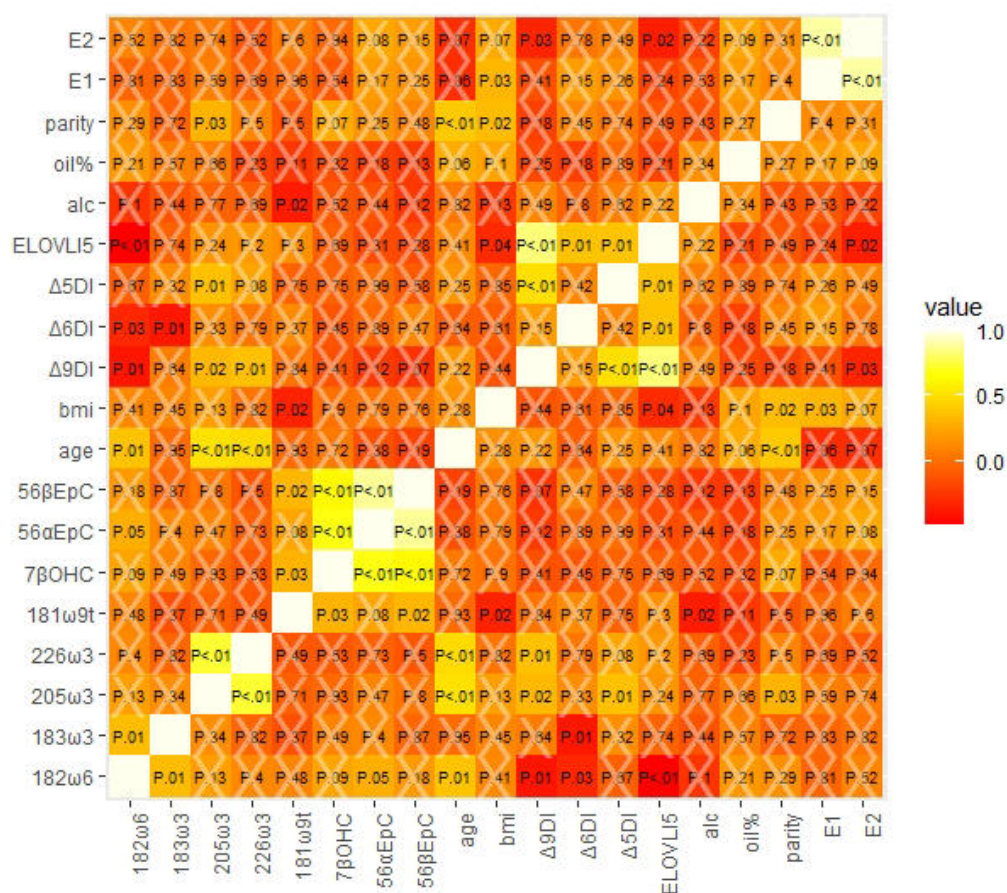
Figure 5.1.: Coloured and clustered heatmap-representation of Spearman-correlation co-efficients. Correlation analysis of desaturase and elongase indexes (Δ9DI, Δ6DI, Δ5DI and ELOVLI5, respectively), ADT FA biomarkers (18:2 $\omega6$, 18:3 $\omega3$, 20:5 $\omega3$, 22:6 $\omega3$ and 18:1 $\omega9$ *trans*, respectively), oxysterol metabolites representing oxidative stress biomarkers (7β-OHC, 5,6α-epoxyC and 5,6β-epoxyC, respectively), endogenous estrogens (E1 and E2, respectively), parity, oil%, alcohol consumption, BMI and age was carried out with data of 40 ADT samples from the study population of ISOCROSS. Numbers inside the cells indicate the respective P-values. Cells with white crosses have P values >0.05. E2 17-β-estradiol; E1 estrone; oil% percentages of oil; alc alcohol consumption; ELOVLI5 elongases of very long chain FAs 5 index; Δ5DI Δ5 desaturase index; Δ6DI Δ6 desaturase index; Δ9DI Δ9 desaturase index, 7β-OHC 7-β-OH-cholesterol; 5,6α-epoxyC 5,6α-epoxy-cholesterol; 5,6β-epoxyC 5,6β-epoxy-cholesterol.

<u>Correlation within DIs and ELOVLI</u>

Significant positive correlations were observed for Δ9DI and Δ5DI, Δ9DI and ELOVLI5, Δ6DI and ELOVLI5 as well as Δ5DI and ELOVLI5 (fig. 5.1; app. A.5). Since indexes shall represent the respective enzyme activities (ch. 2.4), and all mentioned indexes are representative for desaturation and elongation processes in PUFA metabolism, the described correlations in ADT seem plausible. As a consequence for the following multiple linear regression models (ch. 5.1.3), it must be noted, that because of the correlations between the respective indexes, the indexes (and possible influencing *exVar*s of the indexes) might affect each other indirectly by any variable significantly influencing the other indexes.

Because of the significant correlations within FA biomarker and oxidative stress biomarker groups as well as within endogenous estrogens, which might hinder each others selection into the following multiple linear regression models (ch. 5.1.3), PCAs were applied in the next step. Since multiple linear regression models shall be applied for Δ9DI, Δ6DI and Δ5DI as well as ELOVLI5 as dependent variables, no PCA was conducted for investigated indexes.

5.1.2. PCA of possible numerical *exVar*s

In order to reduce the dimensionality of the dataset, while preserving as much variability as possible PCAs were applied for FA biomarkers (ch. 5.1.2.1), oxidative stress biomarkers (ch. 5.1.2.2) and endogenous estrogens (ch. 5.1.2.3). As a dimension reduction technique, PCA aims to explain the variation observed within the data by constructing linear combinations of used variables. The factor loadings used to construct the linear combinations build up the PCs. These factor loadings indicate the correlation of each used variable to the respective PC.

5.1.2.1. PCA of FA biomarkers

Within FA biomarkers, significant correlations were observed for the biomarker groups vegetable oils and nuts (18:2 ω6 and 18:3 ω3), fish (20:5 ω3 and 22:6 ω3) and partially hydrogenated vegetable oils (18:1 ω9 *trans*) (ch. 5.1.1). Therefore PCA was applied and yielded three PCs (fig. 5.2).

The first PC ($PC_{diet}1$) was mainly characterized by high percentages of 20:5 ω3 and 22:6 ω3 (increasing impact on $PC_{diet}1$; fig. 5.2; Annex A.6 and A.7), respectively. No other variable had either a positive or a negative impact on PC_{diet}, therefore $PC_{diet}1$ represented the
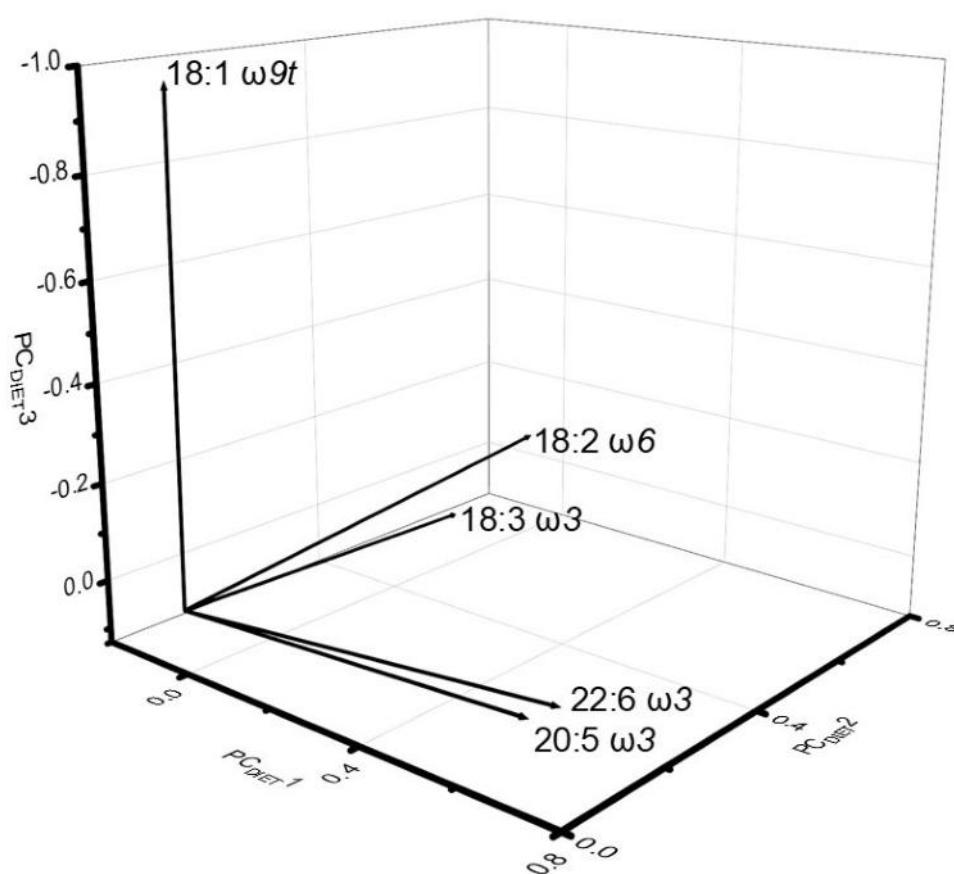
Figure 5.2.: 3D vectorplot designed from the respective factor loadings (Appendix A.6) from selected FA biomarkers (18:2 ω6, 18:3 ω3, 20:5 ω3, 22:6 ω3 and 18:1 ω9 *trans*, respectively) identified by PCA. $PC_{diet}1$ represents dietary fish intake, represented by 20:5 ω3 and 22:6 ω3; $PC_{diet}2$ represents dietary intake of vegetable oils and nuts, represented by 18:2 ω6 and 18:3 ω3; $PC_{diet}3$ represents dietary intake of partially hydrogenated vegetable oils, represented by 18:1 ω9 *trans*. PC principle component.

dietary fish intake. $PC_{diet}2$ was mainly characterized by high percentages of 18:2 ω6 and 18:3 ω3 (increasing impact on $PC_{diet}2$; fig 5.2; Annex A.6 and A.7), respectively. Since these two FAs refer to the biomarker class of vegetarian origin, mainly derived from vegetable oils and nuts (ch. 2.2.3), $PC_{diet}2$ represented the dietary intake thereof. 18:1 ω9 *trans* was the only FA, that had a factor loading >0.50 on $PC_{diet}3$. No further variable had either a positive or a negative impact on $PC_{diet}3$. Due to the large impact of 18:1 ω9 *trans* on $PC_{diet}3$, it represented the dietary intake of partially hydrogenated vegetable oils (ch. 2.2.3). The PCs $PC_{diet}1$, $PC_{diet}2$ and $PC_{diet}3$ explained 84.50% of variance (Appendix A.6).

5.1.2.2. PCA of oxidative stress biomarkers

Due to significant correlations within oxidative stress biomarkers (ch. 5.1.1), PCA was also applied and yielded one PC (fig. 5.3). $PC_{Oxy}1$ was mainly characterized by low ADT levels of 7β-OHC, 5,6β-epoxyC and 5,6α-epoxyC (decreasing impact on $PC_{Oxy}1$; fig. 5.3; Annex A.8 and A.9), respectively, therefore $PC_{Oxy}1$ represented oxidative stress biomarkers. Since all three metabolites arise predominantly in autoxidational processes (ch. 2.4.1), the described $PC_{Oxy}1$ is plausible. $PC_{Oxy}1$ explained 73.33% of variance (Appendix A.8).
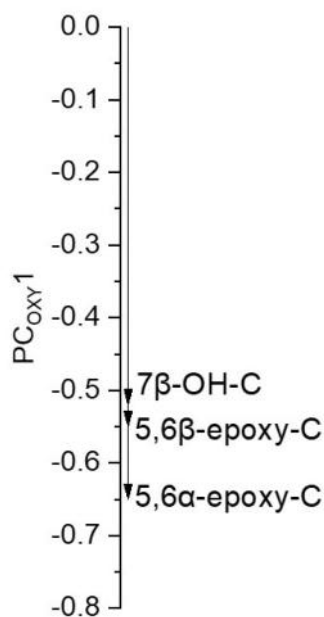
Figure 5.3.: 2D vectorplot designed from the respective factor loadings (Appendix A.8) from selected oxidative stress biomarkers (7β-OHC, 5,6β-epoxyC and 5,6α-epoxyC, respectively) identified by PCA. $PC_{Oxy}1$ represented by 7β-OHC, 5,6β-epoxyC and 5,6α-epoxyC. PC principle component; 7β-OHC 7-β-OH-cholesterol; 5,6α-epoxyC 5,6α-epoxy-cholesterol; 5,6β-epoxyC 5,6β-epoxy-cholesterol.

5.1.2.3. PCA of endogenous estrogens

Due to significant correlations within endogenous estrogens (ch. 5.1.1), PCA was also applied and yielded one PC (fig. 5.4). Since its precursor status of E1 for synthesis of E2 and vice versa (ch. 5.1.1), one PC, representative for endogenous estrogens, is plausible. Therefore, PC_E1 represented endogenous estrogens, and explained 94.59% of variance (Appendix A.10).
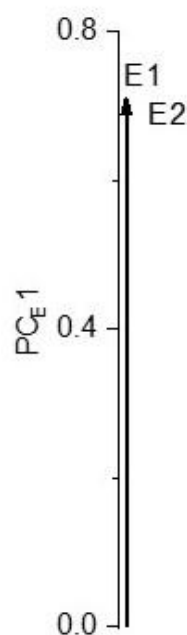
Figure 5.4.: 2D vectorplot designed from the respective factor loadings (Appendix A.10) from endogenous estrogens (E1 and E2, respectively) identified by PCA. PC_E1 represented by E1 and E2. PC principle component; E1 estrone; E2 17-β-estradiol.

5.1.3. Multiple linear regression models

To investigate potential influence of various *exVar*s on desaturase and elongase indexes, stepwise forward multiple linear regression models (ch. 4.2.4.5) were applied for the dependent variables Δ9DI, Δ6DI, Δ5DI and ELOVLI5. The R^2, the number of observations in each final model and the observation to *exVar* ratio after forward selection of variables are given in fig. 5.5. Potential *exVar*s included into the models were age (continuous), parity (continuous), oil% (continuous), smoking habits (categorical), alcohol consumption (continuous), BMI (continuous), $PC_{Oxy}1$ (continuous), $PC_{diet}1$ (continuous), $PC_{diet}2$ (continuous), $PC_{diet}3$ (continuous), PC_E1 (continuous), MP status (categorical) and intake of EADs (categorical). The number of observations in each final model was limited to a maximum of 40 observations. In addition, numbers of removed outliers, the regression coefficients, their confidence intervals, as well as the P-values of each *exVar* selected are given in app. A.12.

Δ9DI- and Δ6DI-model

Smoking habits significantly (P<0.05) and non-significantly (P=0.061) positively influenced Δ9DI and Δ6DI in breast ADT, respectively (fig. 5.5). No influence of alcohol consumption and BMI on Δ9DI and Δ6DI was observed. Neither age nor MP status or PC_E1 influenced Δ9D-index (Δ9DI) and Δ6DI. Furthermore, intake of EADs did not influence Δ9DI and Δ6DI as well. Significantly positive influence of the number of pregnancies was observed for Δ6DI (P<0.05; fig: 5.5). Diverse influences were observed for dietary FA biomarkers. $PC_{diet}2$ significantly (P<0.05) and non-significantly (P=0.103) negatively influenced Δ6DI and Δ9DI in breast ADT, respectively. $PC_{diet}1$ significantly (P<0.05) and non-significantly (P=0.093) positively influenced Δ9DI and Δ6DI, respectively. Significant negative influence (P<0.05) on Δ6DI was observed for $PC_{diet}3$. However, negative eigenvector of 18:1 ω9 *trans* (app. A.6) changed the direction of influence into significantly positive. No influence was observed for $PC_{Oxy}1$ (fig. 5.5).

Δ5DI- and ELOVLI5-model

Similar to Δ9DI and Δ6DI, $PC_{diet}1$ significantly (P<0.05) and non-significantly (P=0.075) positively influenced ELOVLI5 and Δ5DI in breast ADT (fig. 5.5). However, in contrast to Δ9DI and Δ6DI, $PC_{diet}2$ and $PC_{diet}3$ exhibited no influence on either Δ5DI or ELOVLI5. Significant positive influence was observed for the intake of ERD on Δ5DI (P<0.05; fig. 5.5). No influence was observed for ERD on ELOVLI5. Furthermore, neither age nor MP status or PC_E1 significantly influenced Δ5DI and ELOVLI5, respectively. Δ5DI was non-significantly negatively influenced by BMI and alcohol consumption (P=0.187 and P=0.096, respectively). Contrarily, ELOVLI5 was significantly and non-significantly positively influenced by BMI and alcohol consumption (P<0.05 and P=0.115, resepctively; fig. 5.5). The number of pregnancies, smoking habits and $PC_{Oxy}1$ did not significantly influence Δ5DI or ELOVLI5 in breast ADT (fig. 5.5).
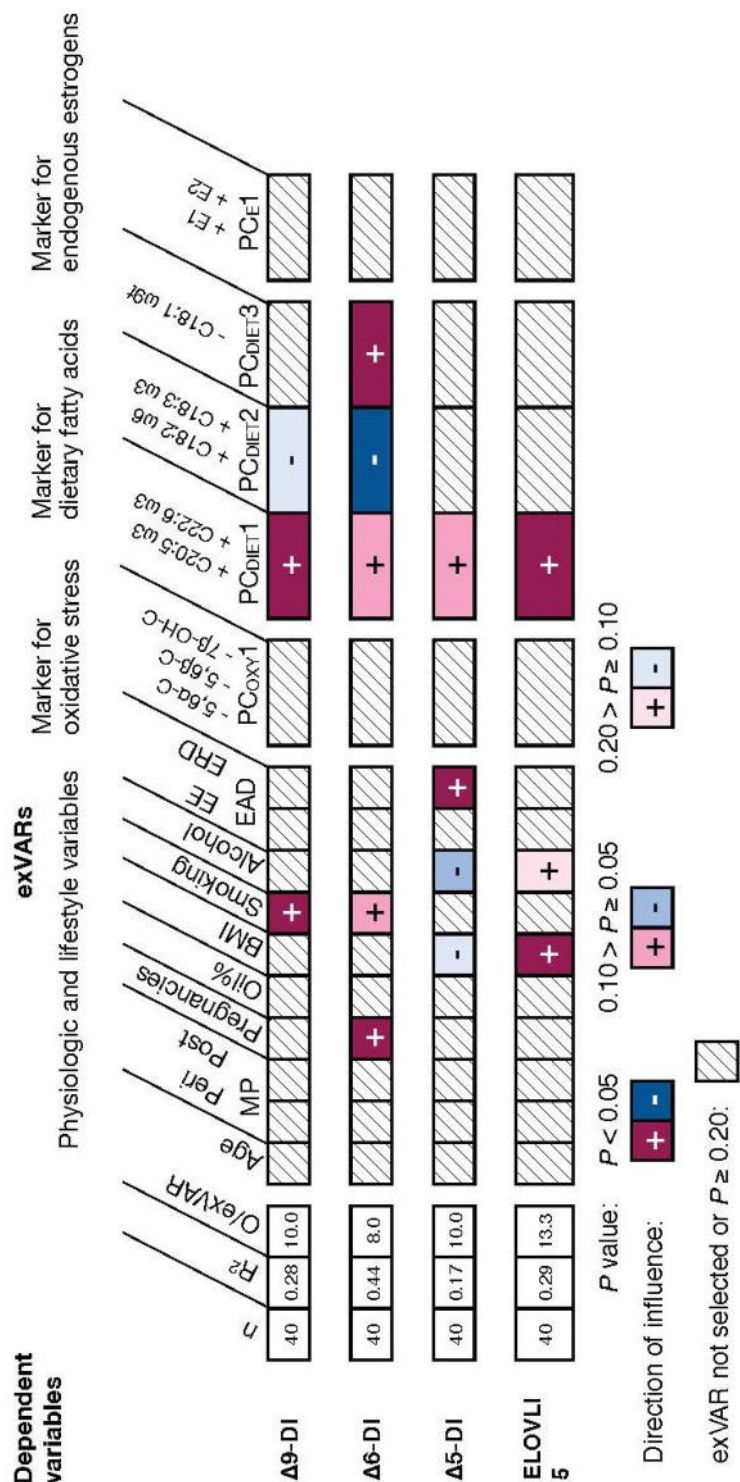
Figure 5.5.: Influence of various *exVars* on Δ9DI, Δ6DI and Δ5DI as well as ELOVLI5 in breast ADT (dependent variables) identified by multiple linear regression models using stepwise forward selection. For each model, the number of observations (n), the adjusted coefficient of determination (R^2) and the ratio of the number of observations to *exVars* after forward selection of variables is given. PostMP postmenopausal status; PeriMP perimenopausal status; EAD estrogen active drugs; EE ethinyl estradiol; ERD estrogen releasing drugs; $PC_{Oxy}1$ including 7β-OHC, 5,6β-epoxyC and 5,6α-epoxyC; $PC_{diet}1$ including 20:5 ω3 and 22:6 ω3; $PC_{diet}3$ including 18:2 ω6 and 18:3 ω3; $PC_{diet}3$ including 18:1 ω9 *trans*; PC_E1 including E1 and E2.

5.1.4. Relevance

In this study of breast ADT from healthy women of the study population of ISOCROSS, the present results encompasses physiologic, lifestyle-related, oxidative stress and dietary FA biomarkers as well as endogenous and exogenous estrogen *exVar*s influencing Δ9DI, Δ6DI, Δ5DI and ELOVLI5 estimated by product-to-precursor ratios of ADT FAs. In accordance with studies investigating either intake of FAs or FA biomarkers in compartments different to ADT (ch. 2.4.1), Δ9DI and Δ6DI were influenced negatively (P=0.103 and 0.004, respectively) by $PC_{diet}2$ supporting a putative beneficial effect of the dietary intake of vegetable oils and nuts (fig. 5.6).

Although $PC_{diet}1$ exhibited putative beneficial significant and non-significant influence on ELOVLI5 and Δ5DI (P=0.025 and 0.075, respectively), it also affected putatively adversely on Δ9DI and Δ6DI (P=0.002 and P=0.093, respectively), suggesting an ambivalent role of the dietary intake of fish on investigated indexes (fig. 5.6). Studies in experimental animals found significantly decreased Δ9DI, Δ6DI, Δ5DI and ELOVLI5 mRNA expressions after supplementation of fish oils and diets high in 18:2 *ω*6 and 18:3 *ω*3 (ch. 2.4.1), however not all product-to-precursor indexes correlate with their respective mRNA expressions (ch. 2.4). It is therefore possible, that opposing results could be obtained when investigating the influence of dietary FA biomarker on either desaturase and elongase indexes or on their respective mRNA expressions. It is assumed, that all PUFAs may downregulate desaturases through interaction with two transcription factors (M. T. Nakamura and Nara 2004). In this study, $PC_{diet}2$ followed this assumption, however conflicting results were obtained for $PC_{diet}1$. Previously, a dietary intervention in healthy subjects with supplementation of 20:5 *ω*3 and 22:6 *ω*3 found significantly higher Δ5DI and lower Δ6DI in serum from healthy men and women (Vessby, Gustafsson, Tengblad, and Berglund 2013), also suggesting an ambivalent role of dietary fish biomarkers. Furthermore, Δ6DI was found positively influenced (P=0.046) by $PC_{diet}3$ supporting a putative adverse effect of the dietary intake of partially hydrogenated vegetable oils (fig. 5.6). Interestingly *trans* fats are regarded as an inhibitor of Δ6D (Das 2006), which is contradictory to the findings in this study. However, it must be noted that sufficient human data is lacking and the positive influence of $PC_{diet}3$ on Δ6DI from this study may differ from results of other studies using mRNA expression of Δ6D because of missing correlation between Δ6D mRNA expression and Δ6DI. Alike its association in compartments different to ADT (ch. 2.4.1), smoking significantly and non-significantly influenced Δ9DI and Δ6DI putatively adversely (P=0.027 and 0.061, respectively) in breast ADT as well (fig. 5.6). Ethanol induces transcriptional activity of a sterol regulatory element-binding protein (You et al. 2002). Recently, hu-
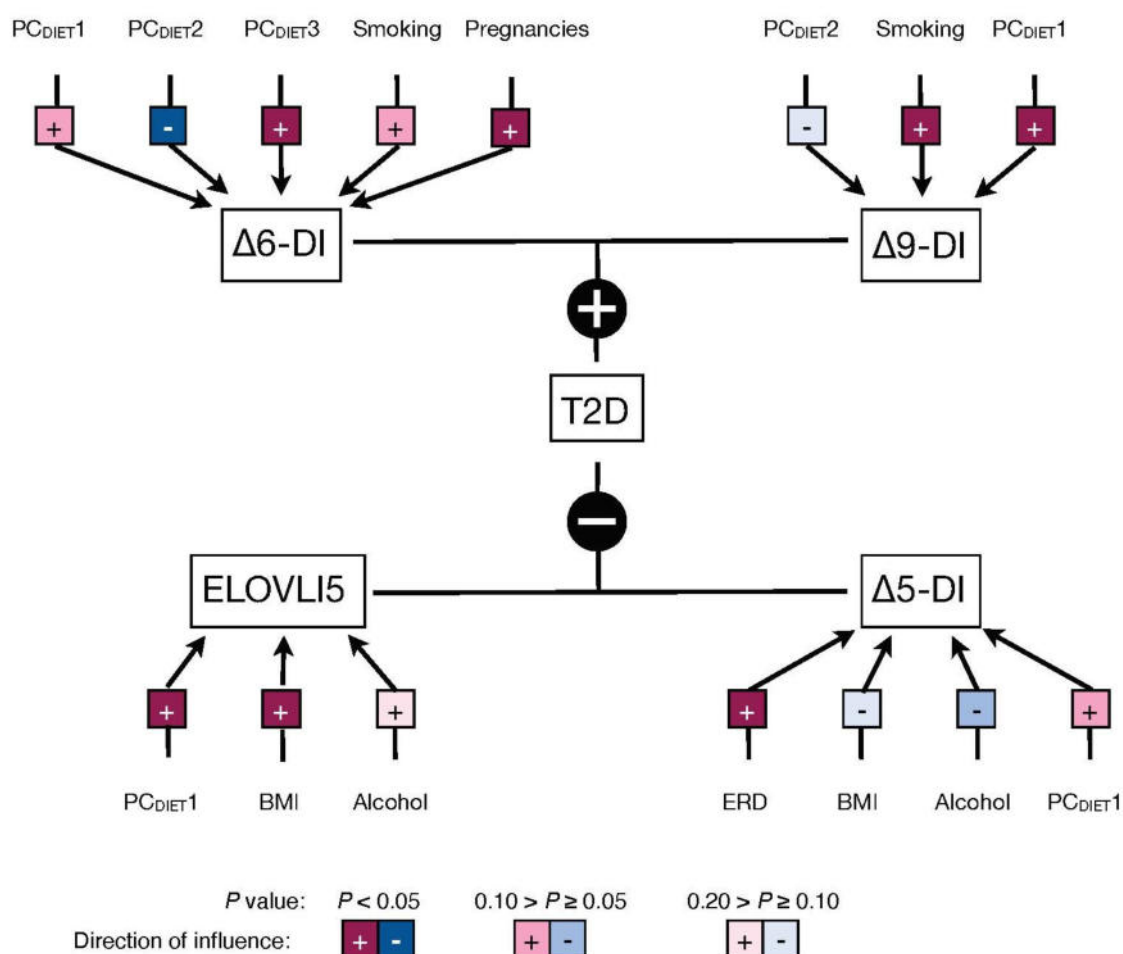
Figure 5.6.: Positive (circled cross) association of Δ9DI and Δ6DI and negative (circle line) association of Δ5DI and ELOVLI5 with T2D described in the literature and *exVar*s influencing selected indexes in ADT with P < 0.05, 0.05 < P < 0.10 or 0.10 < P < 0.20 identified by multiple linear regression models using stepwise forward selection. T2D type 2 diabetes; $PC_{diet}1$ including 20:5 ω3 and 22:6 ω3; $PC_{diet}2$ including 18:2 ω6 and 18:3 ω3; $PC_{diet}3$ including 18:1 ω9 *trans*; ERD estrogen releasing drugs.

man ELOVL5 has been found as a sterol regulatory element-binding protein target gene (Shikama et al. 2015) and therefore it is suggested that alcohol consumption may have a positive influence on ELOVLI5. In this study alcohol consumption exhibited putative beneficial non-significant positive influence on ELOVLI5 (P=0.115, fig. 5.6). Therefore the positive influence of alcohol consumption on ELOVLI5 might reflect ethanol induced *de novo* lipogenesis. However, opposing results were obtained for Δ5DI. Alcohol consumption putatively adversely non-significantly negatively influenced Δ5DI (P=0.096; fig.

5.6). Previous in vitro and in vivo studies suggest, that the influence of alcohol consumption on the elongation/desaturation pattern may depends on the amount and timeframe of alcohol intake (summarized in Pawlosky and Salem 2004). Also putative beneficial significant influence on ELOVL5-index (ELOVLI5) was observed for BMI (P=0.002; fig. 5.6). Interestingly, Δ5DI was non-significant negative influenced by BMI (P=0.187) and thus revealed a putative adverse influence. However, this finding is in agreement with recent studies (ch. 2.4.1). Contrarily to recent studies (ch. 2.4.1), BMI did not significantly influence either Δ9DI or Δ6DI (fig. 5.5). The number of pregnancies influenced Δ6DI putatively adversely (P=0.010; fig. 5.6). During pregnancy, the supply of PUFA, especially 22:6 ω3, is crucial for the development of the fetus, therefore maternal plasma 22:6 ω3 increases (ch. 2.4.1). Consequently, human erythrocytes Δ9DI and Δ6DI increases until delivery (ch. 2.4.1). Since pregnancies significantly positively influenced Δ6DI in ADT, it may be assumed that Δ6DI postpartum could be altered and does not return back to its pregestational stage after pregnancy. Estrogens may affect lipid metabolism through downregulation of lipogenic enzymes (ch. 2.4.1). Studies in experimental animals suggest negative influence of estrogens on desaturase and elongase mRNA expressions (ch. 2.4.1), however inconsistent results were obtained in human biospecimens. Putative beneficial positive influence was observed for the intake of EADs on ELOVLI5 (P=0.006; fig. 5.6). Contrary results to the literature may also be interpreted with the missing correlation between Δ5D mRNA expression and Δ5DI. In postMP women receiving HRT, Δ9D mRNA expression decreased after three months treatment. Contrarily, HRT in two healty postMP populations significantly increased levels of 20:4 ω6 and 22:6 ω3 suggesting an increased activity of elongases and desaturases (ch. 2.4.1).

Avoiding indirect associations in linear regression models, the influence of the other indexes on the current investigated index was not tested in the present results. However, due to correlations between Δ9DI, Δ6DI and Δ5DI as well as ELOVLI5 (ch. 5.1.1) and the influence of *exVar*s on the respective indexes in the present models, the indexes might affect each other indirectly by any variable significantly influencing the other indexes.

It should be noted, that the multiple linear regression models from any investigated index exhibited low R^2 (fig. 5.6) suggesting either important variables missing in the models (which seems likely in this case) or large variations within the data set. Significant influence of polymorphisms in the Δ5D- and Δ6D encoding genes FADS1 and FADS2 on Δ5DI and Δ6DI, calculated from erythrocyte membrane FAs, were observed in a subcohort from the EPIC study (Zietemann et al. 2010). Additionally, only recently, significantly

lower Δ5DI levels were found in postmenopausal women with at least one minor allele (Muzsik et al. 2018), suggesting a putatively influence of polymorphism on DIs. It should further be mentioned that investigated indexes may be confounded by exogenous origins as a consequence of dietary FA intake. However, this may only apply for Δ6DI, since 18:2 ω6 is the only biomarker, which significantly correlated with its dietary uptake in human biospecimens (ch. 2.4.1).

5.1.5. Correlation between indexes and gene expression

It was found, that the gene expression of Δ9D and its respective index (Δ9DI) correlate with each other, whereas no correlation between the gene expression of Δ6D and Δ5D and its respective indexes (Δ6DI and Δ5DI, respectively) was observed (Sjogren et al. 2008). To investigate, if the indexes Δ6DI and Δ5DI correlate with the respective gene expressions of Δ6D and Δ5D in the study population of ISOCROSS, the relative gene expression levels of Δ5D and Δ6D were determined and correlation analysis with their respective indexes (Δ6DI and Δ5DI, respectively) was conducted. The relative gene expression levels of Δ5D and Δ6D were determined in 30 breast ADT samples with commercial TaqMan® gene expression assays and n_0 of the respective desaturase were set into ratio with n_0 of the housekeping-gene HPRT1 (ch. 4.2.5.4). The resulting relative gene expression of Δ5D and Δ6D were correlated with the respective Δ5DI and Δ6DI, calculated as product-to-precursor ratio from the same 30 breast ADT samples (app. A.13). Similar to Sjogren et al. 2008 no correlation was observed for either gene expression of Δ5D and Δ5DI or Δ6D and Δ6DI (p=0.27 and 0.54, respectively). This observation could be due to a few reasons. Firstly, particularly for the calculation of Δ6DI, FA biomarker were used. The respective indexes could therefore be biased through FAs from dietary intake and concludingly did not reflect true enzyme activities. This might be the case for Δ6DI, however, Δ5DI was not calculated with FA biomarkers but with 20:4 ω6 and 20:3 ω6 which could be occur from both, endogenously *de novo* synthesis and exogenously dietary intake. Secondly, it seems possible, especially for FAs used to calculate Δ5DI, that the high bioactivity of the product (in the case of Δ5DI the FA 20:4 ω6, which is a precursor of eicosanoids including prostaglandins, thromboxane and leukotrienes), leads to a biased FA ratio, which not only reflects the Δ5D enzyme activity as such but also further pathways of 20:4 ω6, which may cause altered Δ5DI ratios (Sjogren et al. 2008). Δ6DI and Δ5DI are well-established indirect measures of Δ6D and Δ5D in different human biospecimens in epidemiological studies (ch. 2.4). However, taking this into account, it may be assumed, that the product-to-precursor indexes Δ5DI and Δ6DI do not represent the mRNA expressions of Δ5D and

Δ6D. Consequently, for Δ5DI and Δ6DI, the indexes are falsely established as indirect measures of Δ6D and Δ5D.

5.2. Establishment of FA analysis using GC-MS/FID

For the analysis of FA compositions and biomarkers (ch. 2.2.3) of different tissue specimens, a method was chosen, which was suitable of simultaneous determination of FAs with a high amount of selectivity. Since GC-FID (which was used for the study population of ISOCROSS, ch. 4.2.5.3) is limited in selectivity when applied to complex matrices (ch. 2.3.2), a GC coupled to a FID and MS (ch. 2.3.3) was chosen for FA analysis in the context of analyzed samples of ISOCROSS and WASP in this manuscript (ch. 4.2.3.3). For the establishment of the GC-MS/FID method described in ch. 4.2.3, the authentic standard reference mix (ch. 4.2.3.4) was first tested for sufficient separation of all FAs and if no sufficient separation was achieved, its impact on the method was discussed (ch. 5.2.1). Recoveries and LODs were further determined (ch. 5.2.2). One of the objectives was to compare GLT and ADT samples from the study population of ISOCROSS regarding dietary FA biomarkers (ch. 3). To evaluate, if the FA composition of GLT determined with the method described in ch. 4.2.3 could be compared with already analyzed ADT samples (Mahdiani 2017) from the ISOCROSS study (4.2.5.3) or not, the implemented GC-MS/FID method described in 4.2.3 was applied on three ADT samples from the study population of ISOCROSS and respective FA compositions were compared with the same ADT FA compositions from already analyzed samples from Mahdiani (ch. 5.2.2.4).

5.2.1. Results of the authentic standard reference mix

The main focus for the implementation of the GC-MS/FID method (ch. 4.2.3.3) was to gain as much information as possible regarding a comprehensive FA composition of human biospecimens with special attention to FA biomarkers of dietary intake (ch. 2.2.3). For that, the authenthic standard reference mix (ch. 4.2.3.4) was analyzed according to the GC-MS/FID method (ch. 4.2.3.3) and the following chromatographical and mass spectrometrical key parameters of FAMEs were determind: t_R, MI and respective FIs. A chromatogram from the authentic standard reference mix is depicted in app. A.14. To analyze separation between FAMEs, the resolution R was determined as followed:

$$Resolution\ R = \frac{1.18x\ t_{R2} - t_{R1}}{w_{0.5(1)} + w_{0.5(2)}} \tag{5.1}$$

with t_R retention time and $w_{0.5}$ peakwidth at 50% height of the respective peak. R > 1.5 are considered to be nearly fully separated (which means that the baseline width of the two peaks would overlap by 0.1%; Barth 2020). This subsection shall provide all weaknesses of the implemented GC-MS/FID method and the consequences thereof. Tab. 5.1 provides all

relevant key parameters of FAMEs and FAMEs which were not sufficiently separated (R < 1.5) will be discussed below. Relevant key fragmentation patterns of FAMEs are explained in ch. 2.3.3 and will not be further discussed.

Table 5.1.: Relevant chromatographical (t_R [min]) and mass spectrometrical (MI and respective FIs [m/z]) key parameters of individual FAs. BI basic ion, SI second intensive ion. Indicative fragment patterns of FAMEs are explained in ch. 2.3.3.

FA	t_R	$[M]^+$	$[M-29]^+$	$[M-31]^+$	$[M-43]^+$	$[M-32]^+$	$[M-74]^+$	$[M-116]^+$	$\omega 3$	$\omega 6$	α	$\alpha+27$	BI	SI
4:0		102	73	71	59								74	87
6:0		130	101	99	87								74	87
8:0	8.79	158	129	127	115								74	87
10:0	10.63	186	157	155	143								74	87
11:0	11.86	200	171	169	157								74	87
12:0	13.25	214	185	183	171								74	87
13:0	14.79	228	199	197	185								74	87
14:0	16.43	242	213	211	199								74	87
14:1 $\omega 5$	17.85	240		209		208	166	124					55	69
15:0	18.12	256	227	225	213								74	87
15:1 $\omega 5$	19.56	254		223		222	180	138					55	69
16:0	19.87	270	241	239	227								74	87
16:1 $\omega 7t$	20.67	268		237		236	194	152					55	69
16:1 $\omega 7$	21.05	268		237		236	194	152					55	69
17:0	21.52	284	255	253	241								74	87
17:1 $\omega 7$	22.72	282		251		250	208	166					55	69

cont.

FA	t_R	$[M]^+$	$[M-29]^+$	$[M-31]^+$	$[M-43]^+$	$[M-32]^+$	$[M-74]^+$	$[M-116]^+$	$\omega3$	$\omega6$	α	$\alpha+27$	BI	SI
18:0	23.23	298	269	267	255								74	87
18:1 $\omega9t$	23.95	296		265		264	222	180					55	69
18:1 $\omega7t$	24.02	296		265		264	222	180					55	69
18:1 $\omega9$	24.26	296		265		264	222	180					55	69
18:1 $\omega7$	24.38	296		265		264	222	180					55	69
19:0		312	283	281	269								74	87
18:2 $\omega6t$	25.06	294		263		264	220	178					67	81
18:2 $\omega6$	25.75	294		263		264	220	178					67	81
20:0	26.41	326	297	295	283								74	87
18:3 $\omega6$	26.88	292								150	194		79	67
20:1 $\omega9$	27.34	324		293		292	250	208					55	69
18:3 $\omega3$	27.49	292							108		236		79	67
21:0	27.9	340	311	309	297								74	87
20:2 $\omega6$	28.8	322		291		290	248	206					81	67
22:0	29.41	354	325	323	311								74	87
20:3 $\omega6$	29.86	320								150	222		79	67

cont.

FA	t_R	$[M]^+$	$[M-29]^+$	$[M-31]^+$	$[M-43]^+$	$[M-32]^+$	$[M-74]^+$	$[M-116]^+$	ω3	ω6	α	α+27	BI	SI
22:1 ω9	30.29	352		321		320	278	236					55	69
20:3 ω3	30.45	320							108		264		79	67
20:4 ω6	30.70	318								150	180	207	79	91
23:0	30.70	368	339	337	325								74	87
22:2 ω6	31.71	350		319		318	276	234					81	67
24:0	32.30	382	353	351	339								74	87
20:5 ω3	32.48								108		180	207	79	91
24:1 ω9	33.23	380		349		348	306	264					55	69
22:6 ω3	36.88								108		166	193	79	91

Butyric acid (4:0) Caproic acid (6:0) Caprylic acid (8:0) Capric acid (10:0) Undecylic acid (11:0) Lauric acid (12:0) Tridecylic acid (13:0) Myristic acid (14:0) Myristoleic acid (14:1 ω5) Pentadecylic acid (15:0) 10Z-pentadecenoic acid (15:1 ω5) 16:0 *trans* Palmitoleic acid (16:1 ω7 *trans*) 16:1 ω7 Margaric acid (17:0) Margaroleic acid (17:1 ω7) Stearic acid (18:0) Elaidic acid (18:1 ω9 *trans*) Oleic acid (18:1 ω9) *trans* Vaccenic acid (18:1 ω7 *trans*) Vaccenic acid (18:1 ω7) Nonadecylic acid (19:0) Linolelaidic acid (18:2 ω6 *transtrans*) Linoleic acid (18:2 ω6) Arachidic acid (20:0) γ Linolenic acid (18:3 ω6) Gondoic acid (20:1 ω9) 18:3 ω3 Heneicosylic acid (21:0) Eicosadienoic acid (20:2 ω6) Behenic acid (22:0) Dihomo-gamma-linolenic acid (20:3 ω6) Erucic acid (22:1 ω9) Eicosatrienoic acid (20:3 ω3) Arachidonic acid (20:4 ω6) Tricosylic acid (23:0) Docosadienoic acid (22:2 ω6) Lignoceric acid (24:0) Eicosapentaenoic acid (20:5 ω3) Nervonic acid (24:1 ω9) Docosahexaenoic acid (22:6 ω3).

4:0 and 6:0

The first FAME, which was detectable in the authentic standard reference mix with the method described in ch. 4.2.3 was 8:0 (fig. 5.7 and tab. 5.1). The authentic standard reference mix provides FAMEs with smaller carbon chains than 8:0 (6:0 and 4:0, respectively). However, the FAMEs 4:0 and 6:0 were overlapped by the solvent peak (fig. 5.7).
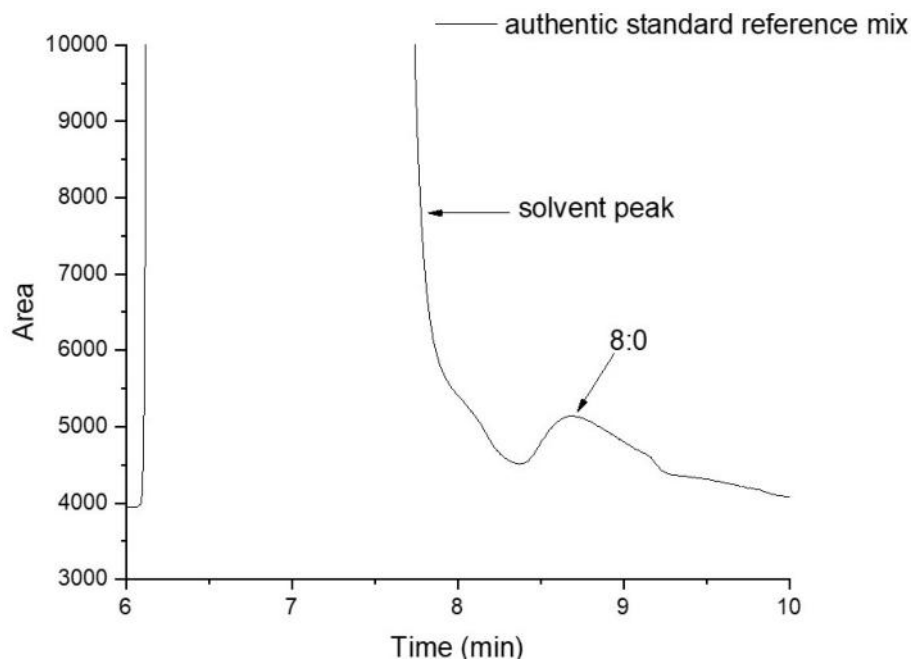
Figure 5.7.: Chromatogram of the authentic standard reference mix from six to ten minutes showing the first identified FAME as 8:0 (t_R= 8.79 min). FAMEs with chain length below eight carbon atoms (6:0 and 4:0, respectively) could not be detected due to the overlapping solvent peak in GC-MS/FID.

Consequently, 4:0 and 6:0 were not detectable with the implemented GC-MS/FID method described in ch. 4.2.3. As a consequence, 4:0 and 6:0 could not be considered as FAMEs for a comprehensive FA composition of human biospecimens.

18:1 ω9 *trans* and 18:1 ω7 *trans*

18:1 ω9 *trans* is expected to be only consumed from (partially) hydrogenated vegetable oils and serves as an indicator of habitual dietary intake from products thereof (ch. 2.2.3.4). In contrast to that, 18:1 ω7 *trans* occurs in dairy and ruminant fat but could also be an isomer from 18:1 *trans* derived from (partially) hydrogenated vegetable oils (Efsa Panel on Dietetic Products 2010). However, recent studies confirm that 18:1 ω7 *trans* may be a potential FA biomarker for the dietary intake of dairy and ruminant fat (ch. 2.2.3.1). Therefore sufficient separation of these two FAMEs in GC-MS/FID analysis should be investigated.

Even though nearly sufficient separation of 18:1 ω9 *trans* and 18:1 ω7 *trans* were achieved in the authentic standard references mix (fig. 5.8, black line, resolution R = 1.33), no separation was achieved when running the analysis on ADT matrix (fig. 5.8, dotted black line). Also no separation was achieved when running the implemented GC-MS/FID method on
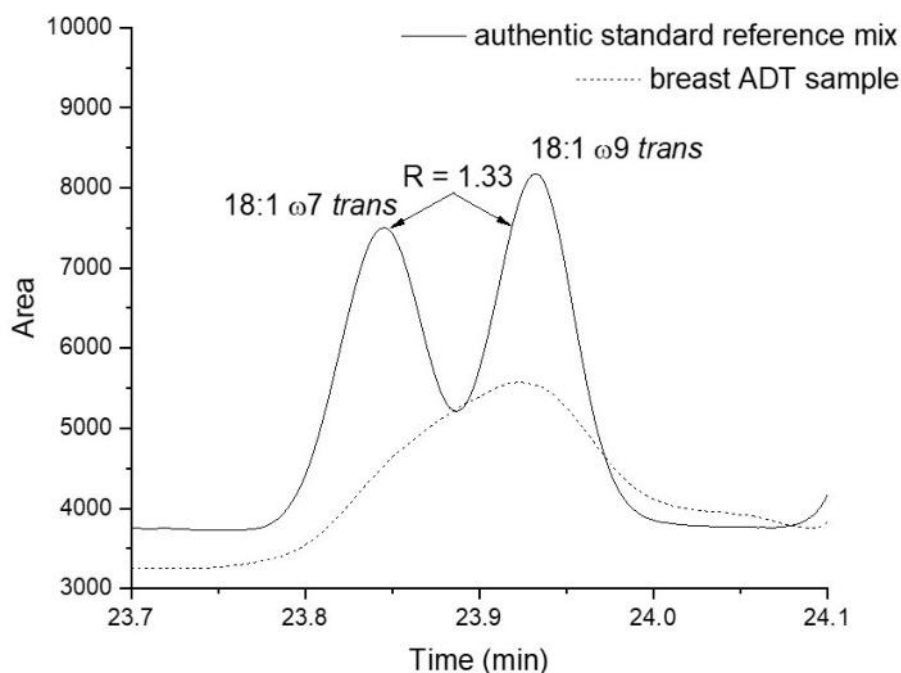
Figure 5.8.: Seperation efficiency of 18:1 ω7 *trans* and 18:1 ω9 *trans* after separation and detection with GC-MS/FID. Even though 18:1 ω7 *trans* and 18:1 ω9 *trans* were seperated in the authentic standard reference mix (t_R=23.84 min and t_R=23.93min , respectively; black line) with the resolution R=1.33, no seperation was achieved, when analysis was performed with breast ADT samples (dotted black line).

GLT matrix (data not shown). Concludingly, this method has the disadvantage that only a sum of all 18:1 *trans* in the authentic standard reference mix (sum of 18:1 ω9 *trans* and 18:1 ω7 *trans*) can be measured. Therefore a pure objective measurement of (partially) hydrogenated vegetable oils can not be obtained, since 18:1 ω9 *trans* is the only objective dietary FA biomarker for (partially) hydrogenated vegetable oils (ch. 2.2.3.4). For dairy intake, not only 18:1 ω7 *trans* serves as a dietary FA biomarker (ch. 2.2.3.1). It is therefore possible to use other FA biomarker for a pure objective measurement of dairy fat. It is common to measure the sum of 18:1 *trans* since separation efficiency between 18:1 *trans* isomers is hard to achieve (Pranger, Joustra, et al. 2019). Putative beneficial or adverse health effects of TFAs from industrial and ruminant origin may vary according to their

origin. Up to now, industrial derived TFAs are found to be more harmful than ruminant derived TFAs (summarized in Pipoyan et al. 2021). It must therefore be taken into account that the sum of 18:1 *trans* (18:1 ω9 *trans* and 18:1 ω7 *trans*) established in the GC-MS/FID method should be used with caution due to the potential bias when apply this variable into potential health outcomes or as an explanatory variable in multivariate statistics.

20:4 ω6 and 23:0

No separation was achieved for 20:4 ω6 and 23:0 (fig. 5.9).
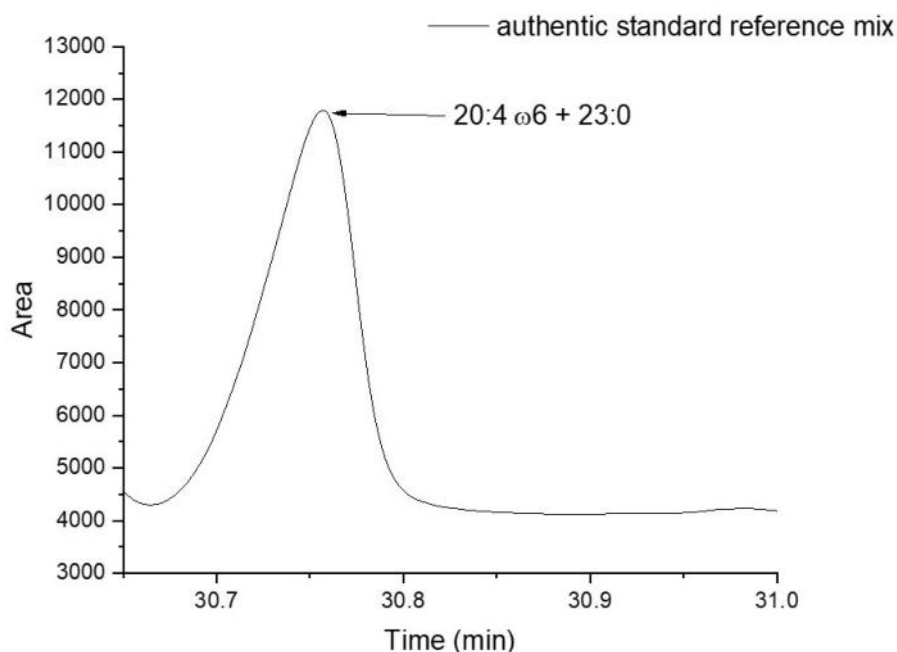
Figure 5.9.: Chromatogram of the authentic standard reference mix from 30.6 to 31.0 minutes showing only one peak for FAMEs 20:4 ω6 and 23:0. No separation was achieved for 20:4 ω6 and 23:0 due to complete overlapping of peaks.

23:0 is expected to be absent in human biospecimens and is used as an IS for FAME analysis (summarized in Chiu and Kuo 2020). Therefore, no consequences were expected for the analysis of 23:0 due to the absence of this FAME in human biospecimens. Also no consequences were expected for the analysis of 20:4 ω6 due to no expected bias triggered by potential coeluation of 23:0.

5.2.2. Implementation of method

The scope of implementation of the GC-MS/FID method for FA analysis included the selection of two IS (ch. 5.2.2.1), the calculation of recoveries (ch. 5.2.2.2), since it was

unknown, if loss of specific FAs occured during extraction of FAs from biospecimens and the calculation of LODs of important FA biomarkers and FAs, which were detected in the lowest range of FA composition (ch. 5.2.2.3). Since one of the objectives was to compare the FA composition of GLT with ADT from the same person from the ISOCROSS stuy (ch. 3), comparison of the method described in this manuscript (ch. 4.2.3) and the implemented method from Mahdiani 2017 (ch. 4.2.5.3) were compared (ch. 5.2.2.4).

5.2.2.1. Choice of IS

13:0 and 19:0 were used as IS due to the following reasons: (I) Neither 13:0 nor 19:0 were expected to be present in human biospecimens due to the odd-chain character. No endogenous biosynthesis and dietary intake of 13:0 and 19:0 were expected, since 13:0 and 19:0 have been established as IS in the past two decades (summarized in Chiu and Kuo 2020). It was therefore hypothesized, that GLT and ADT samples would not contain these IS. (II) t_R of 13:0 and 19:0 (14.79 and 24.75 min, respectively; tab. 5.1) were not biased by possible coeluating FAMEs.

5.2.2.2. Recoveries

Since FAs may vary in polarity due to the length of the respecting alkyl chain (ch. 2.2), it was important to determine whether a trend of preferred extracting of FAs with long or short alkyl chains may occur with the extracting method described in ch. 4.2.3.1, which was used to analyze GLT and ADT samples in this thesis. To analyze recoveries, the IS 13:0 and 19:0 were used. Therefore recoveries were determined as described in ch. 4.2.3.5 for the IS 13:0 and 19:0 and difference between the recoveries of 13:0 and 19:0 are depicted in fig. 5.10 left plot. Furthermore, the difference between the ratio of $\frac{13:0_{(\%)}}{19:0_{(\%)}}$ pre- and postextraction was calculated (fig. 5.10) to evaluate the extraction efficiency for FAs with shorter alkyl chains (13:0) in comparison to FAs with longer chains (19:0) and possible consequences thereof. Mean recovery for 13:0 was $\bar{x}_{13:0} = 90.51\%$ and for 19:0 $\bar{x}_{19:0} = 96.21\%$. Both analytes reached recovery rates >90%. Thus there is no significant loss of FAs with shorter and longer chains to be expected. In comparison to each other, there was a slightly higher recovery rate for 19:0 (difference between recovery rates of 13:0 to 19:0 were all below zero; fig. 5.10 left plot), however no statistical significance was reached for comparison between the recovery rates of 13:0 and 19:0 (P = 0.246; fig. 5.10 left plot). No difference was observed between the ratio of $\frac{13:0_{(\%)}}{19:0_{(\%)}}$ pre- and postextraction (P = 0.252; fig. 5.10 right plot). Consequently, there is no preferred trend observed for extraction efficiency regarding FAs with lower or higher alkyl chains.
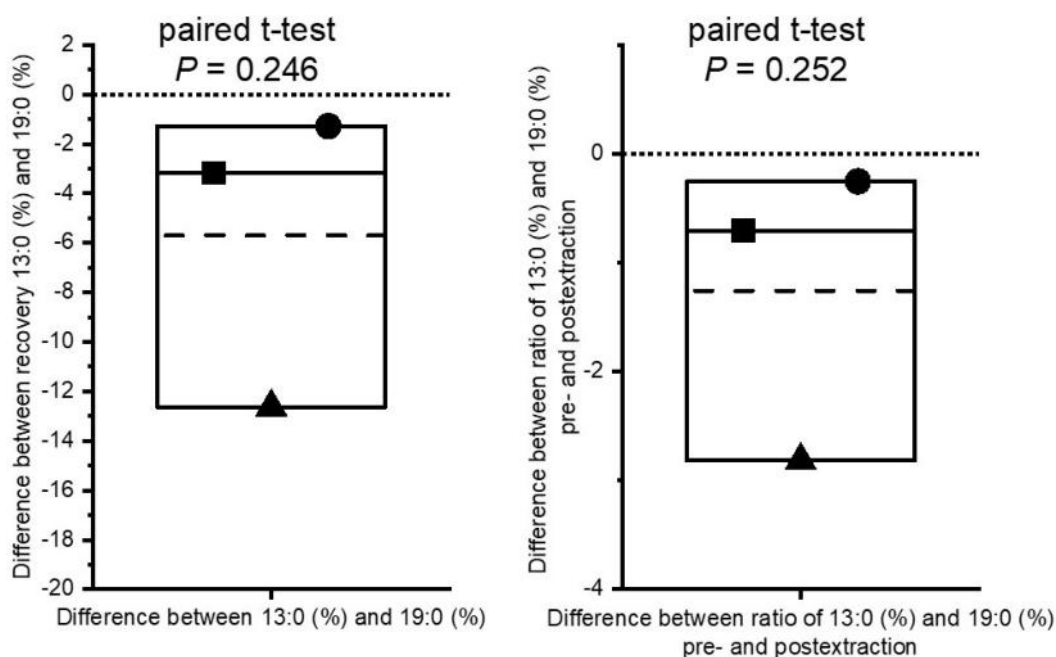
Figure 5.10.: Calculated difference boxplots after extracting method described in ch. 4.2.3.1 for the recoveries of 13:0 (%) and 19:0 (%) pre- and postextraction (left plot) as well as for the ratio of 13:0 (%) and 19:0 (%) pre- and postextraction. Statistical evaluation was performed with paired t-tests and different geometric forms indicate different samples. Boxplot shows data points from 25th to 75th percentile, the dotted line shows the mean of the three samples and the black line shows the median of the three samples. The dotted line at 0 shall be used as an orientation for the location of the respective data points.

5.2.2.3. LODs

LODs were calculated for ADT samples from WASP according to ch. 4.2.3.5. For LOD determination only those FAs were chosen, which were either not detectable at all (21:0, 22:0, 23:0, 24:0, 15:1 ω5, 24:1 ω9, 18:2 ω6 *transtrans*) or in a subset (22:6 ω3) of ADT samples or were found to only small amounts in ADT (18:3 ω6, 20:3 ω6, 20:3 ω3, 20:5 ω3, 22:2 ω6), where LOD determination seemed to be appropriate due to the small proportion of respective FAs on FA composition. For FAs, which were either not detectable at all or in a subset of ADT samples of WASP, LODs of respective FAs were calculated as proportion of FA_{LOD} (%) on total FA composition (ch. 4.2.3.5) and box-plotted (fig. 5.11 and 5.12). FA_{LOD} were determined as LOD_{median} of respective FAs and LOD_{median} obtained for those FAs were $21{:}0_{LOD}= 0.075\%$; $22{:}0_{LOD}= 0.058\%$; $23{:}0_{LOD}= 0.017\%$; $24{:}0_{LOD}= 0.056\%$; $15{:}1\ \omega5_{LOD}= 0.018\%$; $24{:}1\ \omega9_{LOD}= 0.077\%$; $18{:}2\ \omega6\ transtrans_{LOD}=0.028\%$ and $22{:}6\ \omega3_{LOD}= 0.023\%$ (fig.

5.11 and 5.12).
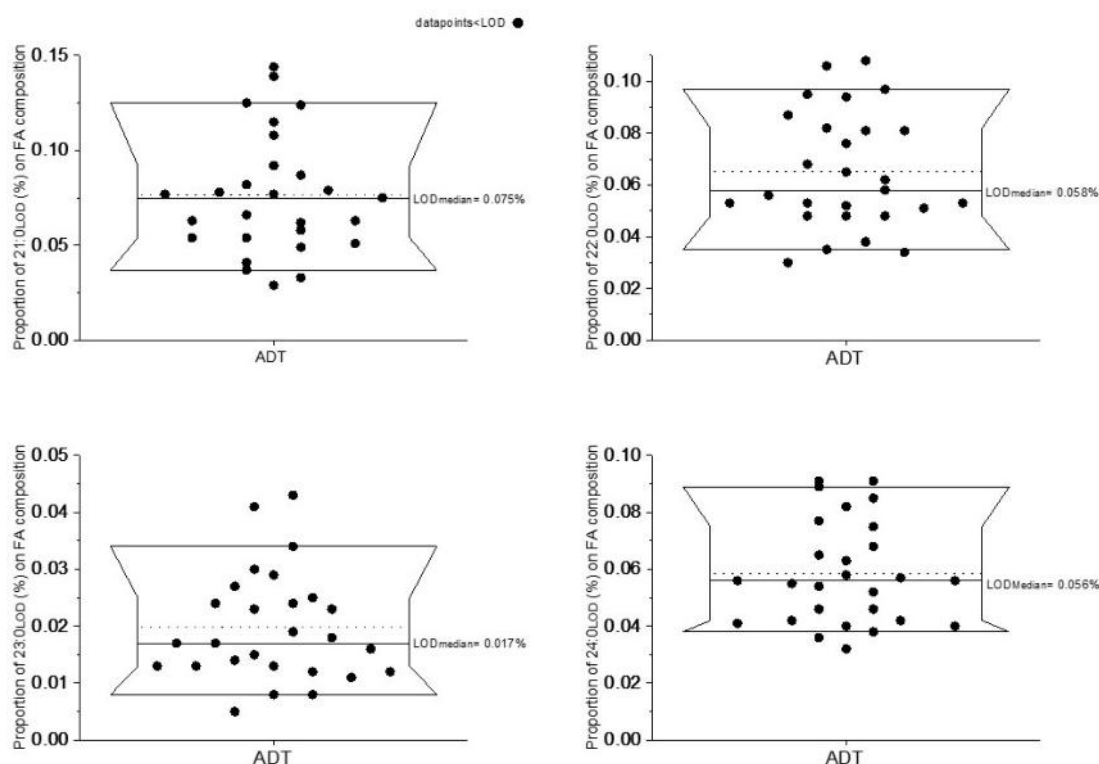
Figure 5.11.: Boxplots of LODs for n=27 ADT samples of WASP for the FAs 21:0, 22:0, 23:0
and 24:0. FA_{LOD} was determined as LOD_{median} of respective FAs. $21{:}0_{LOD}=$
0.075%, $22{:}0_{LOD}= 0.058\%$, $23{:}0_{LOD}= 0.017\%$, $24{:}0_{LOD}= 0.056\%$. Boxplot shows
data points from 10th to 90th percentile, the dotted line shows the mean of
the samples and the black line shows the median of the samples. LODs were
calculated according to ch. 4.2.3.5.

For those FAs ((21:0, 22:0, 23:0, 24:0, 15:1 ω5, 24:1 ω9, 18:2 ω6 *transtrans*), which were not
detectable at all in ADT samples of WASP (n=27 samples), the FA_{LOD} was interpreted as
that proportion of respective FA on total FA composition, which could not be excluded in
ADT due to methodological reasons. In the following chapters FA composition will not
be calculated with those FA_{LODs}, therefore FA_{LODs} from FAs, which were not detectable
at all in ADT samples of WASP will not be contribute to total FA composition. 22:6 ω3
was not detectable in a subset of ADT samples (n= 7 samples). Therefore 22:6 $\omega3_{LOD}$
was determined in that subset and proportion of 22:6 $\omega3_{LOD}$ on total FA composition was
0.023% (fig. 5.12, bottom right plot). Conlcudingly, for those samples in which 22:6 ω3
was detected but the proportion of 22:6 ω3 on total FA composition was below 0.023%,
22:6 $\omega3_{LOD}$ was applied. Consequences on proportion of 22:6 ω3 in overall ADT samples
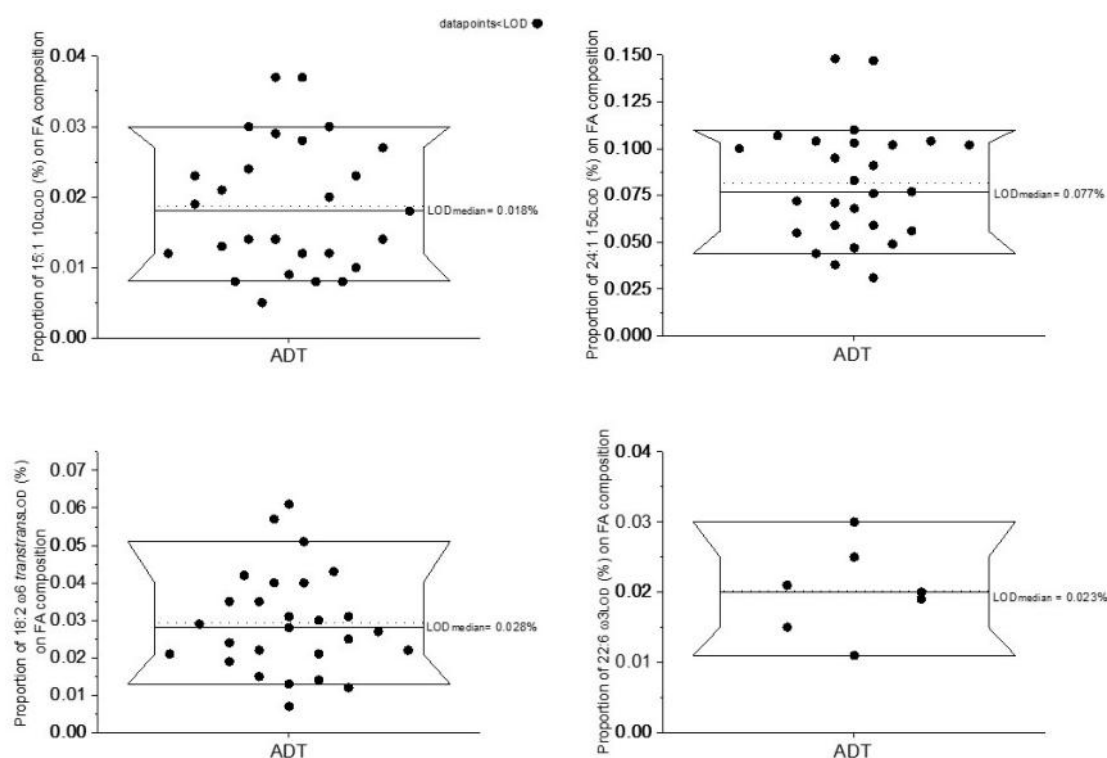
Figure 5.12.: Boxplots of LODs for n=27 ADT samples of WASP for the FAs 15:1 ω5, 24:1 ω9, 18:2 ω6 *transtrans* and 22:6 ω3. For 22:6 ω3, LODs were calculated for n= 7 samples. FA$_{LOD}$ was determined as LOD$_{median}$ of respective FAs. 15:1 ω5$_{LOD}$= 0.018%, 24:1 ω9$_{LOD}$=0.077%, 18:2 ω6 *transtrans*$_{LOD}$= 0.028%, 22:6 ω3$_{LOD}$= 0.023%. Boxplot shows data points from 10th to 90th percentile, the dotted line shows the mean of the samples and the black line shows the median of the samples. LODs were calculated according to ch. 4.2.3.5.

of WASP will be discussed in ch. 5.4.

LODs were further calculated for FAs (18:3 ω6, 20:3 ω6, 20:3 ω3, 20:5 ω3, 22:2 ω6), which were found to only small amounts in ADT. Since all of these mentioned FAs were detected in all ADT samples of WASP, LODs were calculated from detected signals of ADT samples from the 10th percentile and below (ch. 4.2.3.5). Mean FA$_{LOD}$ for 18:3 ω6, 20:3 ω6, 20:3 ω3, 20:5 ω3, 22:2 ω6 are depicted in tab. 5.2.

Concludingly, for those samples in which 18:3 ω6, 20:3 ω6, 20:3 ω3, 20:5 ω3 or 22:2 ω6 were detected but the proportion of respective FA on total FA composition was below the calculated FA$_{LOD}$, respective FA$_{LOD}$ was applied for that sample. Consequences on proportion of 18:3 ω6, 20:3 ω6, 20:3 ω3, 20:5 ω3 or 22:2 ω6 in ADT samles of WASP will be discussed in ch. 5.4.

Table 5.2.: Calculated mean FA_{LOD} for 18:3 ω6, 20:3 ω6, 20:3 ω3, 20:5 ω3 and 22:2 ω6. LODs were calculated according to the ch. 4.2.3.5.

FA	LOD (%)
18:3 ω6	0.009
20:3 ω6	0.031
20:3 ω3	0.004
20:5 ω3	0.007
22:2 ω6	0.011

5.2.2.4. Comparison of methods

One of the objectives was to compare the FA composition of GLT with ADT from the same person from the ISOCROSS study, especially regarding dietary FA biomarkers. Mahdiani 2017 already analyzed a subset of ADT samples regarding FA composition from the ISOCROSS study with her implemented GC-FID method summarized in ch. 4.2.5.3. A subset of GLT samples were analyzed with the GC-MS/FID method described in this thesis (ch. 4.2.3). Comparison between GLT samples, which were analyzed according to the implemented method in this thesis (GC-MS/FID; ch. 4.2.3) and already analyzed ADT samples from Mahdiani 2017 (GC-FID; ch. 4.2.5.3) would only be possible to excute, if no method bias between these methods exist. Therefore it needed to be examined initially, if the implemented GC-MS/FID method according to ch. 4.2.3 and the method of Mahdiani 2017 (summarized in ch. 4.2.5.3) were comparable for FA analysis. Three ADT samples from the ISOCROSS study (ch. 4.2.1.2) were worked up with the method described in ch. 4.2.3 and compared with the same already analyzed samples from Mahdiani (ch. 4.2.5.3) and statistically tested with paired t-tests (ch. 4.2.3.5). The means of the individual FA percentages (n= 27 identified FAs) from the three samples were calculated and compared. Statistical significant differences between individual FAs were found for 12:0, 14:0, 15:0, 16:0, 16:1 ω7 *trans*, 17:0, 17:1 ω7, 18:1 ω9 *trans*, 18:1 ω9, 18:2 ω6, 20:0, 18:3 ω3, 22:0, 20:3 ω6, 20:4 ω6, 22:2 ω6 and 22:6 ω3 (P-value < 0.05 for all; tab. 5.3).

Table 5.3.: Comparison of means of the individual FA percentages from three samples of the ISOCROSS study population from samples analyzed with the method described in this manuscript (ch. 4.2.3; Jaud) and reinjection of already analyzed samples (from Mahdiani 2017). Statistical evaluation was carried out with paired t-tests for every identified FA and significance was reached for $P < 0.05$. $\bar{x}$ indicates the mean of the respective identified FA from the three samples.

FA	$\bar{x}$ Jaud (%)	$\bar{x}$ Mahdiani (%)	P-value	FA	$\bar{x}$ Jaud (%)	$\bar{x}$ Mahdiani	P-value
12:0	0.96	0.87	0.0161	20:0	0.33	0.25	0.0015
14:0	4.07	3.89	0.0441	18:3 ω6	0.05	0.04	0.2254
14:1 ω5	0.36	0.34	0.2254	20:1 ω9	0.76	0.84	0.2327
15:0	0.42	0.38	0.0051	18:3 ω3	0.45	1.06	0.0063
16:0	24.60	23.39	0.0781	20:2 ω6	0.17	0.18	0.7418
16:1 ω7 *trans*	0.31	0.19	0.0068	22:0	0.22	0.08	0.0214
16:1 ω7	4.15	3.95	0.5165	20:3 ω6	0.10	0.16	0.0091
17:0	0.35	0.32	0.0351	22:1 ω9	0.16	0.12	0.1215
17:1 ω7	0.31	0.28	0.0153	20:3 ω3	0.03	0.04	0.2254
18:0	6.60	6.45	0.6395	20:4 ω6	0.17	0.38	0.0624
18:1 ω9 *trans*	1.78	0.74	0.0057	22:2 ω6	0.14	0.05	0.0041
18:1 ω9	42.21	42.89	0.0403	20:5 ω3	0.04	0.04	0.7418
18:1 ω7	1.71	1.77	0.2759	22:6 ω3	0.04	0.11	0.0171
18:2 ω6	9.38	11.13	0.0163				

According to the European Food Safety Authority (EFSA) scientific committee (Committee 2011) significance should only be related to statistical concepts, however relevant effects should be evaluated and interpreted into causal context of the methodology used and importance attributed to the findings. Statistical significance (P-value < 0.05, tab. 5.3) was reached for the majority of FAs in comparison between the two methods. However, statistical significance does not necessarily mean that relevant effects have to be expected. Therefore, possible relevant effects regarding the question whether the comparison of the FA composition of GLT analyzed according to the method described in ch. 4.2.3 and ADT samples from the same female donor analyzed according to the method described in ch. 4.2.5.3 (Mahdiani 2017) would be influenced by the observed statistical significance or not must be considered and evaluated. For the evaluation of possible relevant effects histograms, calculated from the FA percentages from respective compositions of all ADT samples from Mahdiani (Mahdiani 2017), of every FA were plotted. The location of data points from every FA of ADT samples used from Mahdiani (n=50; Mahdiani 2017), the reinjection of ADT samples from Mahdiani (ch. 4.2.3.5) and the ADT samples treated according to ch. 4.2.3 were drawn into the histograms (fig. 5.13, 5.14, 5.15 and app. A.16).

<u>Milk FA biomarker</u>
Even though statistical significance was reached for the comparison of 17:0 (P = 0.0351; fig. 5.13 right plot), the data points from the same samples of Mahdiani (Mahdiani 2017), the reinjection as well as the ADT samples analyzed according to ch. 4.2.3 were located nearly identical. Consequently, no relevant effect of the method for the comparison of 17:0, as an biomarker for milk and ruminant fat (ch. 2.2.3.1), between GLT and ADT was expected. Similar to 17:0, statistical significance was reached for the comparison of 15:0, too (P = 0.0038; fig. 5.13, left plot). However there was a tendency towards higher 15:0 percentages in samples treated according to ch. 4.2.3 observed. Thereby shifts 15:0 from mid located (data from Mahdiani 2017) towards the higher end of the gaussian curve (data from ADT samples analyzed according to ch. 4.2.3). This may be a relevant effect for the comparison of 15:0 in GLT and ADT through potential bias triggered by the observed shift. By that, it may be possible that a putative significant and relevant effect will be observed, even if there exists no effect and vice versa. However, since no effect was expected for 17:0 alone, the assessment and comparison of milk and ruminant fat FA biomarkers (ch. 2.2.3) would be feasible by reinjection of ADT samples from Mahdiani (Mahdiani 2017) and GLT samples from the same women treated according to ch. 4.2.3.
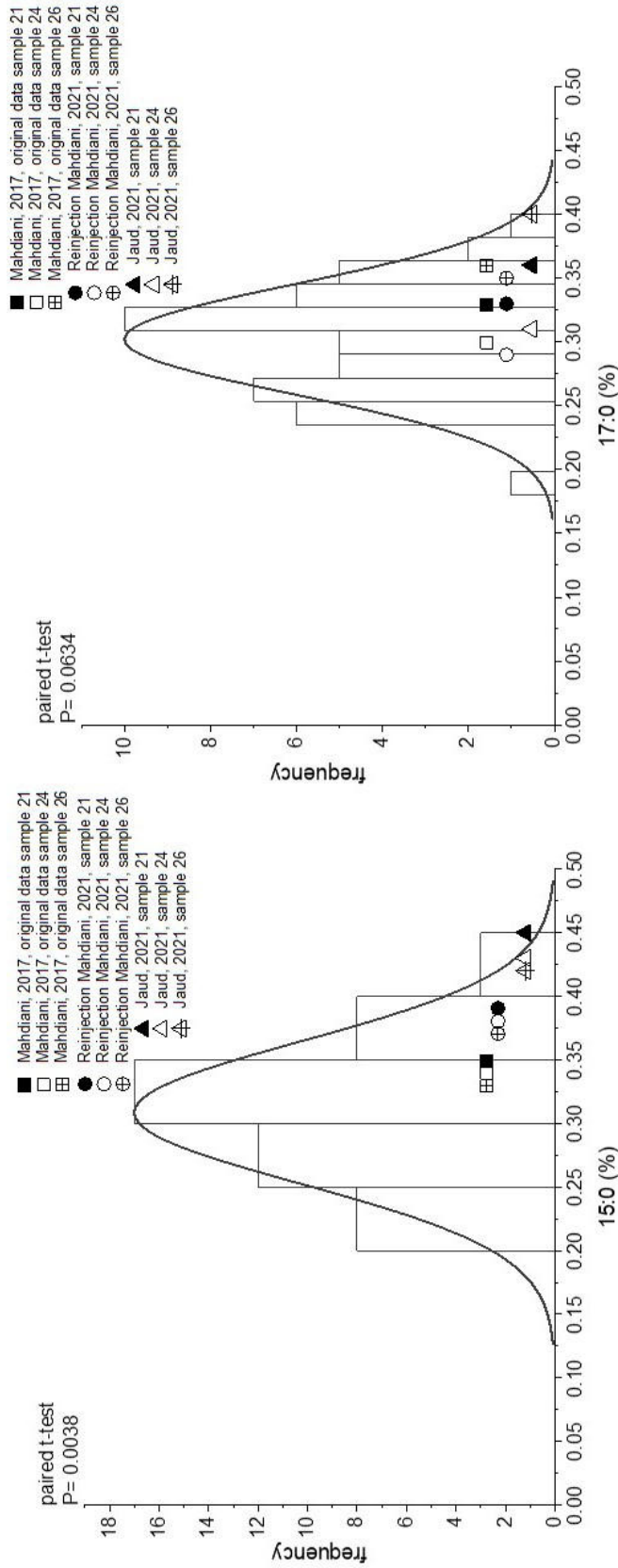
Figure 5.13.: Histograms for the milk FA biomarkers (ch. 2.2.3) 15:0 (left) and 17:0 (right). The plotted distribution was calculated from 15:0 and 17:0 percentages, respectively, from respective composition of all ADT samples from Mahdiani (Mahdiani 2017). The location of data points from sample 21, 24 and 26 of ISOCROSS of 15:0 and 17:0, respectively, were drawn into the histograms from the original data from Mahdiani (Mahdiani 2017), the reinjection of ADT samples from Mahdiani (ch. 4.2.3.5) and the ADT samples treated according to ch. 4.2.3. Prior plotting of the location of data points, the FA composition was calculated only by all FAs Mahdiani identified (Mahdiani 2017; app. A.16). Paired t-tests were calculated between the reinjection of ADT samples from Mahdiani and ADT samples treated according to ch. 4.2.3
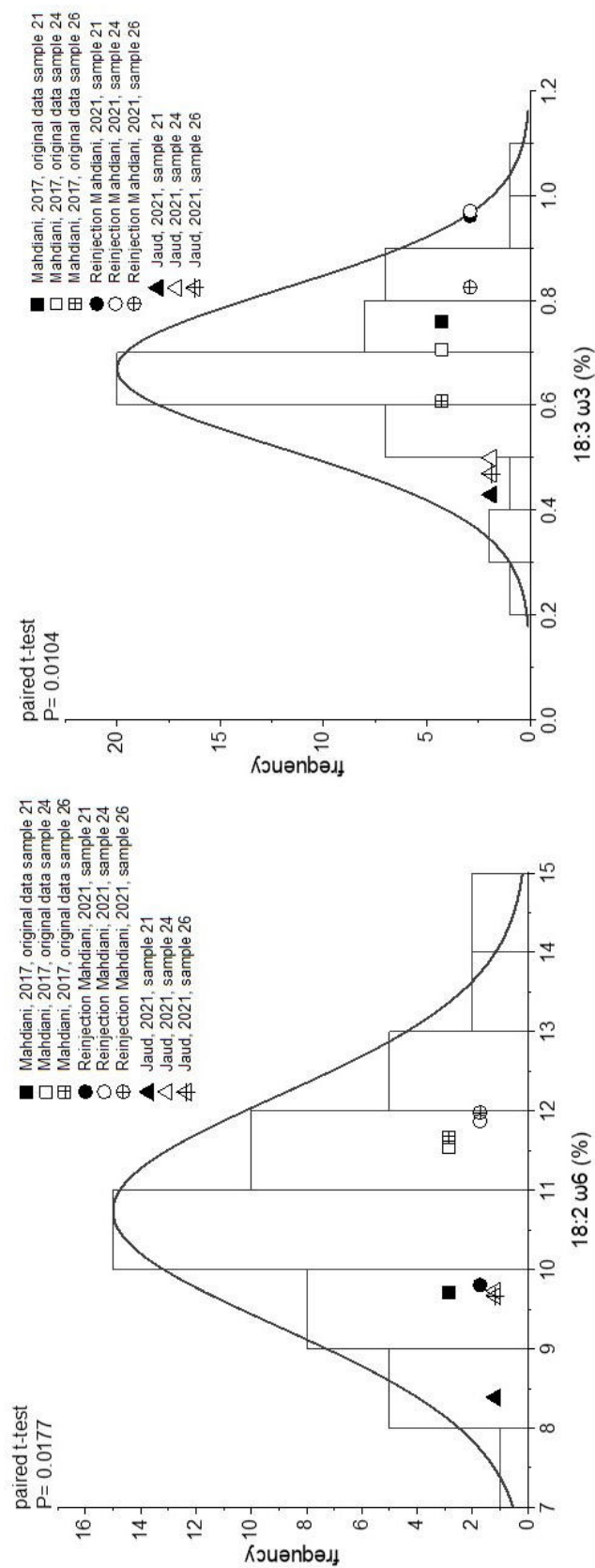
Figure 5.14.: Histograms for the vegetable oils and nuts FA biomarkers (ch. 2.2.3) 18:2 ω6 (left) and 18:3 ω3 (right). The plotted distribution was calculated from 18:2 ω6 and 18:3 ω3 percentages, respectively, from respective composition of all ADT samples from Mahdiani (Mahdiani 2017). The location of data points from sample 21, 24 and 26 of ISOCROSS of 18:2 ω6 and 18:3 ω3, respectively, were drawn into the histograms from the original data from Mahdiani (Mahdiani 2017), the reinjection of ADT samples from Mahdiani (ch. 4.2.3.5) and the ADT samples treated according to ch. 4.2.3. Prior plotting of the location of data points, the FA composition was calculated only by all FAs Mahdiani identified (Mahdiani 2017; app. A.16). Paired t-tests were calculated between the reinjection of ADT samples from Mahdiani and ADT samples treated according to ch. 4.2.3

Figure 5.15.: Histograms for the fish FA biomarkers (ch. 2.2.3) 20:5 ω3 (left) and 22:6 ω3 (right). The plotted distribution was calculated from 20:5 ω3 and 22:6 ω3 percentages, respectively, from respective composition of all ADT samples from Mahdiani (Mahdiani 2017). The location of data points from sample 21, 24 and 26 of ISOCROSS of 20:5 ω3 and 22:6 ω3, respectively, were drawn into the histograms from the original data from Mahdiani (Mahdiani 2017), the reinjection of ADT samples from Mahdiani (ch. 4.2.3.5) and the ADT samples treated according to ch. 4.2.3. Prior plotting of the location of data points, the FA composition was calculated only by all FAs Mahdiani identified (Mahdiani 2017; app. A.16). Paired t-tests were calculated between the reinjection of ADT samples from Mahdiani and ADT samples treated according to ch. 4.2.3

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Figure 5.16.: Histogram for the sum of 18:1 *trans* (18:1 *ω9 trans* and 18:1 *ω7 trans*) (ch. 2.2.3). The plotted distribution was calculated from the sum of 18:1 *trans* percentages from respective composition of all ADT samples from Mahdiani (Mahdiani 2017). The location of data points from sample 21, 24 and 26 of ISOCROSS of 18:1 *ω9+ω7 trans*, respectively, were drawn into the histograms from the original data from Mahdiani (Mahdiani 2017), the reinjection of ADT samples from Mahdiani (ch. 4.2.3.5) and the ADT samples treated according to ch. 4.2.3. Prior plotting of the location of data points, the FA composition was calculated only by all FAs Mahdiani identified (Mahdiani 2017; app. A.16). Paired t-tests were calculated between the reinjection of ADT samples from Mahdiani and ADT samples treated according to ch. 4.2.3

Vegetable oils and nuts FA biomarker

Statistical significance was also reached for the comparison of 18:2 *ω6* and 18:3 *ω3* (P = 0.0177, fig. 5.14 left plot and P = 0.0104, fig. 5.14 right plot, respectively). There was a slightly decrease in 18:2 *ω6* percentages observed in ADT samples treated according to the method described in this manuscript (ch. 4.2.3) in comparison to ADT samples of Mahdiani (Mahdiani 2017) and the reinjection of ADT samples from Mahdiani (ch. 4.2.3.5). However, this effect may be small and putatively not relevant for the comparison of GLT and ADT. Opposite location of data points were observed from ADT samples analyzed according to ch. 4.2.3 (left shifted) and reinjection of ADT samples from Mahdiani (right shifted)

in comparison to the original samples of Mahdiani (Mahdiani 2017) for percentages of 18:3 ω3. This observation might have a high impact on the comparison of 18:3 ω3 in GLT and ADT because the gaussian curve would either shift to lower percentages (if ADT samples would be analyzed according to ch. 4.2.3) or to higher percentages (if reinjection of ADT samples from Mahdiani would be applied). However, because GLT was treated according to the method described in ch. 4.2.3, the ADT samples from the same women from the study population of ISOCROSS should be treated identical for the comparison in regards to vegetable oils and nuts biomarkers (ch. 2.2.3).

Fish biomarker

No significant differences between reinjection of ADT samples from Mahdiani (ch. 4.2.3.5) and ADT samples analyzed according to the method described in ch. 4.2.3 were observed for 20:5 ω3 (P = 1.0000; fig. 5.15, left plot). The data points from the ADT samples of Mahdiani (Mahdiani 2017), the reinjection as well as the ADT samples treated according to ch. 4.2.3 were located nearly identical. Significant differences were observed for the comparison of 22:6 ω3 (P = 0.0438; fig. 5.15, right plot). There was a tendency observed towards lower percentages of 22:6 ω3 in the ADT samples analyzed according to the method described in this manuscript (ch. 4.2.3). However reinjection of ADT samples from Mahdiani were nearly identically located to the data points from ADT samples of Mahdiani (Mahdiani 2017). Similar to 15:0, this may be a relevant effect for the comparison of 22:6 ω3 in GLT and ADT through potential bias triggered by the observed shift to lower percentages of 22:6 ω3. Since no significance was observed for 20:5 ω3 and no relevant effect was expected thereof, the assessment of fish FA biomarkers (ch. 2.2.3) with reinjection of ADT samples from Mahdiani (ch. 4.2.3.5) and GLT samples from the same women from the study population of ISOCROSS analyzed according to ch. 4.2.3 would be feasible.

Sum of 18:1 *trans* (18:1 ω9 *trans* and 18:1 ω7 *trans*)

Significant differences were observed between reinjected samples (ch. 4.2.3.5) and ADT samples analyzed according to the method described in this manuscript (ch. 4.2.3) for percentages of 18:1 ω9+ω7 *trans* (P = 0.0059; fig. 5.16). Both reinjected and ADT samples analyzed according to ch. 4.2.3 showed strong shifts towards higher percentages of 18:1 ω9+ω7 *trans* in comparison to the data points from the ADT samples of Mahdiani (Mahdiani 2017). Furthermore there was also a strong shift towards higher percentages of 18:1 ω9+ω7 *trans* observed for ADT samples analyzed according to the method described in this manuscript (ch. 4.2.3) in comparison to reinjected ADT samples from Mahdiani.

Consequently, relevant methodological effects for the comparison of 18:1 ω9+ω7 *trans* in GLT and ADT from the same women in the study population of ISOCROSS were expected, since different percentages were obtained ($\bar{x}$ of reinjected ADT samples from Mahdiani 0.74% vs. $\bar{x}$ of ADT samples analyzed according to the method described in ch. 4.2.3 1.81%,respectively; app. A.16). Consequently, for the comparison of the sum of 18:1 ω9+ω7 *trans* in GLT and ADT from the same women in the study population of ISOCROSS, samples should be treated identical.

Miscellaneous FAs

For SFAs and MUFAs (app. A.16), no relevant effects were observed. However, for PUFAs (app. A.16) putative relevant effects were observed for 20:3 ω6 (P = 0.0027, left shift of ADT samples analyzed according to the method described in ch. 4.2.3 in comparison to reinjected samples from Mahdiani) and 20:4 ω6 (P = 0.0523, left shift of ADT samples analyzed according to the method described in ch. 4.2.3 and right shift of reinjected ADT samples from Mahdiani in comparison to data points from ADT samples of Mahdiani (Mahdiani 2017)).

Overall

For most of FA biomarkers (except of 20:5 ω3; fig. 5.15, left plot) both statistical significance and relevant effects thereof were observed. Furthermore diverse PUFAs (20:3 ω6 and 20:4 ω6, respectively; app. A.16) were affected by significant differences between ADT samples analyzed according to the method described in this manuscript (ch. 4.2.3) and reinjected ADT samples from Mahdiani (ch. 4.2.3.5) and consequently by relevant methodological effects in relation to the comparison of FAs in GLT and ADT, too. Conclusively ADT and GLT samples from the same women from the study population of ISOCROSS were identically analyzed according to ch. 4.2.3 and will be further compared in ch. 5.3.

5.3. FAs in human breast tissues - comparison of dietary biomarkers from ADT and GLT samples from the study population of ISOCROSS

Since biomarker analysis has been mostly investigated in compartments, which were easy to obtain, FA biomarker analysis of biospecimens such as plasma, serum and ADT are well-known and has been validated against dietary measurement tools (e.g. FFQ; ch. 2.2.3). In general, diet represents an important factor in the development of diverse diseases. Therefore objective marker for diet and dietary intake of nutrients are important for the estimation of diet-disease associations but also in evaluating influence of diet on potential risk factors of known diseases (ch. 2.2.3). Dietary FA biomarker have been established in ADT (ch. 2.2.3). The human female breast is mainly built of ADT and GLT, which are not always available separately often leading to mixed breast tissue samples (either breast ADT samples contaminated with GLT and vice versa; ch. 2.1.2). It is therefore unknown, if differentiation between breast GLT and ADT has a relevant impact on the analysis of dietary FA biomarkers or if the contamination of breast GLT with breast ADT and vice versa is neglectable for the analysis of dietary FA biomarker. Thus, the aim was to determine the relative FA profile in GLT samples from the ISOCROSS study (ch. 4.2.1.2; 5.3.1) and to compare relative concentrations of FAs serving as biomarkers for food intake (ch. 2.2.3) with ADT breast samples from the same women (ch. 5.3.2).

5.3.1. GLT FA composition

The GLT FA compositions (n=5 GLT samples) and the ADT FA compositions from the same women of ISOCROSS are depicted in tab. 5.4 and 5.5 and were calculated as the means of individual FAs from respective samples. Chromatograms and FA compositions from the participants are depicted in app. A.17. In total, 26 FAs were determined, of which were 7 FAs SFA, 8 FAs MUFA, 9 FAs PUFA and 2 FAs TFA (tab. 5.4 and 5.5). For the ADT samples from the same women, the same amount of FAs and respective FA classes were found. MUFA represents the largest FA class (55.28%; tab 5.4) with both endogenous and exogenous FAs. As expected, the largest amount of MUFAs encompasses ω9 FAs with its most dominant representative 18:1 ω9. Since ω9 FAs are both, synthesized endogenously (ch. 2.2.1) and consumed via diet, the proportion of ω9 FAs on MUFA is >80%. Furthermore, ω5 and ω7 FAs were found in GLT. Subsequently after MUFA, SFA class follows as second largest FA class (33.00%, tab. 5.4) with both endogenous and exogenous FAs. 15:0 and 17:0,

representatives of odd-chain milk FA biomarker (ch. 2.2.3.1), were found in the SFA class (tab. 5.4).

Table 5.4.: Mean FA composition of GLT and ADT samples from the same women of the study population ISOCROSS (n=5). Percentages of the respective FA-classes were calculated as the sum of the FA proportions within these FA classes. FA-classes shown in this table: SFA and MUFA.

		FA (%)			FA class (%)	
FA-class	FA	ADT	GLT	P-value	ADT	GLT
SFA	12:0	0.58	0.50	1.000		
	14:0	3.25	2.83	1.000		
	15:0	0.42	0.48	0.572		
	16:0	24.68	23.89	1.000	35.63	33.00
	17:0	0.32	0.30	0.936		
	18:0	6.18	4.88	1.000		
	20:0	0.20	0.13	1.000		
MUFA	14:1 ω5	0.30	0.34	1.000		
	16:1 ω7	4.54	5.22	1.000		
	17:1 ω7	0.34	0.35	1.000		
	18:1 ω9	45.27	45.29	1.000	53.71	55.28
	18:1 ω7	2.53	3.13	1.000		
	20:1 ω9	0.71	0.82	1.000		
	22:1 ω9	0.23	0.12	0.240		

Table 5.5.: Mean FA composition of GLT and ADT samples from the same women of the study population ISOCROSS (n=5). Percentages of the respective FA-classes were calculated as the sum of the FA proportions within these FA classes. FA-classes shown in this table: PUFA and TFA.

FA-class	FA	FA (%)			FA class (%)	
		ADT	GLT	P-value	ADT	GLT
PUFA	18:2 ω6	7.58	9.19	0.282		
	18:3 ω6	0.048	0.036	1.000		
	18:3 ω3	0.34	0.47	0.936		
	20:2 ω6	0.17	0.22	0.406		
	20:3 ω3	0.017	0.021	1.000	8.46	10.22
	20:4 ω6	0.10	0.18	0.153		
	22:2 ω6	0.150	0.054	0.270		
	20:5 ω3	0.041	0.034	0.252		
	22:6 ω3	0.063	0.039	0.936		
TFA	16:1 ω7 *trans*	0.26	0.33	0.485	1.60	1.31
	18:1 9+11 *trans*	1.34	0.98	0.192		

The majority of SFA represents FAs either from *de novo* synthesis and further elongation steps (ch. 2.2.1) or habitual diet. FA biomarker from vegetable oils and nuts as well as fish (ch. 2.2.3.3 and 2.2.3.2) were found in PUFA class (tab. 5.5). Western diets typically have a ratio of ω6 to ω3 essential FAs of 15-16.7 (Simopoulos 2002). Since the german population is expected to have a western-diet behavior (ch. 2.2.2) the $\frac{18:2\ \omega6}{18:3\ \omega3}$ ratio of 19.7 is indicative for the western-diet lifestyle. Therefore, the largest proportion of the PUFA class is represented by ω6 FAs (94.6%), with its most dominant representative 18:2 ω6. Since 18:2 ω6 can not be synthesized endogenously (ch. 2.2.1) it serves as a biomarker of vegetable oils (ch. 2.2.3.2). The smaller part of the PUFA class encompasses ω3 FAs (tab. 5.5), which were all FAs with proportion of total FAs <1%. 18:3 ω3 serves as biomarker for the dietary intake of nuts, whereas 20:5 ω3 and 22:6 ω3 are representative for the dietary intake of fish (ch. 2.2.3.3 and 2.2.3.2). The smallest part of FA class on total FAs encompasses TFAs (tab. 5.5). Since TFAs can not be endogenously synthesized by the human body, all

TFAs are expected to be exclusively exogenous origin (ch. 2.2.3.1 and 2.2.3.4). Interestingly, the sum of 18:1 *trans* (18:1 ω7 *trans* + 18:1 ω9 *trans*), which is representative for both, ruminant fat (18:1 ω7 *trans*) and hydrogenated vegetable oils (18:1 ω9 *trans*) was the most dominant part in this FA class (tab. 5.5). Apart from that, 16:1 ω7 *trans*, which is also representative for ruminant/milk fat (ch. 2.2.3.1) was found (tab. 5.5).

5.3.2. Comparison of GLT FA biomarkers with ADT samples from the study population of ISOCROSS

The comparison between GLT and ADT FA biomarker (tab. 5.4 and 5.5) is depicted in the following subsection devided into the respective FA biomarker classes to determine whether differentiation between GLT and ADT for the analysis of FA biomarker is necessary or not. Each FA biomarker will be discussed in the background of its respective FA biomarker class. The following figures (fig. 5.17, 5.18, 5.19 and 5.20) show the FA biomarker classes with their proportion to total FA found in GLT and ADT (left plots). Percentage differences between GLT and ADT were plotted for matched GLT and ADT pairs (different colours) and statistically significance from 0 was tested with paired t-tests.

5.3.2.1. Milk FA biomarker

Mean proportion of 15:0 were 0.48% and 0.42% in GLT and ADT, respectively (fig. 5.17, left top plot). Differences of the proportion of 15:0 (%) from GLT and matched ADT pairs are distributed only marginal from 0 (fig. 5.17, right top plot). Therefore no statistically significance was observed for 15:0 in GLT and ADT (P=0.572, fig. 5.17). Mean proportion of 17:0 were 0.30% and 0.32% in GLT and ADT, respectively (fig. 5.17, middle left plot). Similar to 15:0, no significant difference was observed for 17:0 in GLT and ADT (P=0.936, fig. 5.17, middle right plot). For 16:1 ω7 *trans*, mean proportion in GLT is slightly higher than in its respective ADT pair from the same female donor (fig. 5.17, bottom left plot, 0.33% and 0.26%, respectively). Concludingly, differences of the proportion of 16:1 ω7 *trans* from GLT and its ADT pairs from the same women of ISOCROSS are all located above 0 (fig. 5.17, bottom right plot). However, the differences from 0 are small and no statistical significance was observed (P=0.485). Therefore, for milk FA biomarker no difference is expected between GLT and ADT.
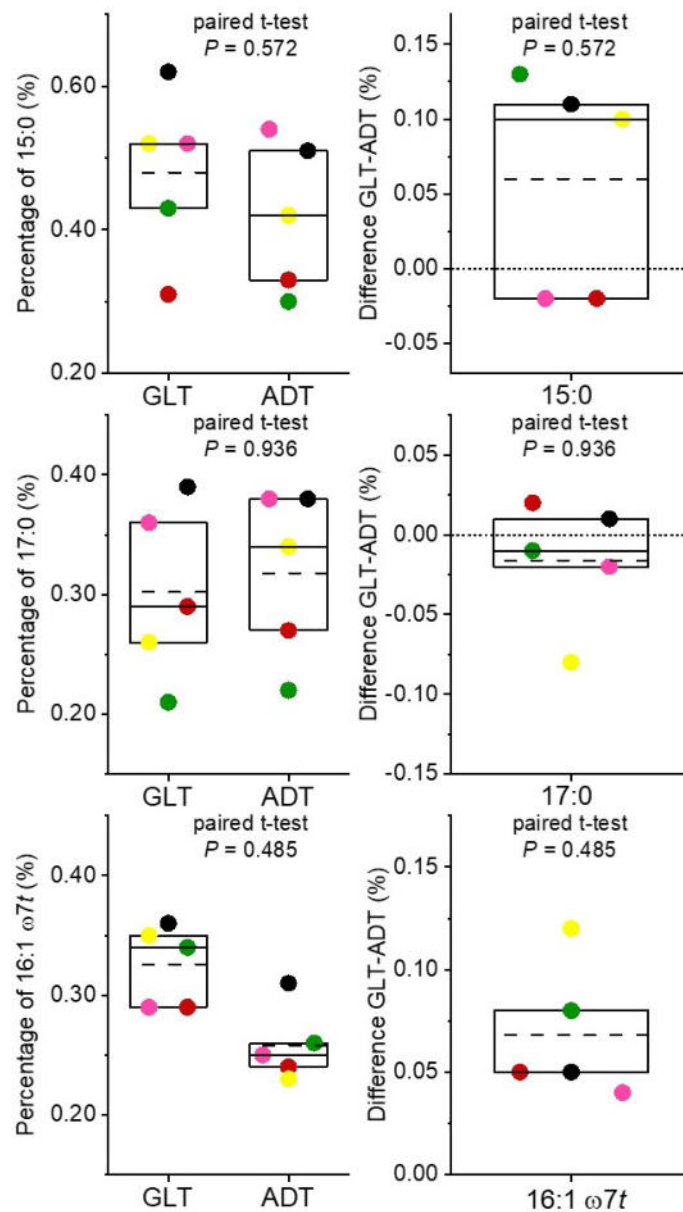
Figure 5.17.: Boxplots of the FA biomarker class milk and ruminant fat with their proportion of 15:0, 17:0 and 16:1 ω7 *trans* to total FA found in GLT and ADT (left plots) and boxplots of percentage differences of 15:0, 17:0 and 16:1 ω7 *trans* between GLT and ADT samples. Percentage differences between GLT and ADT were plotted for GLT-ADT-pairs from the same female donor from the ISOCROSS study (different colours) and statistical significance from 0 was tested with paired t-tests and adjusted P-values (Bonferroni-Holm, n=8) are given.

5.3.2.2. Vegetable oils and nuts FA biomarker

Mean proportion of 18:2 $\omega6$ (%) were 9.19 and 7.58 in GLT and ADT, respectively, whereas for 18:3 $\omega3$ mean proportion (%) were 0.47 and 0.34, respectively (fig. 5.18, left plots). Even
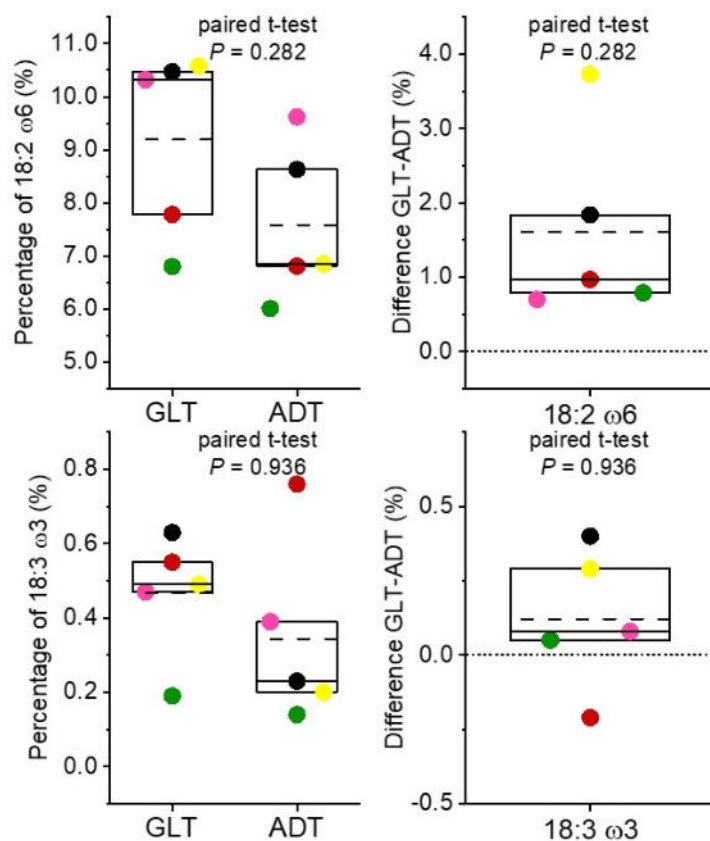
Figure 5.18.: Boxplots of the FA biomarker class vegetable oils and nuts with their proportion of 18:2 $\omega6$ and 18:3 $\omega3$ to total FA found in GLT and ADT (left plots) and boxplots of percentage differences of 18:2 $\omega6$ and 18:3 $\omega3$ between GLT and ADT samples. Percentage differences between GLT and ADT were plotted for GLT-ADT-pairs from the same female donor from the ISOCROSS study (different colours) and statistical significance from 0 was tested with paired t-tests and adjusted P-values (Bonferroni-Holm, n=8) are given.

though slightly higher proportions of 18:2 $\omega6$ (%) were found in GLT in comparison to its ADT pairs and therefore differences of the proportion of 18:2 $\omega6$ from GLT and ADT pairs are all located above 0, no statistical significance was observed (P=0.282, fig. 5.18, top right plot). However for 18:3 $\omega3$, differences of the proportion from GLT and matched ADT pairs are distributed only marginal from 0 (fig. 5.18, right bottom plot). Therefore no

statistically significance was observed for 18:3 ω3 in GLT and ADT (P=0.936). Therefore, for vegetable oils and nuts FA biomarker no difference is expected between GLT and ADT.

5.3.2.3. Fish FA biomarker

Mean proportions of 20:5 ω3 were 0.034% and 0.041%, respectively (fig. 5.19, top left plot). Differences of the proportion of 20:5 ω3 (%) from GLT and ADT pairs from the same
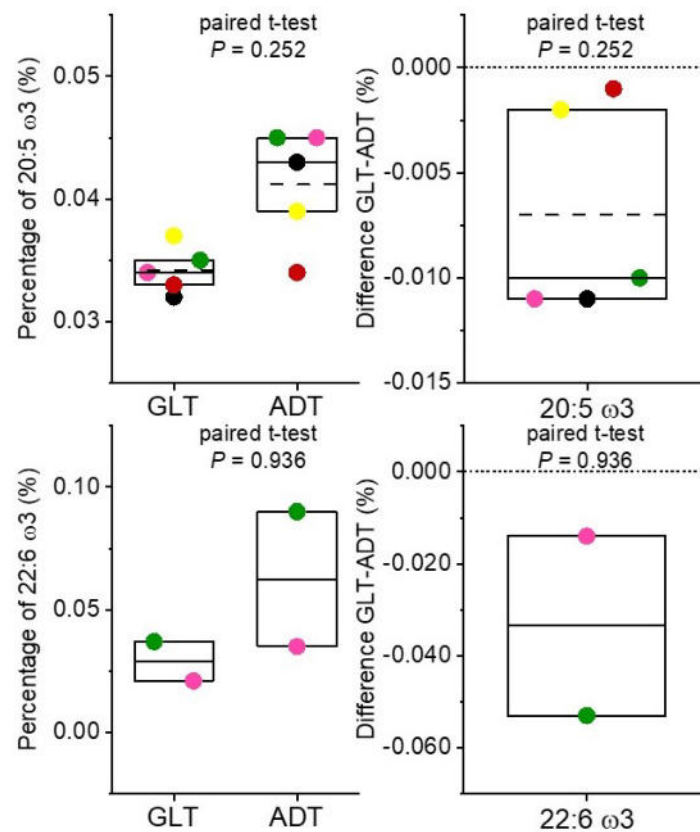
Figure 5.19.: Boxplots of the FA biomarker class fish with their proportion of 20:5 ω3 and 22:6 ω3 to total FA found in GLT and ADT (left plots) and boxplots of percentage differences of 20:5 ω3 and 22:6 ω3 between GLT and ADT samples. Percentage differences between GLT and ADT were plotted for GLT-ADT-pairs from the same female donor from the ISOCROSS study (different colours) and statistical significance from 0 was tested with paired t-tests and adjusted P-values (Bonferroni-Holm, n=8) are given.

female donor are distributed only marginal from 0 (fig. 5.19, right top plot). Therefore no statistically significance was observed for 20:5 ω3 in GLT and ADT (P=0.252, fig. 5.19). For 22:6 ω3, only two ADT samples were > LOD (ch. 5.2.2.3), therefore comparison between

GLT and matched ADT pairs could only be established for two GLT-ADT-pairs. Mean proportion of 22:6 ω3 were 0.039% and 0.062%, respectively (fig. 5.19, bottom left plot). Similar to 20:5 ω3, the difference of the proportion of 22:6 ω3 from GLT and ADT pairs are below 0 (fig. 5.19, bottom right plot). However, no statistically significance was observed for 22:6 ω3 in GLT and ADT (P=0.936). It is worth mentioning, that such a small sample size (n=2) and a large variability (which is the case) will make it more likely to trigger type II errors (Columb and Atkinson 2016).

5.3.2.4. Sum of 18:1 *trans* FAs

Mean proportion of 18:1 *trans* was 0.98% and 1.34% for GLT and the respective ADT pairs from the same female donor, respectively (fig. 5.20, left plot). Even though GLT 18:1
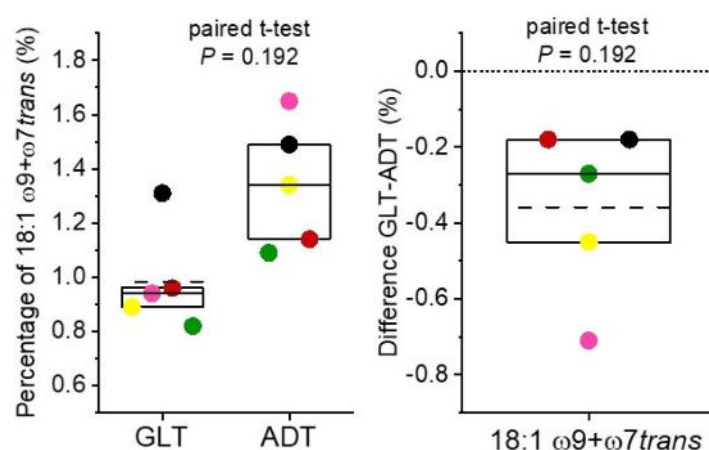
Figure 5.20.: Boxplot of the sum of 18:1 *trans* with the proportion of the sum of 18:1 ω9 *trans* and 18:1 ω7 *trans* to total FA found in GLT and ADT (left plot) and boxplot of percentage differences of the sum of 18:1 *trans* between GLT and ADT samples. Percentage differences between GLT and ADT were plotted for GLT-ADT-pairs from the same female donor from the ISOCROSS study (different colours) and statistical significance from 0 was tested with paired t-tests and adjusted P-values (Bonferroni-Holm, n=8) are given.

trans revealed slightly lower proportion than its respective ADT pair, no differences were observed for 18:1 *trans* in GLT and ADT (P=0.192, fig. 5.20, right plot). Therefore no differences are expected to be between GLT and ADT for the proportion of the sum of 18:1 *trans* (18:1 ω9 *trans* + 18:1 ω7 *trans*).

5.3.2.5. Miscellaneous FAs

Neither for SFA nor for MUFA and PUFA significant differences were observed for GLT and ADT ($P > 0.05$ for all, app. A.18).

5.3.2.6. Overall

For all FAs (biomarkers as well as miscellaneous; fig. 5.17, 5.18, 5.19, 5.20, A.18) no statistical significance were observed ($P > 0.05$ for all). Differentiation between breast GLT and ADT seems not to be necessary for the analysis of FAs serving as biomarkers for the intake of specific food groups. Thus potential influence of mixed breast tissue on FA biomarker analysis seems to be neglectable.

5.4. FA biomarker analysis in the study population of WASP

Analysis of FA composition and especially FA biomarkers will be depicted in the following section for the study population of WASP (ch. 4.2.1.1). Biospecimens were analyzed according to the method described in ch. 4.2.3. To investigate if dietary FA biomarkers could also be used as *exVars* (similar to ch. 5.1) in WASP samples, FA biomarkers (as described in ch. 2.2.3) were analyzed, statistically tested and results were interpreted. For that, the study cohort will be characterized for all necessary information regarding FA analysis (ch. 5.4.1). Secondly, the FA composition especially FA biomarker of the whole study population and consequences of the implemented GC-MS/FID method will be depicted in ch. 5.4.2, following statistical evaluation to provide objective markers of diet and dietary intake of nutrients for the study population of WASP (ch. 5.4.3 and 5.4.4).

5.4.1. Characterization of the study population

For this thesis, 27 fat specimen from healthy participants (26 women and 1 men), who underwent surgery for cosmetic reasons, were analyzed for FA biomarkers and FA composition according to the method described in ch. 4.2.3. Besides acquisition and analysis of fat samples, participating patients gave further information (ch. 4.2.1.1). Nearly 50% of patients were breast surgeries (n=13, fig. 5.21). Breast tissue were either obtained
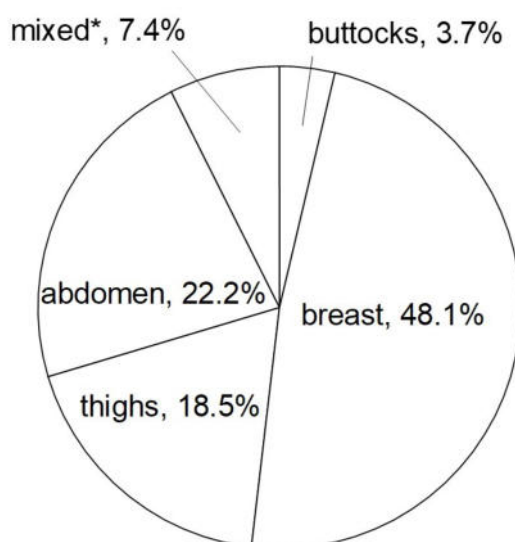
Figure 5.21.: Cake chart of respective type of surgeries (buttocks, breast, thighs, abdomen, mixed*) and their proportion on total number of surgeries (n=27 samples). *mixed indicates ADT samples from different regions of the body.

from mammareduction, mastopexy or mammaaugmentation. Approximately one-fith of surgeries were obtained from either thighs (n=5) or abdomen (n=6), whereas <5% were obtained from the buttock (n=1). Two samples (7%) were mixed tissues from different parts of the body (fig. 5.21). FA analysis was performed for these samples according to the method described in ch. 4.2.3 and will be depicted in the following section.

5.4.2. FA composition of study population

Mean proportion of FAs as well as the 25th and 75th percentile from 27 biospecimens of WASP are depicted in table 5.6 and 5.7. Chromatograms and FA compositions from the participants are depicted in app. A.19. Twenty six FAs were identified. Among these 26 FAs, 7 FAs were SFAs, 7 FAs were MUFAs, 10 FAs were PUFAs and 2 were TFA in ADT.

5.4.2.1. Sum of SFA, MUFA, PUFA and TFA

As expected, in the study population of WASP, mean proportion of SFAs on total FA composition was 34.24% (28.29-38.56%; fig. 5.22). Mean proportion of MUFA and PUFA were 53.81% (48.05-58.77%; fig. 5.22) and 10.69% (7.27-13.73%; fig. 5.22), respectively. Mean TFA was 1.23% (0.82-1.81%; fig. 5.22).
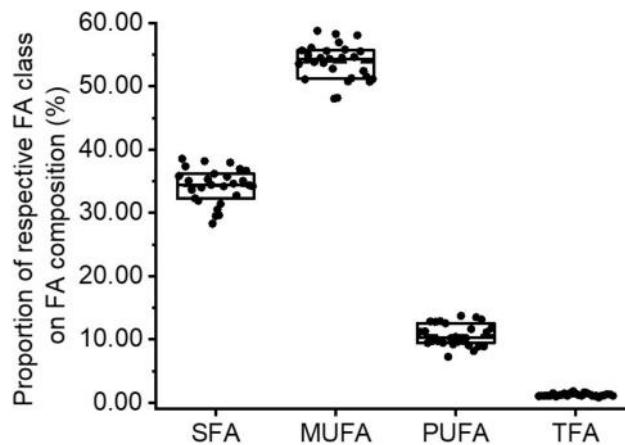
Figure 5.22.: Boxplots of FA-classes SFA, MUFA, PUFA and TFA (%) from the study population of WASP (n=27 participants). Respective FA-classes were calculated by summing all SFAs (n=7), MUFAs (n=7), PUFAs (n=10) and TFAs (n=2).

As expected, the largest proportion of FAs belonged to MUFA (mean=53.81% with its most dominant representative 18:1 ω9, mean=45.56%, tab. 5.6) followed by SFA (mean=34.24%) and PUFA (mean=10.68%). Smallest proportion of FAs belonged to TFA (mean=1.23%),

Table 5.6.: Mean FA proportions on FA composition as well as the 25th and 75th percentile from the study population of WASP (n=27 samples). FA classes shown in this table: SFAs and MUFAs.

FA class	FA	mean FA proportion (%)	25th perc. (%)	75th perc. (%)
SFA	12:0	0.72	0.62	0.84
	14:0	3.77	3.58	4.15
	15:0	0.47	0.42	0.54
	16:0	23.47	22.33	24.61
	17:0	0.34	0.32	0.38
	18:0	5.27	4.46	5.96
	20:0	0.20	0.15	0.24
MUFA	14:1 ω5	0.37	0.29	0.43
	16:1 ω7	4.49	3.45	5.02
	17:1 ω7	0.34	0.30	0.39
	18:1 ω9	45.56	43.87	47.38
	18:1 ω7	2.11	1.96	2.21
	20:1 ω9	0.77	0.65	0.87
	22:1 ω9	0.16	0.11	0.19

however since origin of TFAs are mostly from industrial processed oils and ruminant fat (ch. 2.2.3.4), it was expected that TFA encompasses the smallest FA class in FA composition.

5.4.2.2. SFAs

Since 16:0 is the endproduct of endogenous FA synthesis via FAS (ch. 2.2.1) and is also extremely dominant in dietary intake of SFAs, it was expected, that 16:0 will make up the highest amount of SFAs. In the population of WASP, 16:0 represents the highest proportion of SFA (mean=23.47%; tab. 5.6).18:0 is part of the elongation pathway via ELOVL from 16:0 (ch. 2.2.1) and is also highly presented in dietary intake of SFAs, therefore the second common SFA was 18:0 (mean=5.27%; tab. 5.6). 14:0 (mean=3.77%; tab. 5.6) and 12:0

Table 5.7.: Mean FA proportions on FA composition as well as the 25th and 75th percentile from the study population of WASP (n=27 samples). FA classes shown in this table: PUFAs and TFAs. [a] indicates, that in 1 sample, 18:3 ω6 was not detectable; [b] indicates, that in 13 samples, 20:3 ω6 was not detectable; [c] indicates, that in 6 samples, 20:3 ω3 was not detectable; [d] indicates, that in 3 samples, 22:2 ω6 was not detectable; [e] indicates, that in 3 samples, 22:6 ω3 was not detectable.

FA class	FA	mean FA proportion (%)	25th perc. (%)	75th perc. (%)
PUFA	18:2 ω6	9.58	8.49	10.58
	18:3 ω6[a]	0.043	0.033	0.051
	18:3 ω3	0.48	0.36	0.55
	20:2 ω6	0.18	0.16	0.19
	20:3 ω6[b]	0.17	0.11	0.24
	20:3 ω3[c]	0.037	0.020	0.046
	20:4 ω6	0.15	0.07	0.20
	22:2 ω6[d]	0.070	0.037	0.099
	20:5 ω3	0.034	0.025	0.036
	22:6 ω3[e]	0.061	0.039	0.075
TFA	16:1 ω7 *trans*	0.25	0.23	0.28
	18:1 *trans*	00.98	0.85	1.13

(mean=0.72%; tab. 5.6) are also part of endogenous FA synthesis and present in western diet. The lowest proportion encompasses 20:0 (mean=0.20%; tab. 5.6). The odd chain SFAs 15:0 (mean=0.47%; tab. 5.6) and 17:0 (mean=0.34%; tab. 5.6) were expected to be small, since both SFAs are representatives of milk and ruminant fat intake (ch. 2.2.3.1). 15:0 and 17:0 ranged from 0.3-0.6% and 0.24-0.41%, respectively (fig. 5.23). All SFAs were detectable in 100% of samples.
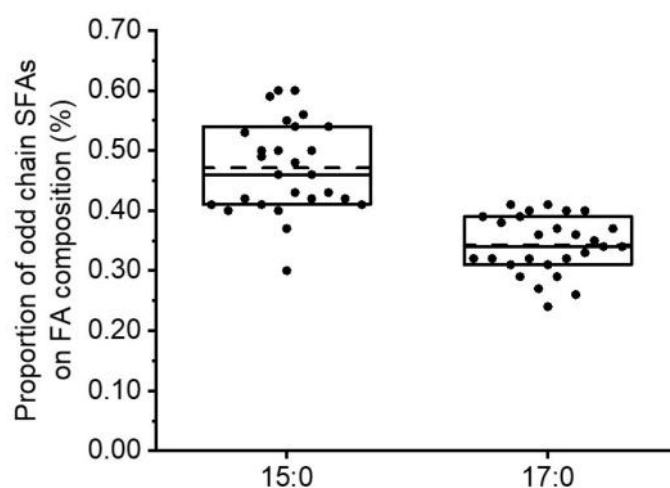
Figure 5.23.: Boxplots of the percentages of odd-chain milk and ruminant fat FA biomarker 15:0 (left plot) and 17:0 (right plot) on total FA composition from the study population of WASP (n= 27 participants).

5.4.2.3. MUFAs

The main MUFA in the study population was 18:1 ω9 (mean=45.56%; tab. 5.6). The high amount of 18:1 ω9 shows the importance of that FA as a dietary fat source and as an endogenous product from desaturation of 18:0 (ch. 2.2.1). Higher amounts of 16:1 ω7 and 18:1 ω7 (4.49% and 2.11%, respectively; tab. 5.6) were expected, since 16:1 ω7 is part of the western diet and 18:1 ω7 is produced by chain elongation of 16:1 ω7 (ch. 2.2.1). Small amounts of MUFAs were observed for 14:1 ω5 (mean=0.37%; tab. 5.6), 17:1 ω7 (mean=0.34%, tab. 5.6), 20:1 ω9 (mean=0.77%, tab. 5.6) and 22:1 ω9 (mean=0.16%; tab. 5.6). The human body has the capacity to endogenously synthesize MUFAs. Therefore MUFAs are not expected to reflect dietary intake (ch. 2.2.3). All MUFAs were detectable in 100% of samples.

5.4.2.4. PUFAs

In the 27 samples of WASP, six ω6 and four ω3 PUFAs were identifed. The sum of all ω6 PUFAs were 10.11% (ranging from 7.03-13.23%) and ω3 PUFAs 0.58% (ranging from 0.25-1.32%). The proportion of ω6 PUFAs were approximately 19.62 (ranging from 7.97-29.49) fold higher than ω3 PUFAs, which typically indicated western diet lifestyle (ch. 2.2.2).

<u>ω6 PUFAs</u>
The most important member of the ω6 group is 18:2 ω6, which is essential for the human

body and therefore expected to be consumed with the diet. It is found abundantly in vegetable oils and is therefore considered as a dietary FA biomarker (ch. 2.2.3.2). 18:2 ω6 had the highest proportion of PUFAs (mean=9.58%; tab. 5.7) and ranged from 6.36-12.94% (fig. 5.24).
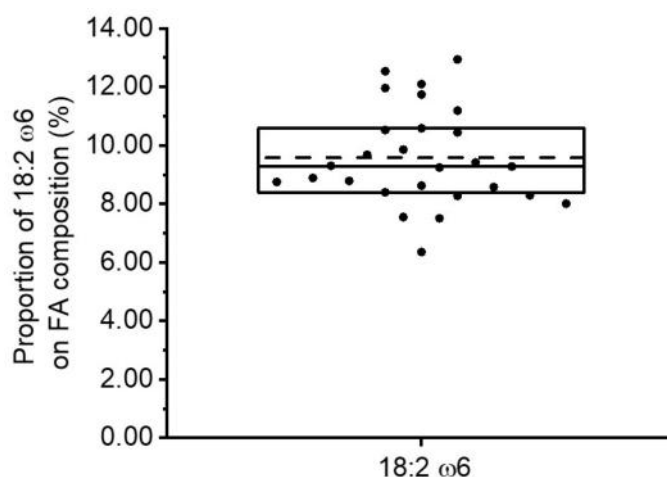
Figure 5.24.: Boxplot of the percentage of ω6 vegetable oils and nuts FA biomarker 18:2 ω6 on total FA composition from the study population of WASP (n= 27 participants).

Mean 18:3 ω6 was 0.043% (tab. 5.7). 18:3 ω6 was not detectable in one sample (app. A.19). 20:2 ω6 was detectable in all samples, whereas 20:3 ω6 was not detectable in 13 samples (app. A.19). 20:4 ω6 (mean=0.15%; tab. 5.7) was also detectable in all samples, whereas 22:2 ω6 (mean=0.070%; tab. 5.7) was not detectable in 3 samples (app. A.19).

ω3 PUFAs

The total ω3 PUFAs reflected the sum of 18:3 ω3, 20:3 ω3, 20:5 ω3 and 22:6 ω3 in the study population of WASP. Dietary FA biomarker are reflected by 18:3 ω3 (mean=0.48%; tab. 5.7) as representative of vegetable oils and nuts (ch. 2.2.3.2) as well as 20:5 ω3 and 22:6 ω3 (mean=0.034 and 0.061%, respectively; tab. 5.7) as representatives of dietary fish intake (ch. 2.2.3.3). 18:3 ω3 ranged from 0.20-1.03%, whereas 20:5 ω3 and 22:6 ω3 ranged from 0.018-0.085% and 0.029-0.140%, respectively (fig. 5.25). Since the german population is part of the western diet society and a low fish consumer country, it was expected that, small proportions of 20:5 ω3 and 22:6 ω3 with small ranges within each FA were observed (fig. 5.25, ch. 2.2.2). 18:3 ω3 and 20:5 ω3 were detectable in 100% of samples, whereas 22:6 ω3 was not detectable in three samples (app. A.19). Furthermore, in 9 samples, proportion of 22:6 ω3 on FA composition was below LOD (22:6 $\omega3_{LOD}$= 0.023%; ch. 5.2.2.3). Conclud-
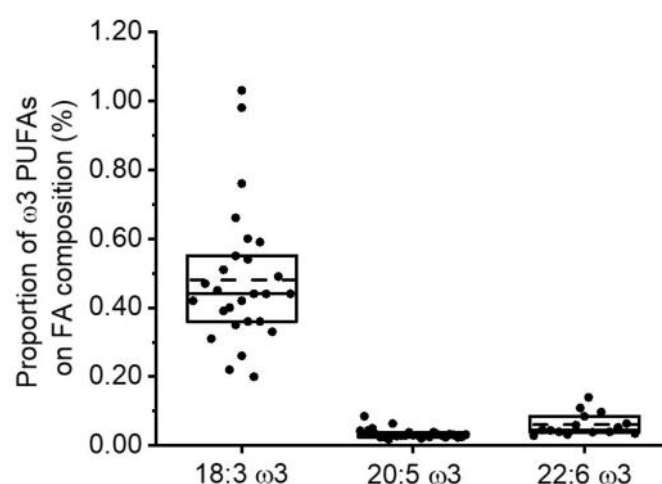
Figure 5.25.: Boxplots of the percentages of ω3 vegetable oils and nuts FA biomarker 18:3 ω3 (left plot) and fish FA biomarker 20:5 ω3 (mid plot) and 22:6 ω3 (right plot) on total FA composition from the study population of WASP (n= 27 participants). For 22:6 ω3 only 15 samples could be analyzed.

ingly, 22:6 ω3 could only be determined in 15 samples of WASP (app. A.19).

5.4.2.5. TFAs

Two TFAs (16:1 ω7 *trans* and the sum of 18:1 ω7 *trans* and 18:1 ω9 *trans*, respectively) were detectable in 100% of samples. 16:1 ω7 *trans* (mean=0.25%; tab. 5.7) is recognized as a dietary FA biomarker for the intake of milk and ruminant fat (ch. 2.2.3.1). Its proportion ranged from 0.18-0.34% (fig. 5.26). Furthermore, the sum of 18:1 ω7 *trans* and 18:1 ω9 *trans* (mean=0.98%; tab. 5.7) was detected and ranged from 0.58-1.47% (fig. 5.26). As described in ch. 5.2.1, the sum of identified 18:1 *trans* FAs can not be used as a objective measurement of dairy or partially hydrogenated vegetable oils intake.

5.4.3. Correlation analysis of dietary FA biomarker

Objective marker for diet and dietary intake of nutrients are important for the estimation of diet-disease associations but also in evaluating influence of diet on potential risk factors of known diseases. To provide objective markers of diet and dietary intake of nutrients, possible colinearities (which might hinder selection and/or influence of variables within multiple linear regression models) between potential dietary FA biomarkers had to be analyzed first. Therefore correlation analysis (ch. 4.2.4.2) was performed between dietary
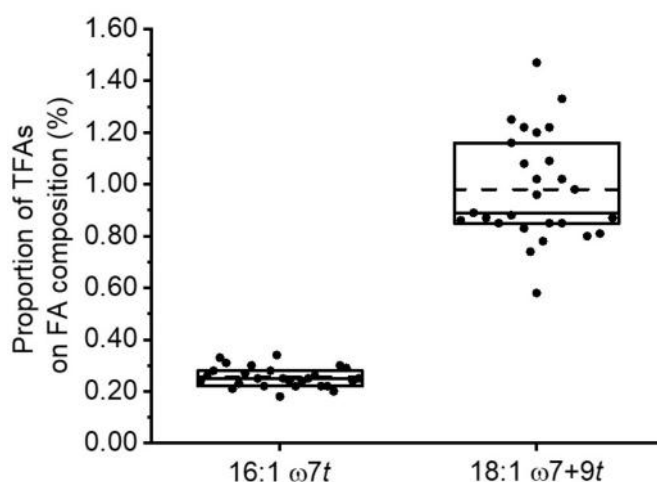
Figure 5.26.: Boxplots of the percentages of milk and ruminant fat FA biomarker 16:1 ω7 *trans* (left plot) and the sum of 18:1 *trans* FAs (18:1 ω9 *trans* and 18:1 ω7 *trans*; right plot) on total FA composition from the study population of WASP (n= 27 participants).

FA biomarkers. For correlation analysis, the amount of FA biomarker levels, which were <LOD was first checked. If more than 33% of the respective FA levels were <LOD, the respective FA biomarker was excluded from correlation analysis. In the case of 22:6 ω3, which reflects the dietary intake of fish, >33% of levels were <LOD (app. A.19), therefore 22:6 ω3 was excluded for correlation analysis. Besides the identified dietary FA biomarker from ch. 5.1 (18:2 ω6, 18:3 ω3, 20:5 ω3 and 18:1 ω9 *trans*) for the dietary intake of vegetable oils and nuts, fish as well as partially hydrogenated vegetable oils, dietary FA biomarker for milk and ruminant fat (15:0, 17:0 and 16:1 ω7 *trans*, ch. 2.2.3.1) were used for correlation analysis. Correlation analysis was performed with 27 samples and the dietary FA biomarkers 15:0, 17:0, 16:1 ω7 *trans*, 18:2 ω6, 18:3 ω3, sum of 18:1 ω9 *trans* and 18:1 ω7 *trans* and 20:5 ω3. In correlation analysis, 7 associations with a P-value <0.05 were observed (app. A.20). The correlation coefficients were displayed in a coloured and clustered heatmap (fig. 5.27).

Within the FA biomarkers group, correlation clusters were identified and allowed the clustering of groups within the FA biomarkers group. 15:0, 17:0 and 16:1 ω7 *trans* were highly positively correlated between each other (P<0.05; fig. 5.27). Since milk and ruminant fat products are an extensive food source in the western population (ch. 2.2.2.3) and the odd chain FAs 15:0 and 17:0 as well as the *trans* FA 16:1 ω7 *trans* may serve as valid biomarkers of these sources (ch. 2.2.3.1), the correlation between 15:0, 17:0 and 16:1 ω7 *trans* seems plausible. Interestingly, 15:0 and 16:1 ω7 *trans*, both, were highly positively correlated
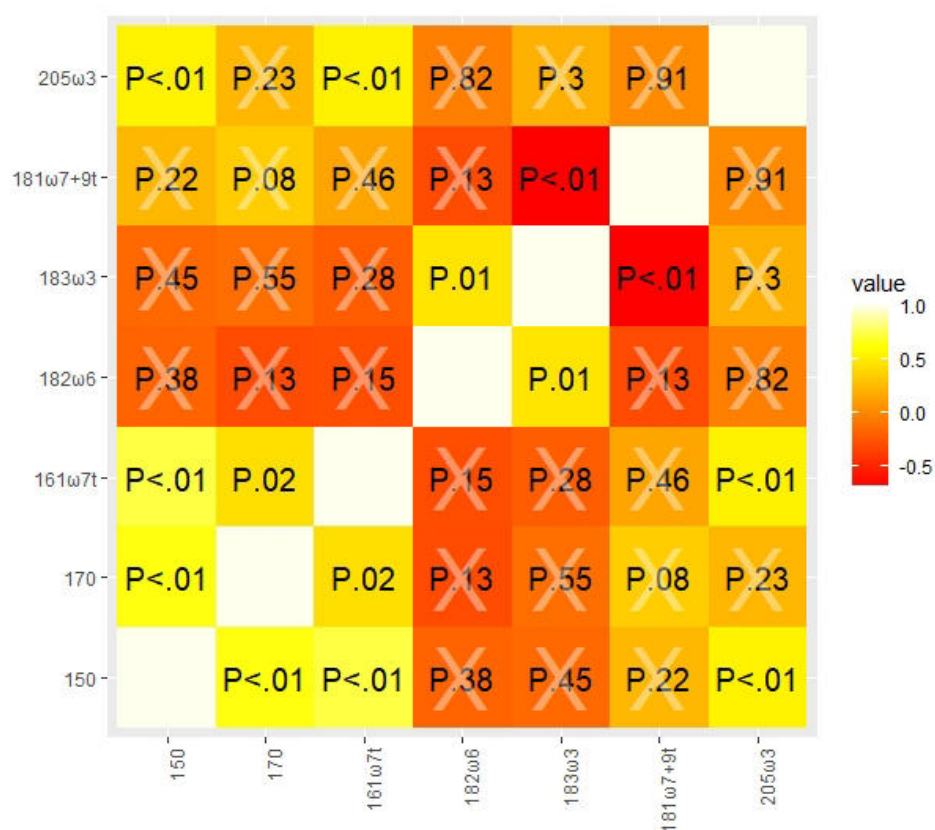
Figure 5.27.: Coloured and clustered heatmap-representation of Spearman-correlation co-
efficients. Correlation analysis of 20:5 ω3, sum of 18:1 *trans* (18:1 ω9 *trans* and
18:1 ω7 *trans*), 18:3 ω3, 18:2 ω6, 16:1 ω7 *trans*, 17:0 and 15:0 was carried out
with data of 27 ADT samples from the study population of WASP. Numbers
inside the cells indicate the respective P-values. Cells with white crosses have
P-values > 0.05.

(P<0.05 for both, fig. 5.27) with 20:5 ω3. Similar to ch. 5.1.1, a significant positive corre-
lation was observed for 18:2 ω6 and 18:3 ω3 (P<0.05; fig. 5.27). Significantly negatively
correlation was observed for sum of 18:1 *trans* and 18:3 ω3 (P<0.05; fig. 5.27). It is possible,
that those participants with a ω3 FA rich diet consume only little 18:1 *trans* FAs. Therefore
correlation of 18:1 *trans* and 18:3 ω3 seems plausible. However, no dietary record was
available to proof this hypothesis. Due to significant correlations within FA biomarkers,
PCA were applied to provide objective markers of diet and dietary intake in the next step.

5.4.4. Principle Component Analysis

Since it was not possible to separate 18:1 ω7 *trans* and 18:1 ω9 *trans* in GC-MS/FID (ch. 5.2.1) and the sum of 18:1 ω7 *trans* and 18:1 ω9 *trans* could not serve as a dietary FA biomarker, PCA was conducted for the dietary FA biomarker 15:0, 17:0, 16:1 ω7 *trans*, 18:2 ω6, 18:3 ω3 and 20:5 ω3. The PCA yielded three PCs (fig. 5.28). The first PC ($PC_{diet}1$)
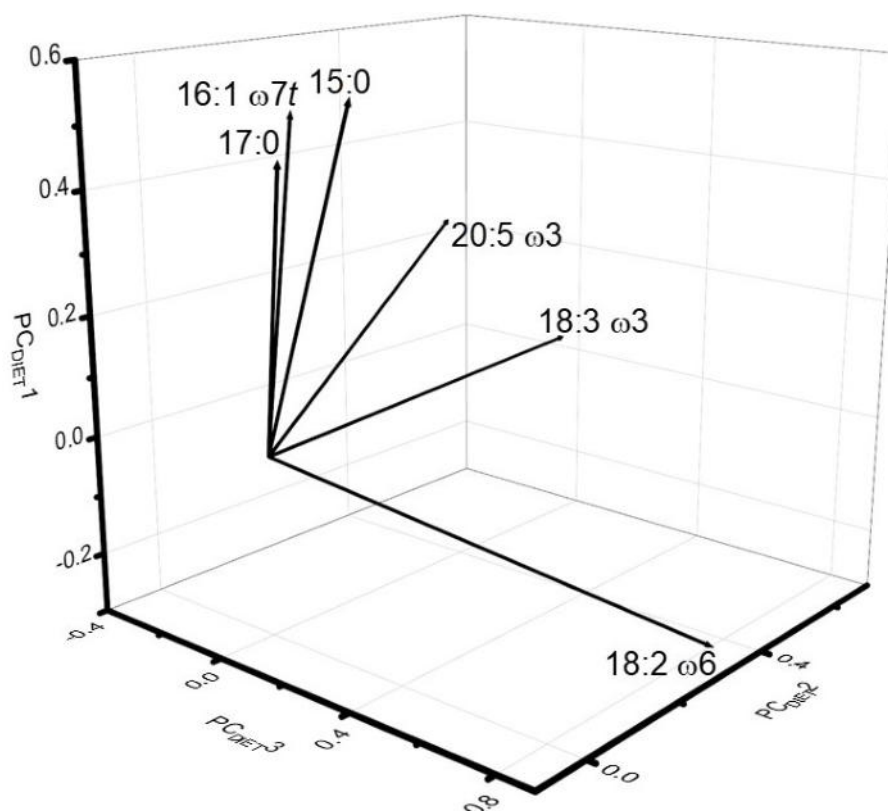
Figure 5.28.: 3D vectorplot designed from the respective factor loadings (app. A.21) from selected FA biomarkers (15:0, 17:0, 16:1 ω7 *trans*, 18:2 ω6, 18:3 ω3 and 20:5 ω3) identified by PCA. $PC_{diet}1$ represents the dietary intake of milk and ruminant fat, represented by 15:0, 17:0 and 16:1 ω7 *trans*. $PC_{diet}2$ represents the dietary intake of ω3 FAs (18:3 ω3 and 20:5 ω3). $PC_{diet}3$ represents the dietary intake of ω6 FAs (18:2 ω6).

was mainly characterized by high impact of 15:0, 17:0 and 16:1 ω7 *trans* (factor loadings >0.50 for all; fig. 5.28; app. A.21). No other FA had either a significantly positive or negative impact on $PC_{diet}1$, therefore $PC_{diet}1$ could interpreted as a PC, which reflects the dietary intake of milk and ruminant fat. $PC_{diet}2$ was strongly impacted by the ω3 FAs 18:3 ω3 and 20:5 ω3 (factor loading >0.50 for both; fig. 5.28; app. A.21). 18:2 ω6 had small impact on $PC_{diet}2$, too (factor loading of 0.32; fig. 5.28; app. A.21). This PC could be interpreted as a

PC, which reflects the dietary intake of $\omega 3$ FAs but not as a PC, which reflects the dietary intake of a specific food group such as fish oder vegetable oils and nuts. Since 18:2 $\omega 6$ and 18:3 $\omega 3$ are both abundant in vegetable oils and nuts (ch. 2.2.3.2), it seems plausible, that 18:2 $\omega 6$ had a small impact on $PC_{diet}2$. Therefore, it could also be possible to interpret $PC_{diet}2$ as a PC, which reflects the dietary intake of total PUFA. That would result into two PCs ($PC_{diet}1$ and $PC_{diet}2$), which only explain 69.00% of variance (app. A.21). $PC_{diet}3$ (additional explained variance of 13.76%, app. A.21) was characterized by high impact of 18:2 $\omega 6$ (factor loading of 0.86; fig. 5.28; app. A.21). Therefore $PC_{diet}3$ would be a better representative of 18:2 $\omega 6$ and could be interpreted as the dietary intake of $\omega 6$ FAs. Neither $PC_{diet}2$ nor $PC_{diet}3$ showed clear pattern for well-established dietary FA biomarker (ch. 2.2.3). $PC_{diet}1$, 2, *and* 3 explained 82.76% of variance (app. A.21).

The aim of this chapter was to provide objective markers for diet and dietary intake of nutrients in the study population of WASP for the influence of diet on potential risk factors of known diseases in multivariate statistical approaches. Only $PC_{diet}1$ could be used as a objective marker of diet and dietary intake of nutrients (in that case for the dietary intake of milk and ruminant fat; fig. 5.28) and could therefore be used as an *exVar* in multivariate statistical approaches. No clear dietary FA biomarker pattern was observed in the study population of WASP for $PC_{diet}2$ and $PC_{diet}3$, which only showed dietary pattern of $\omega 6$ and $\omega 3$ FAs (fig. 5.28). However, 20:5 $\omega 3$ is a well-established FA biomarker for the dietary intake of fish and 18:2 $\omega 6$ and 18:3 $\omega 3$ are well-established biomarker for the dietary intake of vegetable oils and nuts and could therefore be used as a representative of such in multivariate statistical approaches. .

5.5. Scientific background of an application for consumer health

Adverse reactions to food have been recognized since long times. Because of diagnostic lacks, the diagnostic process mainly consists of the adverse reaction history of the patient and placing that history in the context of the dietary habits and the heterogeneous adverse reactions, which can occur from diverse food stuff (summarized in Cox and Sicherer 2020). This multidisciplinary diagnostic process is based on knowledge for the underlying pathogenesis and categories of adverse food reactions. In contrast to widespread diseases such as T2D (ch. 5.1), the prevalence of adverse reactions to food is rather unknown or rare. A rare or orphan disease is defined as a disease affecting not more than five in ten thousand persons (Regulation (EC) No. 141/ 2000). Often, the only choice for patients with adverse reactions to food, as part of a medical and dietary management, is to avoid food stuff and specific food ingredients, which could trigger the adverse reactions. As part of a dietary management tool, the application shall simplify the identification of avoidable food stuff and respective food ingredients and shall therefore improve overall consumer health. For the background of this thesis, scientific information on adverse reactions to food shall be provided. Special focus will be placed on adverse reactions on food ingredients, which could be monitored with this application via GTIN. For this purpose, classification of adverse reaction to food will be placed first (ch. 5.5.1). Secondly, immunologic reactions will be discussed in the context of food allergies and the possibility to monitor special food allergens (ch. 5.5.2). Subsequently, the complex field of non-immunologic reaction will be discussed (ch. 5.5.3). Lastly, an outlook will be provided (ch. 5.5.4).

5.5.1. Classification of adverse reaction to food

Adverse food reactions can be categorized as those events with a non-immunological origin (also referred as food intolerances; fig. 5.29) and those with an immunnological basis (also referred as food allergy; fig. 5.29). Food allergy can further be subdivded into IgE-mediated and non-IgE-mediated reactions (fig. 5.29). To be specified as an IgE-mediated food allergy, the presence of IgE antibodies and a clear relationship between between dietary intake of the food and the clinical symptoms must be proven (summarized in Ortolani and Pastorello 2006). Non-IgE mediated food allergy could consist of diverse immune reactions depending on antibodies and cell-mediated immunity (summarized in Ortolani and Pastorello 2006 and EFSA Panel on Dietetic Products and Allergies 2014). It is also possible, that immuno-logically mediated reactions can be triggered by both, IgE- and non-IgE mediated reactions.
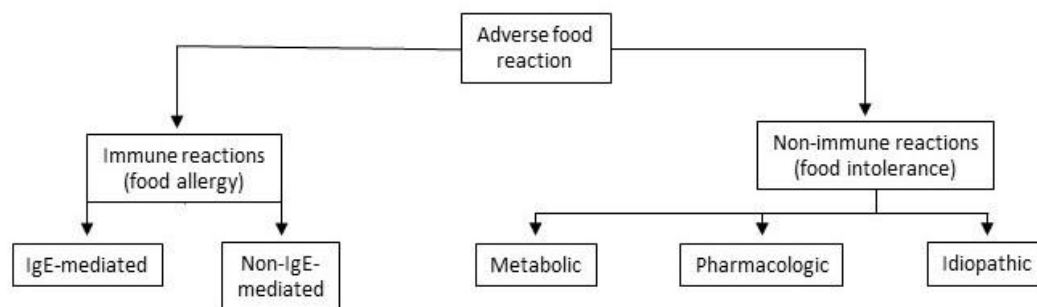
Figure 5.29.: Classification of adverse reactions to food. The classification is further subdi-
vded into immune reactions (food allergy) and non-immune-reactions (food
intolerance), which are further divded into specific reactions. Classification
modified according to Cox and Sicherer 2020.

Non-immunologic adverse food reactions (fig. 5.29; right) may have diverse origins and
include food intolerances that may be due to a special metabolic response of an individual
(e.g. due to enzyme or resorption deficiencies), a response to pharmacological effects of
food ingredients (e.g. biogenic amines) or ideopathic reactions to food ingredients, which
do not have a known origin. In the context of this thesis, focus has been set on special
metabolic responses and one well-known idiopathic reaction to sulfite.

In the following chapters, information will be provided for immunologic and non im-
munologic adverse food reactions. These information will always be placed in the context
of the function of the application and will therefore be discussed for practicability of im-
plementation into the application. Ch. 5.5.2 will provide information about immunological
adverse food reactions, whereas ch. 5.5.3 will take a closer look into the non-immunological
adverse food reactions.

5.5.2. Adverse reaction to food due to immune reactions

5.5.2.1. Overall prevalence of immunological adverse food reactions

In the case of food allergies, many publications reporting the prevalence are available.
However, differences in sample size, inclusion/exclusion criteria and diagnostic methods
may bias the true prevalence in the general population. The prevalence of food allergies is
uncertain (EFSA Panel on Dietetic Products and Allergies 2014). A plausible reason for the
uncertain prevalence may be, that self-reported prevalence, in comparison with the real
prevalence, is highly overestimated (Zuberbier et al. 2004). This was also addressed by the

EFSA report (EFSA Panel on Dietetic Products and Allergies 2014), where self-reported prevalence of food allergies were >10%, whereas prevalence of diagnosed food allergies were smaller, trending to be <1%, when diagnosis of food allergy was confirmed by a food challenge. Current estimations of food allergies in germany, as self-reported doctor diagnosed allergic diseases, showed a lifetime prevalence in adults of 4.7% (Bergmann, Heinrich, and Niemann 2016). However, the study indicated lack of specific food challenge testing due to complexity of performing in the general population. Taking into account, that nearly 5% of the german population are 4 million people, there is a special need for a dietary management tool, such as an application, for consumers health regarding food allergies. Since many foods and food ingredients may trigger adverse reactions the following ch. 5.5.2.2 will provide information on selected food allergens, which may be used in the application.

5.5.2.2. Selected food allergens

A broad range of food and food ingredients may trigger adverse reactions in the context of food allergies. Cross-reactivity between food ingredients and food groups are also possible. Not all possible food allergens play a role in adverse reactions due to the respective prevalence in the general population. Therefore a preselection was made based on the respective Regulation (EU) No. 1169/ 2011. In its Annex II, the Regulation provides information on substances or products causing allergies or intolerances (fig. 5.30). For these substances
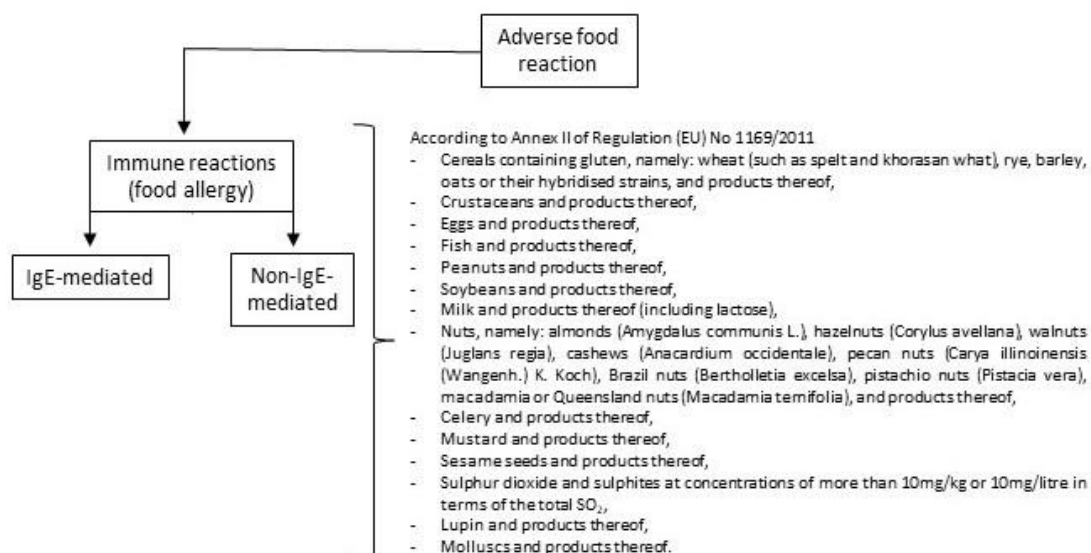
Figure 5.30.: Preselection of food allergies according to the Regulation (EU) No 1169/2011.

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and products, information shall be provided regarding general information and prevalence as well as occurence in food. Where applicable, further information to cross-reactivities with other food stuff will be provided.

Cereals containing gluten

Allergy to cereals containing gluten need to be divided into coeliac disease, an autoimmune systemic disorder triggered by gluten, and general allergy to cereals containing gluten (EFSA Panel on Dietetic Products and Allergies 2014). As an autoimmune disorder, coeliac disease is a life-long disease with permanent gluten intolerance with severe reactions such as diarrhoea and cachexia. It is sometimes accompanied with malabsorption resulting in weight loss. Global prevalence of coeliac disease appeared to be 1:3 000 (EFSA Panel on Dietetic Products and Allergies 2014). For the german population an overall prevalence of 1:270 were observed (Kratzer et al. 2013). The overall prevalence is estimated to be 0.5 to 1% in Europe (EFSA Panel on Dietetic Products and Allergies 2014). Taking this into account, there exists a considerable amount of people in germany (approximately 410.000 to 820.000 people) suffering from coeliac disease. Allergies to cereals containing gluten, which are not coeliac disease, are mainly IgE-mediated allergic reactions (EFSA Panel on Dietetic Products and Allergies 2014). Typical symptoms encompasses oral allergy syndrome, urticaria, atopic dermatitis, respiratory and gastrointestinal adverse reactions as well as anaphylaxis. The overall prevalence of allergies to cereals containing gluten is unknown (EFSA Panel on Dietetic Products and Allergies 2014). Because of the fact, that cereals containing gluten encompasses a broad range of any kind of plant-producing grains, studies investigating the prevalence over the full range are scarce. According to Annex II of Regulation (EU) No 1169/2011 wheat (such as spelt and khorasan wheat), rye, barley, oats or their hybridised strains, and products thereof are considered to be cereals containing gluten. Cereals are a major source of food worldwide. In germany, men and women consume over 300 g and 240 g per day on products consisting of cereals containing gluten (Pfau, Oltersdorf, and Brombach 2008). Therefore, there exists a considerable amount of people consuming cereals containing gluten and products thereof on a daily basis. The prevalence of wheat (a cereal containing gluten) based on clinical history and sensitisation was estimated to be 0.9% for all ages in germany (EFSA Panel on Dietetic Products and Allergies 2014). Based on that data, there exists also a considerable amount of people suffering from allergy to wheat. Taking this into account, there exists a special need for dietary management tools regarding cereals containing gluten. In the context of the application, it is irrespective, if a user is suffering from allergies to cereals containing gluten or coeliac disease because both are triggered by gluten. A high degree of IgE cross-reactivity exists between cereal seeds

and grass pollen regarding allergens. It is therefore possible that grass pollen-allergic patients may also have food allergy to cereals (EFSA Panel on Dietetic Products and Allergies 2014). It is noteworthy, that components different to gluten in cereals may trigger allergic reactions (Scherf 2019), however these components are not covered in Annex II.

Milk and products thereof

According to Annex VII part IV of Regulation (EU) No 1308/ 2013 milk is defined as the produce of the milking of one or more cows. However, milk may be also defined as a substance secreted by the mammary glands of all mammal species. Adverse reactions to milk are a immunological response to specific milk proteins of different mammalian species, mainly derived from cow, goat and ewe (EFSA Panel on Dietetic Products and Allergies 2014). Mainly IgE- and non-IgE-mediated, but also mixed reactions are involved in adverse reactions to milk and products thereof. The clinical manifestation of IgE-mediated reactions encompasses adverse events particular on skin, gastrointestinal tract and respiratory tract. In rare cases also anaphylaxis may occur. Non-IgE-mediated reactions mainly affect the gastrointestinal tract (EFSA Panel on Dietetic Products and Allergies 2014). Prevalence was mainly assessed of cow milk allergy and overall prevalence combining all ages was 0.1% in the german population (EFSA Panel on Dietetic Products and Allergies 2014). Based on self-reported diagnosis of milk allergy, the prevalence was nearly twenty times higher (1.8%, EFSA Panel on Dietetic Products and Allergies 2014). According to Annex II of Regulation (EU) No 1169/2011 milk and all products thereof are considered to be of allergenic potential. As mentioned in ch. 2.2.2.3, the average intake of milk and products thereof (such as yogurt, cheese, quark but also meals like cornflakes with milk) for men and women were over 200g per day. The consumption of milk and products thereof plays therefore an important role in the german population. Taking this into account, there exists a special need for dietary management tools regarding milk and products thereof. It is noteworthy, that, due to high-sequence similarity between milk proteins from different mammalian species, cross-reactions between milk proteins from different species are possible. However, clinical reactions to milk do not tend to be different from each other (EFSA Panel on Dietetic Products and Allergies 2014).

Eggs and products thereof

Female animals of many species lay eggs, but eggs from hens are most frequently consumed (EFSA Panel on Dietetic Products and Allergies 2014). In the german population typical meals consumed are all variations of eggs (e.g. scrambled eggs) but also as an ingredient in salads, cakes et cetera. Mean intake of eggs and meals based on eggs are 21g and 17g

per day for men and women, respectively (Pfau, Oltersdorf, and Brombach 2008). Eggs are used in a broad ranges of meals because of the binding, emulsification, coagulation and adhesion properties. Small amounts of eggs are therefore in a large number of food stuff such as dairy products, cakes, bakery products et cetera, which do not significantly increase the consumption of eggs in the general population but make more meals avoidable to people with egg allergy due to the allergenicity of the resulting meal (EFSA Panel on Dietetic Products and Allergies 2014). Adverse reactions to eggs include clinical symptoms such as anaphylaxis and immediate IgE-mediated reactions of the skin, gastrointestinal and respiratory tracts. Prevalence in the overall german population combining all ages was 0.2% (EFSA Panel on Dietetic Products and Allergies 2014). According to Annex II of Regulation (EU) No 1169/2011 eggs and all products thereof are considered to be of allergenic potential. Since there is a broad range for using eggs in the food industry, there exists a special need for dietary management tools regarding eggs and products thereof.

Nuts

According to Annex II of Regulation (EU) No 1169/2011 nuts encompasses almonds (Amygdalus communis L.), hazelnuts (Corylus avellana), walnuts (Juglans regia), cashews (Anacardium occidentale), pecan nuts (Carya illinoinensis (Wangenh.) K. Koch), Brazil nuts (Bertholletia excelsa), pistachio nuts (Pistacia vera), macadamia or Queensland nuts (Macadamia ternifolia), and products thereof and are therefore considered to be of allergenic potential. As indicated in ch. 2.2.2.1, the average intake of nuts in the german population is identically low in both women and men (2g per day). Nuts trigger a wide range of adverse reactions after consumption, ranging from oral allergy syndrome to anaphylaxis (EFSA Panel on Dietetic Products and Allergies 2014). Even though mentioned nuts are collected together in one group, this group do not form a taxonomic group. Therefore prevalence data on overall nuts allergy are not available (EFSA Panel on Dietetic Products and Allergies 2014). Prevalence of hazelnut allergy based on clinical history plus sensitisation and on clinical history plus food challenge were 4.5 and 2.2% in the german population, respectively (EFSA Panel on Dietetic Products and Allergies 2014). For walnut, prevalence were 1.4 and 2.2% (EFSA Panel on Dietetic Products and Allergies 2014). It is noteworthy, that a broad range of cross-reactivity among nuts, between nuts and peanuts as well as nuts and other food exists (EFSA Panel on Dietetic Products and Allergies 2014).

Peanuts and products thereof

According to Annex II of Regulation (EU) No 1169/2011 are peanuts (Arachis hypogea) and products thereof considered to be of allergenic potential. Peanut is a member of the legume

family and its consumption increased recent years due to its wide uses in processed foods but also as a snack or spread. Prevalence of sensitisation to peanut, based on positive skin prick tests, was 6.8% in german adults (EFSA Panel on Dietetic Products and Allergies 2014). No data on the prevalence based on food challenges exists for the german population. Peanut allergy is one of the most common forms of IgE-mediated adverse reactions to food. Typically anaphylaxis occur as a clinical symptom (EFSA Panel on Dietetic Products and Allergies 2014). Since peanut belongs to the legume family cross-reactivity between peanuts and other legumes (such as lupin, soybean and pea) are possible. Further, as mentioned above, cross-reactivity is also possible between peanuts and tree nuts (EFSA Panel on Dietetic Products and Allergies 2014).

Soybean and products thereof

According to Annex II of Regulation (EU) No 1169/2011 soy (soybean; Glycine max) and products thereof are considered to be of allergenic potential. As a source of protein, consumption of soy and products thereof is widespread in Asia and has increased in Europe during the past decade (EFSA Panel on Dietetic Products and Allergies 2014). The consumption of soy and products thereof in the german population was estimated as 2-3 g per day (Pfau, Oltersdorf, and Brombach 2008). However, the last estimation of habitual diet in the german population was 2008. Current estimations of habitual diet in the german population are ongoing. Soy is consumed in a broad way as e.g. oil, flour, drink or fermented (such as miso or tofu). Because of its excellent technological properties soy products are used in the food industry as emulsifiers (e.g. soy lecithin) or texturisers (e.g. soyprotein, EFSA Panel on Dietetic Products and Allergies 2014). Clinical similarities of soy allergy were observed for milk allergy. Therefore clinical manifestation encompasses adverse events particular in the gastrointestinal tract but also anaphylaxis is possible (EFSA Panel on Dietetic Products and Allergies 2014). Prevalence based on sensitisation rates assessed by positive skin prick tests among adults in the german population was 1.7% (EFSA Panel on Dietetic Products and Allergies 2014). However, no data on the prevalence based on food challenges exists for the german population. Cross-reactivities against other legumes in soy allergic inidividuals are possible (such as for peanuts, green pea, lima bean, string bean). Also cross-reactivity to birch pollen allergy is possible (EFSA Panel on Dietetic Products and Allergies 2014).

Fish and products thereof

According to Annex II of Regulation (EU) No 1169/2011 fish and products thereof are considered to be of allergenic potential. As water-living non-mammalian vertebrates,

fish are a common food in Europe, however dietary intake depends on region, traditions and culture (EFSA Panel on Dietetic Products and Allergies 2014). As described in ch. 2.2.2.4, the german population is a low fish consumer society, which is also typical for the western diet. Clinical symptoms can be severe and sometimes fatal. Dietary intake of fish and products thereof often provoke anaphylaxis (EFSA Panel on Dietetic Products and Allergies 2014). The prevalence of sensitisation to fish was 0.9% in young adults of the german population (EFSA Panel on Dietetic Products and Allergies 2014). However, no data on the prevalence based on food challenges exist for the german population. Fish species share many cross-reactivities among each other (EFSA Panel on Dietetic Products and Allergies 2014).

Crustaceans and products thereof

According to Annex II of Regulation (EU) No 1169/2011 are crustaceans and products thereof considered to be of allergenic potential. Similar to fish consumption, the dietary intake depends on region, traditions and culture. No estimations of dietary intake of only crustaceans and products thereof were made in germany, yet. Dependent on the route of exposure crustaceans food allergy can mainly affect skin or the respiratory tract (EFSA Panel on Dietetic Products and Allergies 2014). Main crustaceans consumed are decapod crustaceans (e.g. shrimp, prawn, crab, lobster). Therefore these food items are the main interest concerning allergenic food. In the german adult population, estimated sensitisation rates to crab, based on positive skin prick tests was 1.9% (EFSA Panel on Dietetic Products and Allergies 2014). However, prevalence of allergy to crustaceans based on clinical history and positive skin prick tests were lower (0.2%, EFSA Panel on Dietetic Products and Allergies 2014). Cross-reactivities are possible among crustaceans, but also between crustaceans and molluscs as well as crustaceans and non-molluscan invertebrates and crustaceans and vertebrates (EFSA Panel on Dietetic Products and Allergies 2014).

Molluscs and products thereof

According to Annex II of Regulation (EU) No 1169/2011 are molluscs and products thereof considered to be of allergenic potential. Molluscs, as such, live in salt water, fresh water and on land. Only three classes of molluscs play an important role as food (gastropos, bivalves and cephalopods, EFSA Panel on Dietetic Products and Allergies 2014). Similar to fish and crustaceans consumption, the dietary intake of molluscs and products thereof depends on region, traditions and culture (EFSA Panel on Dietetic Products and Allergies 2014). However, no estimations of dietary intake were made in german, yet. No prevalence studies using food challenges are available. Zero prevalence was reported based on positive

skin prick tests and clinical history in the german adult population (EFSA Panel on Dietetic Products and Allergies 2014). Clinical manifestation include mostly anaphylactic reactions (EFSA Panel on Dietetic Products and Allergies 2014). Cross-reactivities among molluscs are known as well as potential cross-reactivities between molluscs and crustaceans. Furthermore cross-reactivities between molluscs and non-crustaceans invertebrates as well as molluscs and vertebrates are possible (EFSA Panel on Dietetic Products and Allergies 2014).

Celery and products thereof

Celery (Apium graveolens) plants are composed of a root and of an aerial part, the sticks. Both are consumed raw, cooked but also dried as spice. It is a common ingredient as spice in highly processed food, it occurs thereof in a broad range of food stuff (EFSA Panel on Dietetic Products and Allergies 2014). According to Annex II of Regulation (EU) No 1169/2011 are celery and products thereof considered to be of allergenic potential. As such, celery can induce allergic reactions with clinical manifestations ranging from oral contact urticaria to anaphylaxis (EFSA Panel on Dietetic Products and Allergies 2014). Based on positive skin prick tests, the prevalence in german adults was 9.1% (EFSA Panel on Dietetic Products and Allergies 2014). The prevalence was lower (2.7%) in the german population, when prevalence was estimated based on positive skin prick tests and clinical history of allergy to celery (EFSA Panel on Dietetic Products and Allergies 2014). Cross-reactivities between celery and pollen (such as birch pollen) are possible (EFSA Panel on Dietetic Products and Allergies 2014).

Lupin and products thereof

According to Annex II of Regulation (EU) No 1169/2011 are lupin and products thereof considered to be of allergenic potential. Lupin is a legume with a wide range of species. Typically lupin are used as flour or as lupin derived drinks. Due to its technological values similar to eggs, it is used as egg substitute (EFSA Panel on Dietetic Products and Allergies 2014). Furthermore high protein values and low costs make lupin a food with high nutritional and economical value. In the past decade, development of replacing wheat with lupin flour resulted into the usage of lupin flour in biscuits, pasta, sauces and also soy substitutes. The prevalence of allergy to lupin in the general population is unknown (EFSA Panel on Dietetic Products and Allergies 2014). Cross-reactivities are possible between several legumes (e.g. peanut and soybean) and lupin (EFSA Panel on Dietetic Products and Allergies 2014).

Sesame seeds and products thereof

According to Annex II of Regulation (EU) No 1169/2011 sesame (sesamum indicum L.) seeds and products thereof are considered to be of allergenic potential. Sesame seeds are mainly used in products such as bakery products, fast-food and vegetarian products (EFSA Panel on Dietetic Products and Allergies 2014). Its oil is used for cooking and salad dressing. Prevalence based on positive skin prick tests and clinical history was 1.7% in the german population (EFSA Panel on Dietetic Products and Allergies 2014). Sesame may trigger anaphylaxis as a clinical manifestation (EFSA Panel on Dietetic Products and Allergies 2014).

Mustard and products thereof

According to Annex II of Regulation (EU) No 1169/2019 mustard and products thereof are considered to be of allergenic potential. Main types of mustard used in the kitchen are white/yellow (sinapis alba L.), black (Brassica nigra L.) and brown/oriental mustard (Brassica juncea L, EFSA Panel on Dietetic Products and Allergies 2014). Mustard is mostly used in spices, sauces and salads. No data was available on the prevalence of allergy to mustard in the german population. Mustard may trigger anaphylaxis but also urticaria and bronchial asthma (EFSA Panel on Dietetic Products and Allergies 2014).

Sulphur dioxide and sulphites

According to Annex II of Regulation (EU) No 1169/2011 sulphur dioxide and sulphites are at concentrations of more than 10 mg/kg or 10 mg/l in terms of the total sulphur dioxide considered to be of allergenic potential. Interestingly, sulfite sensitivity is not a typical IgE-mediated allergy but is well known to provoke wheezing in individuals with asthma (summarized in Cox and Sicherer 2020). As defined in Annex II, sulphites and sulphiting agents are defined as sulphur dioxide. Sulphites can occure naturally due to fermentation but are mostly used as preservatives. Their technological property is to inhibit enzymatic as well as non-enzymatic browning when added to food. Furthermore, sulphites have antimicrobial activities and bleaching effects (EFSA Panel on Dietetic Products and Allergies 2014). Consequently, sulphites are used in a broad range of food stuff such as alcoholic beverages, pies and pizza crust. The prevalence of sensitivity to sulphiting agents in the german population is unknown (EFSA Panel on Dietetic Products and Allergies 2014). Bronchospasm is the most likely to get clinical feature after ingestion of sulphite-containing food. Unlike non-asthmatic people, most people with asthma are sensitive to inhaled sulphur dioxide. Therefore, it may be concluded, that among non-asthmatics, the appearance of sensitivity against sulphur dioxide seems to be rare (EFSA Panel on Dietetic Products and Allergies 2014).

5.5.2.3. Implementation into the application

Article 9 Paragraph 1 littera c) of the Regulation (EU) No 1169/2011 indicates that »any ingredient or processing aid listed in Annex II or derived from a substance or product listed in Annex II causing allergies or intolerances used in the manufacture or preparation of a food and still present in the finished product, even if in an altered form «is mandatory to specify on the respective packaging of the food. Typically, that is implemented in the respective list of ingredients (according to Article 9 Paragraph 1 littera b) of Regulation (EU) No 1169/2011). Thereby shall »the name of the substance or product as listed in Annex II be emphasised through a typeset that clearly distinguishes it from the rest of the list of ingredients, for example by means of the font, style or background color. «(According to Article 21 Paragraph 1 littera b) of Regulation (EU) No 1169/2011). Since all mentioned food allergens are mandatory to specify on the respective packaging, it is possible to implement those food allergens into the application. Therefore, when scanning the respective GTIN from the food, information of the list of ingredients with all food allergens included will be provided for the user. Depending on the respective user profile the application will send an alert, if included allergen in the respective food stuff and individual profile settings are matching. This is only possible in the case, that a respective list of ingredient exists for the food item scanned. According to Article 19 of Regulation (EU) No 1169/2011 there exists an omission of the list of ingredients for specific food stuff. However labelling of certain substances or products causing allergies or intolerances are furthermore mandatory. Article 21 Paragraph 1 subparagraph 1 of Regulation (EU) No 1169/2011 creates basis for such special cases as followed: »In the absence of a list of ingredients, the indication of the particulars referred to in point (c) of Article 9(1) shall comprise the word 'contains' followed by the name of the substance or product as listed in Annex II. «That means, that not only information regarding the ingredients list is needed but also information on the word 'contains' and the respective allergen concerned. However, for that special case, there also exists an exception according to Article 21 Paragraph 1 subparagraph 3 of Regulation (EU) No 1169/2011 which lays down, that »the indication of the particulars referred to in point (c) of Article 9(1) shall not be required in cases where the name of the food clearly refers to the substance or product concerned. «That means, that food stuff, which name clearly indicate, that the respective food allergen is inside that respective food stuff, does not need to be labelled in terms of food allergens. Fig 5.31 shows all information and possibilities needed, to implement all mentioned food allergens into the application. To fully implement all mentioned allergens into the application, the following information need to be provided by the scans of the respective food stuff: in case 1 (fig. 5.31), the
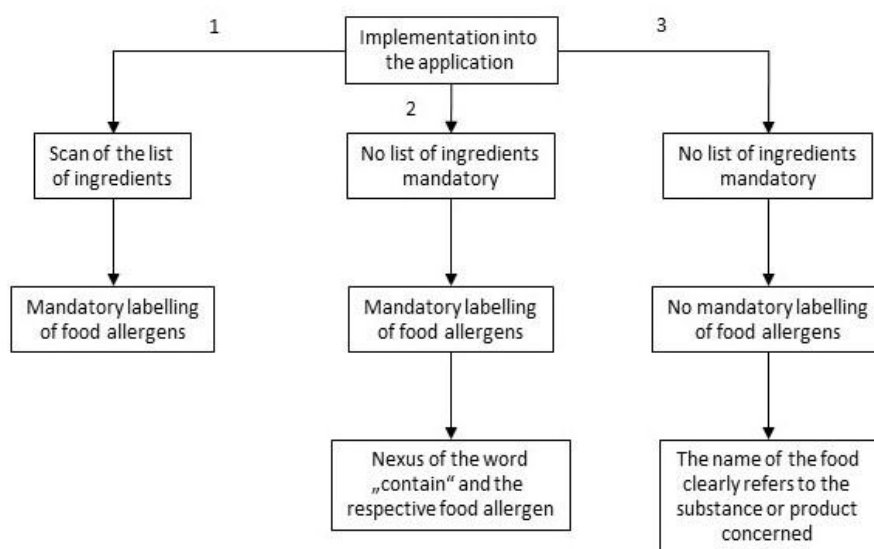
Figure 5.31.: Information needed to fully implement food allergens into the application.

respective food stuff has a list of ingredients. Therefore potential allergens will be labelled in the respective list. For case 1, information from the list of ingredients are sufficiently to provide all information. In case 2 (fig. 5.31) no list of ingredients is mandatory for the respective food stuff. Allergen labelling in the list of ingredients is not applicable. However allergen labelling is mandatory. Information on the respective food allergen will be provided on the package by nexus of the word 'contain' and the respective food allergen (according to Annex II). Concludingly, the scan shall not only provide the list of ingredients but also the logical nexus 'contain' and the food allergen concerned. In case 3 (fig. 5.31) also no list of ingredients is mandatory. Additionally, no labelling of food allergens is mandatory because the name of the food clearly refers to the substance or product concerned (the food allergen). For this case, the scan shall further provide information on the name of the respective food stuff.

To sum up, for the implementation of mentioned food allergens into the application, it is necessary to provide information on the list of ingredients, the nexus 'contain' and the respective food allergen as well as information on the name of the product.

5.5.3. Adverse reaction to food due to non-immune reactions

In difference to food allergens, food intolerances do not have regulatory requirements regarding labelling of specific food ingredients. Therefore, no list of food intolerances

exists, which states out the necessity for implementation into the application. As stated out
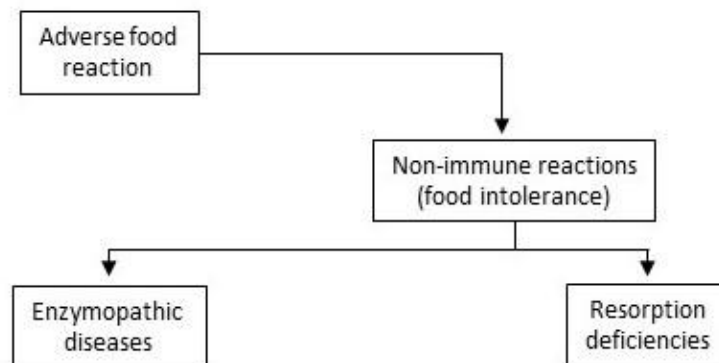
Figure 5.32.: In the context of this manuscript, food intolerances shall comprise enzymo-pathic diseases and resorption deficiencies.

in ch. 5.5.1 food intolerances may have different origins and include food intolerances that may be due to a special metabolic response of an individual, a response to pharmacological effects of food ingredients or ideopathic reactions to food ingredients, which do not have a known origin. This chapter shall provide special focus on food intolerances due to a special metabolic response (Fig. 5.32). Metabolic responses in the context of this manuscript shall comprise enzymopathic diseases and resorption deficiencies (fig. 5.32). Focus was set on these two kinds of metabolic responses due to their scientifical and medical evidence. In germany, reliable diagnosis concerning some enzymopathic diseases is performed by the neonatal screening according to §17 of the Kinder-Richtlinie (Richtlinie des Gemeinsamen Bundesausschusses über die Früherkennung von Krankheiten bei Kindern). According to §17, neonatals will be screened for the following diseases (tab. 5.8). Some of these may be triggered through food ingredients and as a consequence of these diseases, food restrictions need to be applied. For the context of this thesis, special focus was set for Galactosemia, Hyperphenylalaninemia/Phenylketonuria, Maple Syrup Urin Disease, Medium-Chain Acyl-CoA Dehydrogenase deficiency, Long-chain 3-Hydroxyacyl-CoA Dehydrogenase deficiency, Very Long-chain Acyl-CoA-Dehydrogenase deficiency, Carnitine-Palmitoyl-Transferase I and II deficiency, Carnitin-Acylcarnitine Translocase deficiency, Glutaric Acidemia Typ I, Isovaleric Acidemia and Tyrosinemia because these disease are triggered through food and food ingredients and therefore specific food ingredients have to be avoided (tab. 5.8 bold highlighted diseases). Besides diseases from the neonatal screening, focus was set on food intolerances regarding carbohydrates, since these are known to be very common in the german population (Raithel et al. 2013). For that, lactose intolerance,

Table 5.8.: Diseases, which are screened in the neonatal screening according to §17 Kinder-Richtlinie. Bald highlighted diseases indicate inclusion into this manuscript.

Disease	Disease
Hypothyroidism	**Very Long-Chain Acyl-CoA-Dehydrogenase deficiency**
Congenital Adrenal Hyperplasia	**Carnitine Palmitoyl Transferase I deficiency**
Biotinidase Deficiency	**Carnitine Palmitoyl Transferase II deficiency**
Galactosemia	**Carnitine-Acylcarnitine Translocase deficiency**
Hyperphenylalaninemia	**Glutaric Acidemia Type I**
Phenylketonuria	**Isovaleric Acidemia**
Maple Syrup Urine Disease	**Tyrosinemia**
Medium-Chain Acyl-CoA Dehydrogenase deficiency	Cystic Fibrosis
Long-chain 3-Hydroxyacyl-CoA Dehydrogenase deficiency	Severe Combined Immunodeficiency

intestinal fructose intolerance, hereditary fructose intolerance, sorbitol intolerance and sucrase-isomaltase deficiency were chosen for further investigation.

5.5.3.1. Containment according to prevalence

As mentioned in ch. 5.5, prevalence of adverse reactions to food and diseases associated with the intake of special food ingredients are low. Often, orphan disease status is labelled for such adverse reactions. In the context for the implementation of possible food-associated diseases, prevalences for chosen diseases were analyzed first (tab. 5.9) and cut-off criterion for possible admission into the application was set on a prevalence rate of 1:50.000. Most of the investigated diseases in the context of the application had a prevalence lower than 1:50.000 (tab. 5.9). From the neonatal screening, Hyperpheny-

lalaninemia (prevalence 1:5.153; tab. 5.9), Phenylketonuria (prevalence 1:13.88; tab. 5.9) and Medium-chain Acyl-CoA Dehydrogenase deficiency (prevalence 1:9.849, tab. 5.9) were included into further investigations regarding implementation into the application. Food intolerances associated with adverse reactions to carbohydrates (lactose intolerance, intestinal fructose intolerance, hereditary fructose intolerance, sorbitol intolerance and sucrase-isomaltase deficiency) were all included into further investigations regarding implementation into the application (tab. 5.9).

Table 5.9.: Prevalence of selected food intolerances.

Disease	Prevalence	Literature
Galactosemia	1:77.809	Screeningreport-Deutschland 2019
Hyperphenylalaninemia	**1:5.153**	Screeningreport-Deutschland 2019
Phenylketonuria	**1:13.188**	Screeningreport-Deutschland 2019
Maple Syrup Urin Disease	1:194.523	Screeningreport-Deutschland 2019
Medium-chain Acyl-CoA Dehydrogenase deficiency	**1:9.849**	Screeningreport-Deutschland 2019
Long-chain 3-Hydroxyl-Acyl CoA Dehydrogenase deficiency	1:259.363	Screeningreport-Deutschland 2019
Very long-chain Acyl-CoA Dehydrogenase deficiency	1:97.261	Screeningreport-Deutschland 2019
Carnitin-Palmitoyl-Transferase I deficiency	not available	Screeningreport-Deutschland 2019
Carnitin-Palmitoyl-Transferase II deficiency	1:389.045	Screeningreport-Deutschland 2019
Carnitine-Acylcarnitine-Translocase deficiency	not available	Screeningreport-Deutschland 2019
Glutaric Acidemia Typ I	1:194.523	Screeningreport-Deutschland 2019
Isovaleric Acidemia	1:111.156	Screeningreport-Deutschland 2019
Tyrosinemia	1:129.682	Screeningreport-Deutschland 2019
Lactose intolerance	**widespread**	Storhaug, Fosse, and Fadnes 2017
Intestinal fructose intolerance	**widespread**	Raithel et al. 2013
Hereditary fructose intolerance	**1:20.000**	Raithel et al. 2013
Sorbitol intolerance	**widespread**	Raithel et al. 2013
Sucrase-isomaltase deficiency	**1:5.000**	Orphanet 2022

5.5.3.2. Selected food intolerances

In the following subchapter selected food-associated diseases will be discussed in the context of the implementation into the application. First, the medical basics of the respective diseases will be briefly depicted. Subsequently, the association between the selected diseases and food ingredients, which may trigger these diseases will be discussed. Finally, it will be discussed, whether implementation into the application is possible or not.

Hyperphenylalaninemia and Phenylketonuria

Phenylketonuria is an inborn error in aminoacid metabolism and result into hyperphenylalaninemia, by which blood phenylalanin concentrations are increased (Wegberg et al. 2017 and Blau, Spronsen, and Levy 2010). It is characterised by mutations of the phenylalanine hydroxylase gene. Phenylalanine hydroxylase hydroxylates phenylalanine into tyrosine. For this conversion, phenylalanin hydroxylase requires the cofactor tetrahydrobipterin, oxygen and iron (Blau, Spronsen, and Levy 2010). A deficiency in phenylalanine hydroxylase leads to accumulation of phenylalanin in blood and brain (Wegberg et al. 2017). This accumulation leads to phenylketonuria which is characterized by irreversible intellectual disability which is accompanied by additional symptoms. As part of the neonatal screening, it is possible to detect phenylketonuria as early as possible. As part of the therapy, dietary restrictions will be applied, when phenylketonuria is diagnosed. The basis of the therapy encompasses a phenylalanine-free diet with strong restrictions in foods with high protein content (such as eggs, cheese, nuts). Furthermore, patients with phenylketonuria have to avoid foods and drinks containing aspartame because phenylalanine is part of the respective dipeptide. In general, low-protein food can be eaten. The required amount of daily protein is obtained most of the time from food for medical purposes developed to satisfy the nutritional requirements of phenylketonuria patiens (Blau, Spronsen, and Levy 2010). Foods containing aspartame/aspartame-acesulfam salt as sweetener shall label »contains aspartame (a source of phenylalanine) «on the respective packaging if aspartame is listed in the ingredients list (according to Annex III no. 2.3. of Regulation (EU) No. 1169/2011). In the background of the application, it could be possible to monitor food stuff and beverages in the context of the use of aspartam. As a food additive (sweetener, according to Regulation (EU) No. 1333/2008), aspartam could be labelled as its chemical name but also as its respective E-number. When a manufacturer uses aspartame, he is obliged to label »contains aspartam (a source of phenylalanine) «. The scan of the respective food stuff or beverage provides information for users who are suffering from phenylketonuria. However, this is only possible if aspartame is used. Since a lot of food

stuff contains a high amount of protein (and therefore phenylalanine), the implementation of phenylketonuria into the application would result into rejection of all scanned food stuff with a higher protein content. The aim of the application is not to reject as many food stuff as possible but to provide information for potential adverse reactions with food ingredients. Thus, as a consequence, phenylketonuria could not be implemented into the application. It is noteworthy, that a possible implementation into the application would be applicable with a professional dietary concept, which was not scope of this dissertation.

Medium-chain Acyl-CoA Dehydrogenase deficiency

Medium-chain acyl-CoA dehydrogenase is an enzyme in the mitochondrial β-oxidation of MCFAs. It is one of the key enzymes involved in mitochondrial FA β-oxidation. Medium-chain acyl-CoA dehydrogenase deficiency is, after hyperphenylalaninemia, the most common orphan disease from the neonatal screening (tab. 5.9) and the most common disorder of FA β oxidation (summarized in J Lawrence Merritt and Chang 2019). Hypoketotic hypoglycemia is the major clinical symptom. This is often accompanied with vomiting and other gastrointestinal symptoms which may progress to lethargy, seizures and coma (summarized in J Lawrence Merritt and Chang 2019). As part of the neonatal screening, it is possible to detect medium-chain acyl-CoA dehydrogenase deficiency as early as possible. As part of the therapy, dietary restrictions will be applied, when medium-chain acyl-CoA dehydrogenase deficiency is diagnosed. Hypoglycemia is the most important condition to avoid. Therefore, the major for prevention of clinical symptoms, is avoidance of catabolic conditions using a regular feeding regimen. Furthermore, avoidance of MCFAs as primary source of fat is the most important step restriction due to the deficiency of the medium-chain acyl-CoA dehydrogenase (summarized in J Lawrence Merritt and Chang 2019). In the context of the application, similar problems would occur for medium chain acyl-CoA dehydrogenase deficiency as for phenylketonuria. A lot of food stuff contain considerable amount of MCFAs. Therefore the implementation into the application would result into rejection of all scanned food stuff with a higher fat content (and therefore with a higher MCFA content) when this disease would be applied in the user settings. Thus, as a consequence, Medium-chain Acyl-CoA Dehydrogenase deficiency could not be implemented into the application. It is noteworthy, that a possible implementation into the application would be applicable with a professional dietary concept, which was not scope of this dissertation.

Lactose intolerance

Lactose intolerance is the most frequent cause of enzyme deficiency and occurs across

different populations (Storhaug, Fosse, and Fadnes 2017). Lactase, as a *β*-galactosidase, hydrolyses lactose into glucose and galactose. Lactase is especially important for newborn babies to absorb lactose from milk. Deficiency of lactase result into non-hydrolysed lactose, which is osmotically active. Therefore, when non-hydrolysed lactose passes through the intestines without being absorbed, it is fermented by intestinal bacteria which may induce gastrointestinal symptoms such as diarrhea, abdominal pain and flatulence (summarized in Raithel et al. 2013). The vast majority of people with lactose intolerance tolerate up to 12 g of lactose in a single dose (Efsa Panel on Dietetic Products and Allergies 2010). Therefore the severity of symptoms is dependent on the activity of lactase and also on the patients individual threshold. Up to now, one of the possible treatments of lactose intolerance is the restriction of lactose containing food in the diet. In the context of the application, it could be possible to implement lactose intolerance. As indicated in fig. 5.30 milk and products thereof including lactose have to be labelled on the respective packaging of the scanned food (according to Annex II of Regulation (EU) No 1169/2011). Therefore, in general, the scheme of implementation shown in fig. 5.31 could also be feasible for lactose intolerance. However, due to patients individual threshold to lactose, the implementation into the application would result into rejection of all scanned food stuff with the trigger substance lactose irrespective of the amount of lactose in the scanned food. This would result in an overestimation of rejected food stuff. As a consequence, lactose intolerance could not be implemented into the application but a possible implementation into the application would be applicable with (personalized) thresholds, which would result into rejection of food stuff on a personalized basis. For that, a professional dietary concept would be necessary, which was not scope of this dissertation.

Intestinal fructose intolerance and Hereditary fructose intolerance

Intestinal fructose intolerance and hereditary fructose intolerance are two disorders which both result in fructose-free diet. In the case of intestinal fructose intolerance, the cause of the disease is the failure to effectively absorb fructose through the enterocytes lining the small intestine. Because of the disturbed absorption, fructose accumulates in the intestinal lumen. As a consequence, osmotic pressure causes water influx into the lumen (summarized in Benardout et al. 2022 and Ebert and Witt 2016). Hereditary fructose intolerance is distinct from intestinal fructose intolerance, in which fructose transporters in the small intestine are dysfunctional. Hereditary fructose intolerance is the inherited inability to digest fructose due to a deficiency of activity of the enzyme fructose-1-phosphate aldolase (summarized in Gaughan, Ayres, and Peter R. Baker 2021). Different to intestinal fructose intolerance, there is no problem in resorption but in metabolism of fructose

metabolites. Due to loss in activity of fructose-1-phosphate aldolase, accumulation of fructose-1-phosphate in liver, kidney and small intestine occur (summarized in Gaughan, Ayres, and Peter R. Baker 2021). However some symptoms may overlap between intestinal fructose intolerance and hereditary fructose intolerance such as diarrhea, bloating and increased flatulence. For both, intestinal fructose intolerance and hereditary fructose intolerance, the standard treatment regarding diet is the dietary restriction to fructose-containing food and a strict fructose-free diet. Furthermore dietary restrictions should be extended to further oligo-, di-, monosaccharides and polyols (such as sucrose and sorbitol; summarized in Gaughan, Ayres, and Peter R. Baker 2021 and Benardout et al. 2022). In the context of the application, only limited implementation regarding intestinal fructose intolerance and hereditary fructose intolerance would be possible. First, it would be possible to monitor food ingredients, which are listed in the respective ingredients list. However, especially in the context of intestinal fructose intolerance, the absorption capacity for fructose is highly individual (summarized in Ebert and Witt 2016). Therefore information about possible trigger substances in the application may not be sufficient for some users suffering from either intestinal fructose intolerance or hereditary fructose interolance. Since fructose (and further oligo-, di-, monosaccharides and polyols) is widely used especially in highly processed food, the implementation into the application would result into rejection of all scanned food stuff with all trigger substances irrespective of their in amount in the scanned food. This would result in an overestimation of rejected food stuff. As a consequence, intestinal and hereditary fructose intolerance could not be implemented into the application but a possible implementation into the application would be applicable with (personalized) thresholds, which would result into rejection of food stuff on a personalized basis. For that, a professional dietary concept would be necessary, which was not scope of this dissertation.

Sorbitol intolerance

Sorbitol occurs naturally in a wide range of fruits such as banana, orange, apple and pear. Furthermore, as a sugar alcohol, sorbitol is used as a food additive (E420). Its usage encompasses sugar substitution, as a vehical for other substances and as a humectant (summmarized in Raithel et al. 2013). Because no specific transport system for sorbitol absorption exists, sorbitol interferes with fructose absorption in humans (summarized in Fernández-Bañares, Esteve, and Viver 2009). Sorbitol inhibits GLUT5, therefore clinical symptoms are the same as for intestinal fructose intolerance. As part of the treatment strategy, the patients are restricted to a sorbitol-low diet (summarized in Raithel et al. 2013). In the context of the application, only limited implementation regarding sorbitol intolerance

would be possible. First, implementation would be possible when using the application for scanning of food stuff where sorbitol is used as a food additive. Food additives have to be labelled in the respective ingredients list, therefore information regarding sorbitol used as a food additive would be easy to get through scanning of the respective food stuff. However, the absorption capacity for sorbitol is highly individual. Therefore, the same problems would occur as for intestinal fructose intolerance. Furthermore, sorbitol as a natural occuring sugar alcohol would not be traceable in food stuff, which consists of ingredients, which have natural occuring sorbitol. This would result in an underestimation of rejected food stuff and therefore a potential risk of users, who are suffering from sorbitol intolerance. As a consequence, sorbitol intolerance could not be implemented into the application but a possible implementation into the application would be applicable with (personalized) thresholds, which would result into rejection of food stuff on a personalized basis. For that, a professional dietary concept would be necessary, which was not scope of this dissertation.

Sucrase-isomaltase deficiency

Sucrase-isomaltase, a disaccharidase, is a glucoprotein localized to the brush border membrane of the small intestine (summarized in Cohen 2016). Sucrase-isomaltase deficiency is the disability to hydrolyze sucrose, maltose, short 1,4-linked glucose oligomers and starch (summarized in Treem 2012). Variants in either sucrase or isomaltase subunits of sucrase-isomaltase can occur, resulting in different enzyme activities of respective subunits (summarized in Cohen 2016). Sucrase-isomaltase deficiency lead to accumulation of undigested sugars in the lumen of the small intestine. Clinical manifestation of accumulation of these nutrients in the lumen of the small intestine encompasses osmotic diarrhea with bloating and abdominal distention, malabsorption of other nutrients and increased flatulence (summarized in Treem 2012). As part of the treatment regimen, patients with sucrase-isomaltase deficiency have to follow a strict sucrose- and starch-restricted diet. Regular consultation with a dietician is strongly recommended (summarized in Treem 2012). Similar as for sorbitol intolerance, implementation of sucrase-isomaltase deficiency would not be applicable without a professional dietary concept and personalized thresholds.

5.5.4. Outlook

This section provided scientific background of an application for consumer health. The overall aim of this application is to provide information about potential risks outgoing from scanned food because of accordance between food ingredient(s) and the respective

profile setting of the user. For food allergens, which have regulatory requirements in the context of labelling, potential proposals for implementation into the application were made. For food intolerances, which do not have regulatory requirements, limits were shown in the context of the application. If mentioned food intolerances shall be implemented into the application, a professional dietary concept has to be developed for every food intolerance because of the complexity of the implementation. However, implementation of such professional dietary concepts was not scope of this manuscript.

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A. Appendix

A.1. Work flow scheme of FA extraction procedure
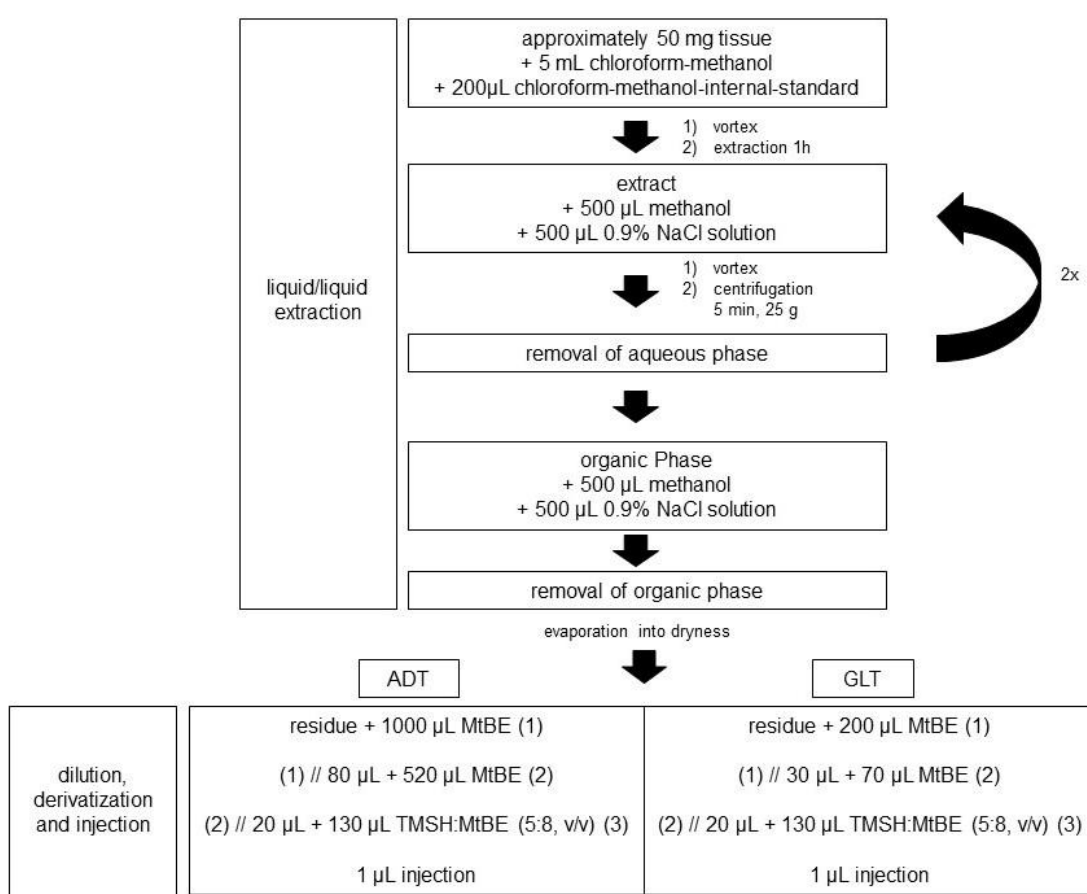
Figure A.1.: Work flow scheme of FA analysis procedure described in this manuscript (ch. 4.2.3).

A.2. Relevant GC-MS/FID parameters

Relevant method parameters from the GC-MS/FID method (ch. 4.2.3.3) to provide consistent data regarding FA analysis.

Autosampler

Sampling	Sample Volume	1.00
	Plunger	3
	Viscous	No
	Sampling depth in Vial	Bottom
Injection	Injection	Standard
	Pre-Injection	0.00
	Post-Injection	0.00
Pre-Inj.	Solvent	A
	Cycles	3
Sample	Rinses	1
Post-Inj.	Solvent	A
	Cycles	5

Trace 1300 series GC

oven

#	Rate (°C/min)	Temperature (°C)	Hold times (min)
Initial		120.0	0.00
1	4.0	240.0	15.00
Data	oven run	45.00 min	
Options	Max.	350.0°C	
	oven on	yes	
	Prep-run	10.00 min	
	Equlbrillation	0.50	
	Ready	0.00	
S/SL	S/SL mode	splitless	
	Carrier	constant pressure	
Carrier	Pressure	yes	500.0 kPa
Carrier	Vacuum		
	Carrier Gas	yes	
	Gas saver	5.0 ml/min	
	Gas saver	2.00 min	
Inlet	Temperature	yes	250°C
	Split flow		5.0 ml/min
	Split ratio		
	Splitless time		1.00 min
Septum	Purge flow	5.0 ml/min	
	Constant	yes	
	Stop purge	0.00 min	

FID (front)

Detector	Flame on	yes	
	Temperature	yes	250℃
	Ignition	0.5 pA	
Gas flow	Air	yes	250 ml/min
	Hydrogen	yes	35.0 ml/min
	Makeup Gas		
Signal	yes		
	GC Peak	Standard	yes
		Fast	

ISQ

Method	Method type	Acquisition - General	
	MS Transfer	250℃	
	Ion Source	250℃	
	Acquisition		
	Ionisation	EI	
Run	yes	GC run	
Scans	Time (min)	Mass List	Dwell
	2.50	50-450	0.2

A.3. Relevant Integration parameters

Relevant integration parameters from the GC-MS/FID method (ch. 4.2.3.3) to provide consistent data regarding FA analysis.

Peak Parameter

Baseline window	500
Area noise factor	3
Peak noise factor	3

Advance

Noise method	Repetitive noise
Min peak width	3
Multiplet Resolution	15
Area tail extension	5
Area scan window	0

A.4. Relevant numerical and categorical data from the study ISOCROSS

In the following tables, all relevant numerical and categorical data from the 40 study participants of the study population of ISOCROSS will be depicted. The no. indicates the real numbers from the study participants. Following numerical and categorical data will be depicted: age (numerical), menopausal status (categorical; possible status pre-, peri- and postmenopausal; MP), parity (numerical; number of pregnancies), oil% (numerical, percentag), smoking habits (categorical; non smoker NS and smoker S), alcohol consumption (numerical, consumption in g alcohol per week; alc), BMI (numerical), intake of estrogen releasing drugs (categorical; no intake, intake of EE ethinylestradiol or intake of ERD estrogen releasing drugs), individual FA percentages on total FA composition (numerical; for 20:5 ω3, 22:6 ω3, 18:2 ω6, 18:3 ω3, 18:1 ω9 *trans*, 16:1 ω7, 16:0, 18:3 ω6, 20:4 ω6 and 20:3 ω6), levels of 7β-OHC, 5,6β-epoxyC and 5,6α-epoxyC (numerical; ng/g), levels of E1 and E2 (numerical; fmol/g), Δ5DI (numerical, ratio of 20:4 ω6 and 20:3 ω6), Δ6DI (numerical, ratio of 18:3 ω6 and 18:2 ω6), Δ9DI (numerical, ratio of 16:1 ω7 and 16:0) and ELOVLI5 (numerical, ratio of 20:3 ω6 and 18:3 ω6).

Table A.1.: Relevant data from the study participants 1-20 from the study population of ISOCROSS. The no. indicates the real numbers from the study participants. MP menopausal status; smoke smoking habits; alc alcohol consumption; ERD intake of estrogen releasing drugs.

no.	age	MP	parity	oil%	smoke	alc	BMI	ERD
1	29	pre	0	59.8	NS	0	27	no
2	49	peri	0	80.9	S	140	24.5	ERD
3	30	pre	1	67	S	0	24	no
5	51	peri	3	80.5	NS	0	26	no
6	51	peri	0	71.8	NS	10	28	no
7	52	peri	1	62.4	NS	0	24	no
8	51	peri	0	62.6	NS	0	24	no
10	51	peri	2	82.3	NS	0	28	no
11	51	peri	2	92.1	NS	0	25	no
12	42	pre	3	81	NS	10	27	no
13	22	pre	1	71	NS	0	26	no
14	49	peri	2	85.4	NS	0	26	no
16	27	pre	0	84.4	S	20	22	EE
18	35	pre	3	94	NS	0	28	no
20	27	pre	0	72.2	S	100	23	EE
21	22	pre	0	86.3	S	10	22	EE
24	56	post	2	81.8	NS	60	24	no
25	47	peri	0	95.9	NS	50	31.4	no
26	44	pre	0	97.2	S	10	23	no
27	54	post	2	85.5	NS	140	24	no

Table A.2.: Relevant data from the study participants 21-40 from the study population of ISOCROSS. The no. indicates the real numbers from the study participants. MP menopausal status; smoke smoking habits; alc alcohol consumption; ERD intake of estrogen releasing drugs.

no.	age	MP	parity	oil%	smoke	alc	BMI	ERD
28	44	pre	2	84.1	NS	20	28	no
29	32	pre	1	69	NS	10	26.9	no
31	54	post	2	88.2	NS	30	24	no
32	33	pre	0	78.5	NS	0	26.8	no
33	18	pre	0	85.5	S	233	26.1	EE
34	40	pre	3	84	NS	0	27.3	no
35	49	peri	3	86.9	NS	50	28.9	ERD
36	35	pre	3	79.6	NS	20	26.2	no
37	24	pre	0	87.3	S	0	31.6	no
38	18	pre	0	88.2	NS	13	25.6	no
39	61	post	1	85.4	NS	0	32.3	no
41	33	pre	0	73.4	S	140	19.6	EE
42	39	pre	2	83	NS	40	23.9	no
43	66	post	1	88.8	NS	30	32.8	no
44	44	pre	2	93.2	NS	0	45.6	no
45	49	peri	2	89	NS	20	26.6	no
46	36	pre	0	90.2	NS	10	26.7	EE
47	57	post	7	92.9	S	20	28.3	no
48	42	pre	3	84.8	NS	2	33.4	no
49	47	peri	2	93.2	NS	1	25	no

Table A.3.: Relevant data from the study participants 1-20 from the study population of ISOCROSS. The no. indicates the real numbers from the study participants. Respective FA proportions are provided as percentages of total FA composition.

no.	20:5 ω3	22:6 ω3	18:2 ω6	18:3 ω3	18:1 ω9t	16:1 ω7	16:0	18:3 ω6	20:4 ω6	20:3 ω6
1	0.03	0.09	11.7	0.76	0.44	3.3	22.16	0.04	0.35	0.21
2	0.05	0.11	8.78	0.54	0.43	6.8	21.5	0.04	0.41	0.19
3	0.03	0.12	10.01	0.68	0.47	3.44	24.26	0.04	0.29	0.21
5	0.04	0.09	9.2	0.63	0.59	4.72	23.66	0.04	0.28	0.16
6	0.07	0.2	10.25	0.64	0.37	7.58	18.92	0.04	0.47	0.29
7	0.1	0.3	11.3	0.67	0.37	5.97	23.07	0.05	0.5	0.29
8	0.04	0.17	14.12	0.68	0.39	3.56	21.97	0.04	0.39	0.21
10	0.07	0.26	12.61	0.62	0.43	3.47	23.67	0.04	0.52	0.33
11	0.04	0.18	10.81	0.58	0.47	5.18	23.82	0.05	0.58	0.34
12	0.08	0.18	10.88	0.65	0.31	3.99	20.59	0.04	0.55	0.27
13	0.03	0.12	8.7	0.5	0.39	2.45	25.25	0.04	0.34	0.22
14	0.04	0.09	10.85	0.39	0.56	2.11	24.8	0.07	0.63	0.5
16	0.03	0.09	11.53	1.07	0.39	2.56	24.16	0.04	0.26	0.14
18	0.04	0.11	10.72	0.53	0.32	2.83	22.82	0.04	0.39	0.25
20	0.03	0.14	9.61	0.53	0.27	3.76	23.75	0.04	0.39	0.33
21	0.03	0.09	9.99	0.84	0.37	4.07	25.12	0.04	0.25	0.17
24	0.07	0.18	12	0.78	0.37	4.68	21.65	0.07	0.53	0.24
25	0.06	0.19	11.5	0.6	0.31	1.87	25.11	0.04	0.55	0.38
26	0.04	0.08	12.15	0.67	0.41	2.68	23.72	0.04	0.34	0.16
27	0.08	0.25	9.85	0.63	0.29	3.62	24.58	0.03	0.37	0.39

Table A.4.: Relevant data from the study participants 21-40 from the study population of ISOCROSS. The no. indicates the real numbers from the study participants. Respective FA proportions are provided as percentages of total FA composition.

no.	20:5 ω3	22:6 ω3	18:2 ω6	18:3 ω3	18:1 ω9t	16:1 ω7	16:0	18:3 ω6	20:4 ω6	20:3 ω6
28	0.03	0.07	10.14	0.69	0.39	3.3	22.95	0.04	0.29	0.24
29	0.08	0.31	9.48	0.37	0.35	5.01	23.2	0.05	0.62	0.27
31	0.04	0.15	10.72	0.64	0.25	3.78	22.91	0.04	0.37	0.17
32	0.02	0.13	9.89	0.44	0.18	2.19	22.56	0.04	0.23	0.18
33	0.03	0.08	10.15	0.61	0.25	4.52	25.28	0.05	0.41	0.28
34	0.07	0.17	11.35	0.77	0.26	3.44	23.04	0.05	0.78	0.45
35	0.06	0.13	8.27	0.68	0.22	4.12	28.01	0.03	0.37	0.17
36	0.05	0.13	10.57	0.74	0.28	2.62	23.79	0.03	0.29	0.18
37	0.06	0.11	9.65	0.89	0.27	5.62	24.11	0.03	0.39	0.25
38	0.05	0.07	10.45	0.68	0.3	3.61	22.97	0.04	0.32	0.17
39	0.07	0.25	11.78	0.82	0.32	3.53	21.2	0.03	0.48	0.32
41	0.02	0.13	8.99	0.67	0.22	4.55	23.36	0.02	0.32	0.16
42	0.04	0.1	11.03	0.53	0.27	2.83	25.12	0.04	0.31	0.24
43	0.03	0.1	14.56	0.63	0.19	2.22	20.42	0.04	0.31	0.23
44	0.05	0.13	13.11	0.95	0.23	2.2	24.77	0.03	0.54	0.36
45	0.03	0.08	11.35	0.65	0.43	2.98	23.01	0.03	0.29	0.23
46	0.02	0.08	8.35	0.7	0.26	5.78	25.19	0.02	0.31	0.16
47	0.06	0.16	7.6	0.25	0.22	7.08	21.52	0.06	1.04	0.54
48	0.03	0.05	12.01	0.75	0.22	1.95	24.39	0.05	0.35	0.25
49	0.03	0.08	11.35	0.65	0.42	2.98	23.1	0.03	0.3	0.22

Table A.5.: Relevant data from the study participants 1-20 from the study population of ISOCROSS. The no. indicates the real numbers from the study participants.

no.	7β-OHC	5,6β-epoxyC	5,6α-epoxyC	E1	E2	Δ5DI	Δ6DI	Δ9DI	ELOVLI5
1	45.61	269.81	85.88	771	229	1.667	0.003	0.149	5.25
2	2.85	74.29	16.88	1415	184	2.158	0.005	0.316	4.75
3	3.47	80.61	23.8	1035	318	1.381	0.004	0.142	5.25
5	6.63	624.2	82.51	452	82	1.750	0.004	0.199	4
6	2.99	101.41	20.53	776	73	1.621	0.004	0.401	7.25
7	2.38	66.43	16.72	376	66	1.724	0.004	0.259	5.8
8	5.82	304.74	86.37	563	157	1.857	0.003	0.162	5.25
10	3.28	90.76	17.17	530	94	1.576	0.003	0.147	8.25
11	4.71	139.11	27.87	2472	1226	1.706	0.005	0.217	6.8
12	37.2	263.79	110.94	2876	1642	2.037	0.004	0.194	6.75
13	5.47	283.26	37.49	1344	830	1.545	0.005	0.097	5.5
14	9.02	184.22	40.04	479	112	1.260	0.006	0.085	7.14
16	18.25	159.81	76.96	2869	1515	1.857	0.003	0.106	3.5
18	4.92	186.45	39.43	4567	3075	1.560	0.004	0.124	6.25
20	0.97	41.95	13.61	578	107	1.182	0.004	0.158	8.25
21	2.22	121.6	26.55	695	291	1.471	0.004	0.162	4.25
24	10.42	181.32	44.22	627	96	2.208	0.006	0.216	3.43
25	3.11	195.29	49.32	2156	1509	1.447	0.003	0.074	9.5
26	2.01	146.97	41.35	1297	478	2.125	0.003	0.113	4
27	26.71	164.17	45.85	368	60	0.949	0.003	0.147	13

Table A.6.: Relevant data from the study participants 21-40 from the study population of ISOCROSS. The no. indicates the real numbers from the study participants.

no.	7β-OHC	5,6β-epoxyC	5,6α-epoxyC	E1	E2	Δ5DI	Δ6DI	Δ9DI	ELOVLI5
28	3.84	117.58	31.1	1556	455	1.208	0.004	0.144	6.000
29	3.23	161.8	40.16	1710	1223	2.296	0.005	0.216	5.400
31	6.66	51.74	17.07	404	41	2.176	0.004	0.165	4.250
32	2.67	147.16	29.5	1444	292	1.278	0.004	0.097	4.500
33	3.54	210.63	38.65	807	58	1.464	0.005	0.179	5.600
34	3.2	100.49	31.77	3708	809	1.733	0.004	0.149	9.000
35	2.56	48.28	14.65	2809	1237	2.176	0.004	0.147	5.667
36	3.52	180.65	42.21	660	201	1.611	0.003	0.110	6.000
37	5.53	152.78	34.84	1292	581	1.560	0.003	0.233	8.333
38	1.29	59.25	17.92	1051	419	1.882	0.004	0.157	4.250
39	2.52	81.51	24	755	147	1.500	0.003	0.167	10.667
41	1.26	25.7	9.87	249	40	2.000	0.002	0.195	8.000
42	3.5	166	49.32	861	421	1.292	0.004	0.113	6.000
43	5.75	43.66	18.22	325	97	1.348	0.003	0.109	5.750
44	2.64	160.86	29.47	2536	1081	1.500	0.002	0.089	12.000
45	4.11	149.9	36.82	3454	1726	1.261	0.003	0.130	7.667
46	4.05	132.31	22.97	602	99	1.938	0.002	0.229	8.000
47	1.58	40.29	19	2121	177	1.926	0.008	0.329	9.000
48	3.23	161.23	34.81	1560	613	1.400	0.004	0.080	5.000
49	10.6	68.13	16.61	513	164	1.364	0.003	0.129	7.333

A.5. Correlation coefficients of correlation analysis of possible *exVars* in the study population of ISOCROSS

Variable 1	R	Variable 2	Variable 1	R	Variable 2
18:2 ω6	0.35	18:3 ω3	18:1 ω9 *trans*	-0.33	BMI
18:2 ω6	0.29	5,6α-epoxyC	18:1 ω9 *trans*	-0.36	alc
18:2 ω6	0.38	age	7β-OHC	0.69	5,6α-epoxyC
18:2 ω6	-0.38	Δ9DI	7β-OHC	0.62	5,6β-epoxyC
18:2 ω6	-0.31	Δ6DI	5,6α-epoxyC	0.88	5,6β-epoxyC
18:2 ω6	-0.48	ELOVLI5	age	0.41	parity
18:3 ω3	-0.40	Δ6DI	BMI	-0.30	ELOVLI5
20:5 ω3	0.74	22:6 ω3	BMI	0.35	parity
20:5 ω3	0.51	age	BMI	0.33	E1
20:5 ω3	0.34	Δ9DI	Δ9DI	0.51	Δ5DI
20:5 ω3	0.37	Δ5DI	Δ9DI	0.84	ELOVLI5
20:5 ω33	0.32	parity	Δ9DI	-0.32	E2
22:6 ω3	0.48	age	Δ6DI	0.40	ELOVLI5
22:6 ω3	0.37	Δ9DI	Δ5DI	0.38	ELOVLI5
18:1 ω9 *trans*	0.32	7β-OHC	ELOVLI5	-0.35	E2
18:1 ω9 *trans*	0.32	5,6β-epoxyC	E1	0.90	E2

A.6. PCA of selected FA biomarkers in the study population ISOCROSS

Composition of PCs calculated in R using 18:2 ω6, 18:3 ω3, 20:5 ω3, 22:6 ω3 and 18:1 ω9 *trans* in human ADT derived from 40 healthy women and resulting values of PC ($PC_{diet}1$, $PC_{diet}2$ and $PC_{diet}3$) used in multiple linear regression models. Percentages of variation explained by the PCs are provided and eigenvectors of prominent variables influencing the PCs are indicated with bold characters. Breast ADT levels of 18:2 ω6, 18:3 ω3, 20:5 ω3, 22:6 ω3 and 18:1 ω9 *trans* derived from 40 healthy women of the ISOCROSS study were calculated as the relative conentration of each FA using the FID signal of the FA in relation to the sum of all FA-related signal (n=25).

*used in multiple linear regression models on Δ9DI, Δ6DI and Δ5DI as well as ELOVLI5 in human breast ADT.

Table A.7.: Results of PCA of selected FA biomarkers 18:2 ω6, 18:3 ω3, 20:5 ω3, 22:6 ω3 and 18:1 ω9 *trans*. Eigenvectors of prominent variables influencing the PCs are indicated with bold characters and percentages of variation explained by the PCs are provided. *PC indicates, that PC was used in multiple linear regression models.

Variable	Eigenvectors				
	$PC_{diet}1^*$	$PC_{diet}2^*$	$PC_{diet}3^*$	$PC_{diet}4$	$PC_{diet}5$
18:2 ω6	0.01	**0.71**	-0.10	0.68	-0.10
18:3 ω3	-0.17	**0.69**	0.13	-0.67	0.18
20:5 ω3	**0.69**	0.11	0.01	-0.23	-0.68
22:6 ω3	**0.70**	0.04	-0.1	0.05	0.71
18:1 ω9 *trans*	-0.02	0.02	**-0.99**	-0.16	0.02
variance (%)	36.61	27.67	20.22	11.99	3.50

Table A.8.: Factorloadings of $PC_{diet}1$, $PC_{diet}2$ and $PC_{diet}3$ of individual study participants (n=40) from the study population of ISOCROSS.

No.	$PC_{diet}1$	No.	$PC_{diet}1$	No.	$PC_{diet}2$	No.	$PC_{diet}2$	No.	$PC_{diet}3$	No.	$PC_{diet}3$
1	-1.24	21	-1.39	1	0.87	21	-0.21	1	-1.02	21	-0.47
2	-0.11	22	3.32	2	-1.39	22	-1.56	2	-0.89	22	-0.29
3	-0.85	23	-0.08	3	-0.28	23	-0-09	3	-1.28	23	0.86
4	-0.80	24	-0.76	4	-0.83	24	-1.53	4	-2.46	24	1.43
5	1.48	25	-1.16	5	-0.09	25	-0.59	5	-0.30	25	0.87
6	3.59	26	1.04	6	0.78	26	0.98	6	-0.34	26	0.84
7	0.10	27	0.33	7	1.75	27	-0.96	7	-0.75	27	1.37
8	2.17	28	-0.07	8	0.99	28	0.35	8	-1.09	28	0.66
9	0.27	29	-0.12	9	-0.26	29	0.63	9	-1.42	29	0.96
10	1.61	30	-0.67	10	0.29	30	-0.01	10	0.27	30	0.42
11	-0.65	31	1.85	11	-1.73	31	1.48	11	-0.54	31	0.25
12	-0.52	32	-1.03	12	-1.14	32	-0.91	12	-2.48	32	1.29
13	-1.57	33	-0.51	13	2.18	33	-0.47	13	-0.24	33	0.54
14	-0.41	34	-0.91	14	-0.59	34	1.59	14	0.06	34	1.19
15	-0.43	35	-0.27	15	-1.18	35	2.49	15	0.63	35	1.17
16	-1.33	36	-1.23	16	0.40	36	0.19	16	-0.13	36	-0.99
17	1.12	37	-1.63	17	1.37	37	-1.09	17	-0.30	37	0.96
18	1.09	38	1.13	18	0.25	38	-3.23	18	0.17	38	1.04
19	-0.89	39	-1.62	19	0.72	39	0.90	19	-0.82	39	1.17
20	2.39	40	-1.23	20	-0.25	40	0.19	20	0.52	40	-0.89

A.7. Scree plot of PCA of selected FA biomarkers in the study population ISOCROSS
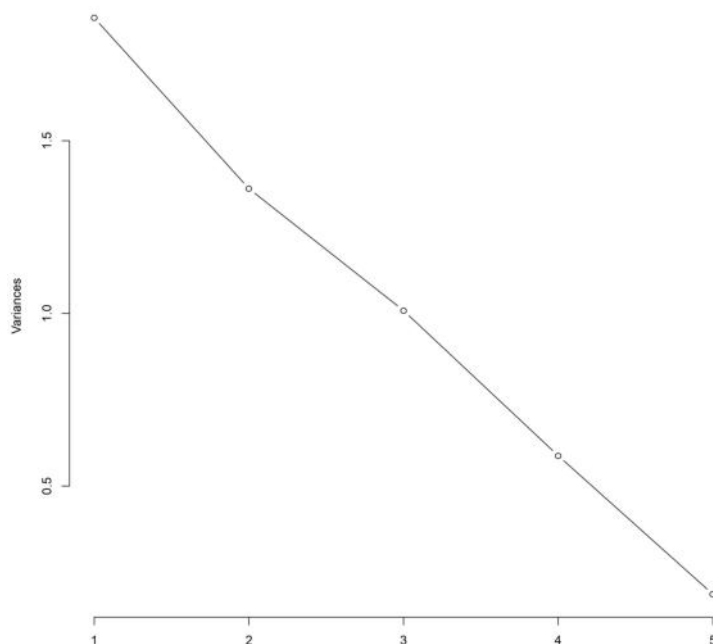
Figure A.2.: Scree plot of PCA of selected FA biomarkers (18:2 ω6, 18:3 ω3, 20:5 ω3, 22:6 ω3 and 18:1 ω9 *trans*, respectively). The "Elbow point" is located at 3 PCs. The Kaiser-Criterion is in accordance with the scree plot. X-axes, numbers of PCs.

A.8. PCA of selected oxidative stress biomarkers in the study population of ISOCROSS

Composition of PCs calculated in R using 7β-OHC, 5,6β-epoxyC and 5,6α-epoxyC in human breast ADT derived from 40 healthy women and resulting values of PC ($PC_{Oxy}1$) used in multiple linear regression models. Percentages of variations explained by the PCs are provided and eigenvectors of prominent variables influencing the PCs are indicated with bold characters. Breast ADT levels of 7β-OHC, 5,6β-epoxyC and 5,6α-epoxyC used for multiple linear regression models have been published previously (Wunder et al. 2022). *used in multiple linear regression models of Δ9DI, Δ6DI and Δ5DI as well as ELOVLI5 in human ADT.

Table A.9.: Results of PCA of selected oxidative stress biomarkers 7β-OHC, 5,6β-epoxyC and 5,6α-epoxyC. Eigenvectors of prominent variables influencing the PCs are indicated with bold characters and percentages of variation explained by the PCs are provided. *PC indicates, that PC was used in multiple linear regression models.

Variable	Eigenvectors		
	$PC_{Oxy}1$*	$PC_{Oxy}2$	$PC_{Oxy}3$
7β-OHC	**-0.52**	0.75	0.41
5,6β-epoxyC	**-0.55**	-0.66	0.51
5,6α-epoxyC	**-0.65**	-0.04	-0.76
variance (%)	73.33	22.64	4.03

Table A.10.: Factorloadings of $PC_{Oxy}1$ of individual study participants (n=40) from the study population of ISOCROSS.

No.	$PC_{Oxy}1$	No.	$PC_{Oxy}1$
1	-4.20	21	0.49
2	1.17	22	0.03
3	0.91	23	1.08
4	-3.82	24	0.44
5	0.92	25	-0.21
6	1.25	26	0.59
7	-2.19	27	1.39
8	1.05	28	-0.15
9	0.41	29	0.09
10	-4.41	30	1.31
11	-0.67	31	0.95
12	-0.41	32	1.72
13	-1.84	33	-0.27
14	-0.18	34	1.14
15	1.54	35	0.37
16	0.68	36	0.14
17	-0.59	37	0.63
18	-0.40	38	1.36
19	0.14	39	0.18
20	-1.45	40	0.78

A.9. Scree plot of PCA of selected oxidative stress biomarkers in the study population of ISOCROSS
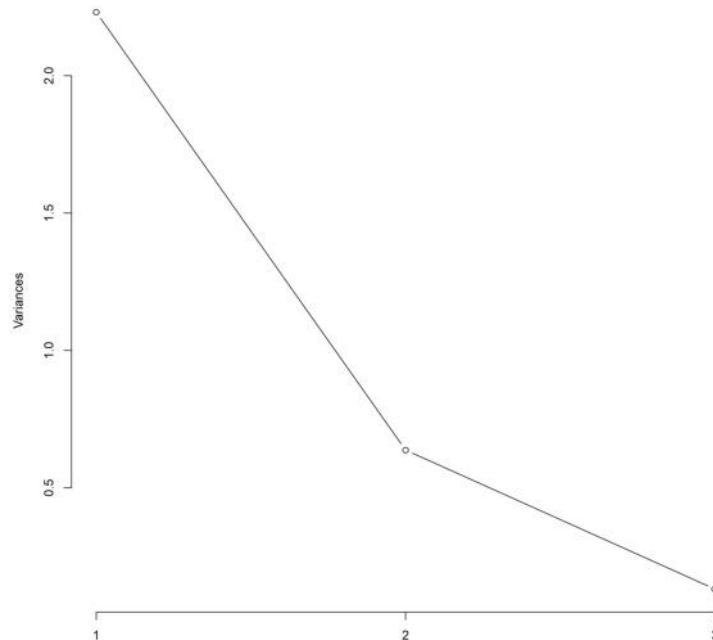
Figure A.3.: Scree plot of PCA of selected oxidative stress biomarkers (7β-OHC, $5,6\beta$-epoxyC and $5,6\alpha$-epoxyC, respectively). The "Elbow point" is located at 1 PC. The Kaiser-Criterion is in accordance with the scree plot. X-axes, numbers of PCs.

A.10. PCA of endogenous estrogens in the study population of ISOCROSS

Composition of PCs calculated in R using E2 and E1 in human breast ADT derived from 40 healty women and resulting values of PC (PC_E1) used in multiple linear regression models. Percentages of variations explained by PCs are provided and eigenvectors of prominent variables influencing the PCs are indicated with bold characters. Breast ADT levels of E1 and E2 used for multiple linear regression models have been published previously (Pemp, Geppert, et al. 2020).

*used in multiple linear regression modls on Δ9DI, Δ6DI and Δ5DI as well as ELOVLI5 in human breast ADT.

Table A.11.: Results of PCA of selected endogenous estrogens E1 and E2. Eigenvectors of prominent variables influencing the PCs are indicated with bold characters and percentages of variation explained by the PCs are provided. *PC indicates, that PC was used in multiple linear regression models.

Variable	Eigenvectors	
	PC_E1*	PC_E2
E1	**0.71**	-0.71
E2	**0.71**	0.71
variation (%)	94.59	5.41

Table A.12.: Factorloadings of PC_E1 of individual study participants (n=40) from the study population of ISOCROSS.

No.	PC_E1	No.	PC_E1
1	-0.74	21	0.02
2	-0.36	22	0.95
3	-0.47	23	-1.19
4	-1.11	24	-0.23
5	-0.91	25	-0.90
6	-1.18	26	1.83
7	-0.96	27	1.70
8	-1.05	28	-0.85
9	1.46	29	-0.02
10	2.18	30	-0.35
11	0.29	31	-0.84
12	-1.06	32	-1.29
13	2.04	33	-0.48
14	4.85	34	-1.18
15	-1.00	35	1.35
16	-0.73	36	2.66
17	-0.98	37	-0.99
18	1.56	38	0.09
19	-0.12	39	0.20
20	-1.19	40	-0.98

A.11. Scree plot of PCA of endogenous estrogens
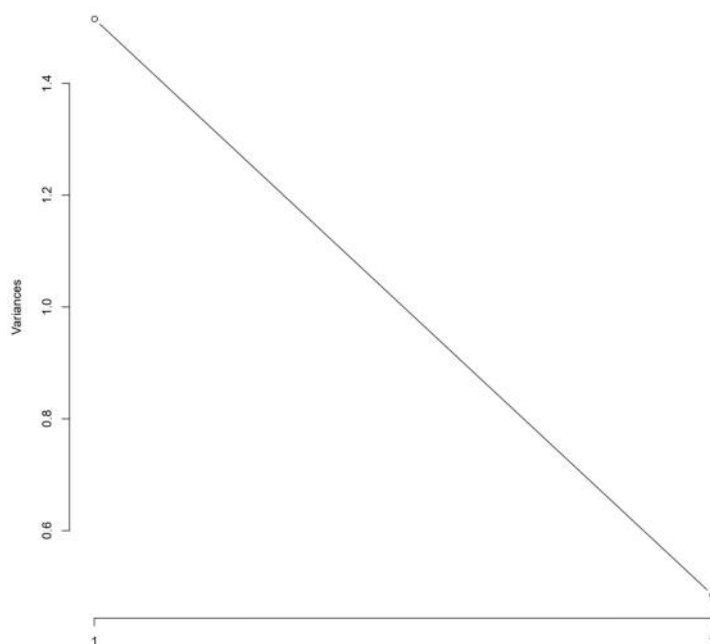
Figure A.4.: Scree plot of PCA of endogenous estrogens (E1 and E2, respectively). The "Elbow point" is located at 1 PC. The Kaiser-Criterion is in accordance with the scree plot. X-axes, numbers of PCs.

A.12. Setup and outcome of multiple linear regression models using stepwise forward selection

To test the association of every possible *exVar* with the dependent variable, the variable explaining the dependent variable best is chosen by an automatic procedure. Subsequently, all possible *exVar*s are added one after another to the first one, ultimately choosing the one improving the model most, applying the Akaike information criterion. This is repeated until the model can not be further improved by adding *exVar*s. Thus, every *exVar* selected into the model contributes to modeling the dependent variable. Significance of the association is expressed by P values and magnitude of impact is expressed by coefficients of regressions. To identify variables influencing Δ9DI, Δ6DI and Δ5DI as well as ELOVLI5 in human breast ADT, 13 *exVar*s (continuous and categorical) were tested as possible *exVar*s. Variables tested were: Age (continuous), menopausal status (categorical, reference group premenopausal, PreMP, n= 22), and further groups perimenopausal (PeriMP, n= 12) and postmenopausal (PostMP, n= 6), number of pregnancies (continuous), proportion of compounds soluble in organic solvent (oil%, continuous), body mass index (BMI, continuous), smoking habits (binary, reference group non-smoker, (n= 30, smoker n= 10), alcohol consumption (continuous) and intake of estrogen active drugs (EAD, categorical, reference group no intake of EAD, n= 32) and further groups intake of ethinyl estradiol (EE, n= 6), and intake of E2-releasing drugs (ERD, n=2). Since a multitude of Spearman correlations between *exVar*s was observed, to minimize collinearity, levels of FA biomarkers, estrogens and oxyChOLs caused by oxidative stress in breast ADT were grouped in PCs $PC_{diet}1$, $PC_{diet}2$, $PC_{diet}3$, PC_E1 and $PC_{Oxy}1$. Number of PCs, which were calculated for specific groups of *exVar*s, were chosen using the scree plots according to the elbow criteria and all PCs considered explained at least 73% of the variation of the original variables. If in the computed model observations with Cook's Distance >1 appeared, they were removed and the model was computed anew. This process was repeated until no conspicuous observations (CO) remained. To achieve normal distribution, all dependent variables except for Δ6DI and Δ5DI were logarithmized. Data distributions were evaluated in Quantile-Quantile plots with simulated confidence bands. Constant standard deviations of the errors were evaluated using scale-location plots. To check the model assumption in independent identically distributed errors, the residual vs. fitted values plot was used. For every final model, adjusted coefficient of determination (R^2), the numbers of CO removed, the numbers of observations contributing to the final model (n, maximum 40). Low R^2 of the respective linear regression models indicate lack of variables contributing to the dependent variable and/or large variations in the data. For every *exVar* selected, the

regression coefficients (which represent the mean changes in the dependent variables for one unit of change in the respective *exVar* while holding other predictors in the models constant), their confidence interval (CI), as well as the P values were given. Spearman's rank correlation analysis was performed to identify collinearity between *exVar*s which might hinder each others selection and/or influence each other P values within the models. Correlations with P values < 0.001 were listed below the table of each model with the respective correlation coefficient (R) and P value. If such *exVar*s were selected for the final model, a comment addressing possible consequences were added. If *exVar*s have P values <0.05, significant influences are identified. +/- positive/negative association; n.a. not applicable; black colored *exVar*selected, P values < 0.05; blue colored *exVar* selected, P values between 0.05 and 0.10; light-blue colored *exVar* selected, P values between 0.10 and 0.20; grey colored *exVar* selected, P values > 0.20.

Δ9¹-Desaturase Index

CO = 0; n = 40; O/exVAR = 13.3; R^2 = 0.28

exVAR	Regression Coefficient		Influence via PC		CI 2.5%		CI 97.5%	P Value
Age			n.a.					
PeriMP			n.a.					
PostMP			n.a.					
Number of pregnancies			n.a.					
Oil%			n.a.					
BMI			n.a.					
Smoking	+	0.2949	n.a.	+	0.0356	+	0.5542	0.0269
Alcohol			n.a.					
EAD:EE			n.a.					
EAD:ERD			n.a.					
PC_DIET1	+	0.1377	+ 20:5 ω3 + 22:6 ω3	+	0.0551	+	0.2204	0.0017
PC_DIET2	-	0.0767	+ 18:2 ω6 + 18:3 ω3	-	0.1697	+	0.0161	0.1027
PC_DIET3			n.a.					
PC_E1			n.a.					
PC_OXY1			n.a.					

Comment:
Due to correlations between Δ9-DI and Δ5-DI it is possible that Δ9-DI might be affected by any variable significantly influencing Δ5-DI [1].

Note:
[1], Correlation of Δ9-DI and Δ5-DI: R= 0.53, P<0.001 (3.8x10-4).

Figure A.5.: Results of multiple linear regression model with Δ9DI as dependent variable. n.a. not applicable; CI confidence interval; CO conspicuous observation; O/exVar observation per *exVar*.

Δ6-Desaturase Index

CO = 0; n = 40; O/exVAR = 8.0; R^2 = 0.44

exVAR	Regression Coefficient		Influence via PC	CI 2.5%		CI 97.5%	P Value
Age			n.a.				
PeriMP			n.a.				
PostMP			n.a.				
Number of pregnancies	+	0.0003	n.a.	0.00007	+	0.00048	0.0101
Oil%			n.a.				
BMI			n.a.				
Smoking	+	0.0006	n.a.	0.00003	+	0.00137	0.0609
Alcohol			n.a.				
EAD:EE			n.a.				
EAD:ERD			n.a.				
PC$_{DIET}$1	+	0.0002	+ 20:5 ω3 + 22:6 ω3	0.00003	+	0.00040	0.0927
PC$_{DIET}$2	-	0.0004	+ 18:2 ω6 + 18:3 ω3	0.00064	-	0.00013	0.0038
PC$_{DIET}$3	-	0.0003	- 18:9 ω9t	0.00006	-	0.000006	0.0455
PC$_E$1			n.a.				
PC$_{OXY}$1			n.a.				

Figure A.6.: Results of multiple linear regression model with Δ6DI as dependent variable. n.a. not applicable; CI confidence interval; CO conspicuous observation; O/exVar observation per *exVar*.

$\Delta 5$-Desaturase Index

CO = 0; n = 40; O/exVAR = 8.0; R^2 = 0.17

exVAR	Regression Coefficient		Influence via PC		CI 2.5%	97.5%		P Value
Age			n.a.					
PeriMP			n.a.					
PostMP			n.a.					
Number of pregnancies			n.a.					
Oil%			n.a.					
BMI	0.0164	-	n.a.	-	0.0412	+	0.0084	0.1868
Smoking			n.a.					
Alcohol	0.0020	-	n.a.	-	0.0044	+	0.0004	0.0959
EAD:EE	0.2095	+	n.a.	-	0.1542	+	0.5732	0.2498
EAD:ERD	0.7067	+	n.a.	+	0.2183	+	1.1951	0.0059
PC$_{DIET}$1	0.0724	+	+ 20:5 ω3 + 22:6 ω3	-	0.0077	+	0.1526	0.0751
PC$_{DIET}$2			n.a.					
PC$_{DIET}$3			n.a.					
PC$_E$1			n.a.					
PC$_{OXY}$1			n.a.					

Comment:
Due to correlations between $\Delta 5$-DI and $\Delta 9$-DI it is possible that $\Delta 5$-DI might be affected by any variable significantly influencing $\Delta 9$-DI ([1]).

Note:
[1], Correlation of $\Delta 5$-DI and $\Delta 9$-DI: R= 0.53, P<0.001 (3.8×10-4).

Figure A.7.: Results of multiple linear regression model with $\Delta 5$DI as dependent variable. n.a. not applicable; CI confidence interval; CO conspicuous observation; O/exVar observation per *exVar*.

Elongase 5 Index
CO = 0; n = 40; O/exVAR = 13.3; R^2 = 0.29

exVAR	Regression Coefficient		Influence via PC		CI 2.5%	97.5%		P Value
Age			n.a.					
PeriMP			n.a.					
PostMP			n.a.					
Number of pregnancies			n.a.					
Oil%			n.a.					
BMI	+	0.0364	n.a.	+	0.0148	+	0.0579	0.0015
Smoking			n.a.					
Alcohol	+	0.0144	n.a.	−	0.0007	+	0.0032	0.1150
EAD:EE			n.a.					
EAD:ERD			n.a.					
PC$_{DIET}$1	+	0.0761	+ 20:5 ω3 + 22:6 ω3	+	0.0100	+	0.1421	0.0252
PC$_{DIET}$2			n.a.					
PC$_{DIET}$3			n.a.					
PC$_E$1			n.a.					
PC$_{OXY}$1			n.a.					

Figure A.8.: Results of multiple linear regression model with ELOVLI5 as dependent variable. n.a. not applicable; CI confidence interval; CO conspicuous observation; O/exVar observation per *exVar*.

A.13. Data of gene expression levels of Δ5D and Δ6D and their indexes Δ5DI and Δ6DI

In the following tables A.13 and A.14 the gene expression of Δ5D and Δ6D as well as their respective product-to-precursor indexes Δ5DI and Δ6DI from the study population of ISOCROSS will be depicted. Gene expression levels of Δ5D and Δ6D were calculated according to ch. 4.2.5.4. The respective product-to-precursor indexes Δ5DI and Δ6DI were calculated according to ch. 4.2.3.7. Raw data of the respective gene expressions are depicted in the enclosed CD under the section of gene expression. The no. indicates the real numbers from the study participants.

In table A.15 the correlation coefficients and the P-values of the correlation analysis of the transcript level of Δ5D relative to HPRT1 and its respective product-to-precursor ratio Δ5DI as well as the transcript level of Δ6D relative to HPRT1 and its respective product-to-precursor ratio Δ6DI will be depicted.

Table A.13.: Relevant data from the study participants from the study population of ISOCROSS regarding gene expression levels of Δ5D and the respective index Δ5DI for correlation analysis. The no. indicates the real numbers from the study participants. Transcript levels of Δ5D relative to HPRT1 were determined acc. to ch. 4.2.5.4. The product-to-precursor ratios of Δ5DI were calculated acc to. ch. 4.2.3.7. Δ5D indicates the transcript level of Δ5D relative to HPRT1. Δ5DI indicates the product-to-precursor ratio of 20:4 ω6 and 20:3 ω6.

no.	Δ5D	no.	Δ5D	no.	Δ5DI	no.	Δ5DI
1	8.24	29	4.15	1	1.67	29	2.30
2	7.66	31	11.71	2	2.16	31	2.18
5	4.48	33	6.86	5	1.75	33	1.46
6	5.71	34	2.58	6	1.62	34	1.73
7	2.34	35	10.25	7	1.72	35	2.18
10	2.38	36	1.58	10	1.58	36	1.61
12	3.49	37	8.45	12	2.04	37	1.56
13	4.14	38	31.81	13	1.55	38	1.88
14	4.30	39	1.52	14	1.26	39	1.50
18	4.33	41	19.55	18	1.56	41	2.00
21	24.79	43	5.41	21	1.47	43	1.35
24	12.58	44	3.08	24	2.21	44	1.50
26	1.82	46	5.18	26	2.13	46	1.94
27	2.06	48	4.81	27	0.95	48	1.40
28	6.71	49	6.55	28	1.21	49	1.36

Table A.14.: Relevant data from the study participants from the study population of ISOCROSS regarding gene expression levels of Δ6D and the respective index Δ6DI for correlation analysis. The no. indicates the real numbers from the study participants. Transcript levels of Δ6D relative to HPRT1 were determined acc. to ch. 4.2.5.4. The product-to-precursor ratios of Δ6DI were calculated acc to. ch. 4.2.3.7. Δ6D indicates the transcript level of Δ6D relative to HPRT1. Δ6DI indicates the product-to-precursor ratio of 18:3 ω6 and 18:2 ω6.

no.	Δ6D	no.	Δ6D	no.	Δ6DI	no.	Δ6DI
1	14.25	29	6.66	1	0.00342	29	0.00527
2	10.15	31	11.86	2	0.00456	31	0.00373
5	8.33	33	15.45	5	0.00435	33	0.00493
6	19.91	34	5.28	6	0.00390	34	0.00441
7	6.92	35	16.05	7	0.00442	35	0.00363
10	4.77	36	3.18	10	0.00317	36	0.00284
12	8.78	37	27.57	12	0.00368	37	0.00311
13	6.57	38	37.74	13	0.00460	38	0.00383
14	11.85	39	5.35	14	0.00645	39	0.00255
18	12.54	41	18.91	18	0.00373	41	0.00222
21	30.64	43	7.87	21	0.00400	43	0.00275
24	18.68	44	5.63	24	0.00583	44	0.00229
26	2.19	46	6.91	26	0.00329	46	0.00240
27	13.07	48	7.86	27	0.00305	48	0.00416
28	16.23	49	12.65	28	0.00394	49	0.00264

Table A.15.: Correlation coefficients and P-values of the correlation analysis of the transcript levels of Δ5D relative to HPRT1 and its respective product-to-precursor ratio Δ5DI as well as the transcript level of Δ6D relative to HPRT1 and its respective product-to-precursor ratio Δ6DI. Transcript levels of Δ5D and Δ6D relative to HPRT1 were determined acc. to ch. 4.2.5.4. The product-to-precursor ratios of Δ5DI and Δ6DI were calculated acc to. ch. 4.2.3.7. Δ5D and Δ6D indicate the transcript levels of Δ5D and Δ6D relative to HPRT1. Δ5DI and Δ6DI indicate the product-to-precursor ratio of 20:4 ω6 and 20:3 ω6 for Δ5DI and 18:3 ω6 and 18:2 ω6 for Δ6DI.

Variable 1	R	P	Variable 2
Δ5D	0.21	0.27	Δ5DI
Δ6D	0.12	0.54	Δ6DI

A.14. Chromatogram authentic standard reference mix

In the following fig. A.9, A.10 and A.11, chromatograms of the authentic standard reference mix acc. to ch. 4.2.3.4 will be depicted. For the sake of clarity, the chromatogram of the authentic standard reference mix was divided in time frames. Fig. A.9 shows all FAMEs from the authentic standard reference mix from the time frame of 8 to 20 minutes whereas fig. A.10 shows all FAME, which were detected in the time frame of 20 to 27 minutes. Fig. A.11 shows all FAMEs, which were detected in the time frame of 27 to 37 minutes. For respective t_Rs and mass spectrometrical data, the author refers to ch. 5.2.1 and 2.3.3.
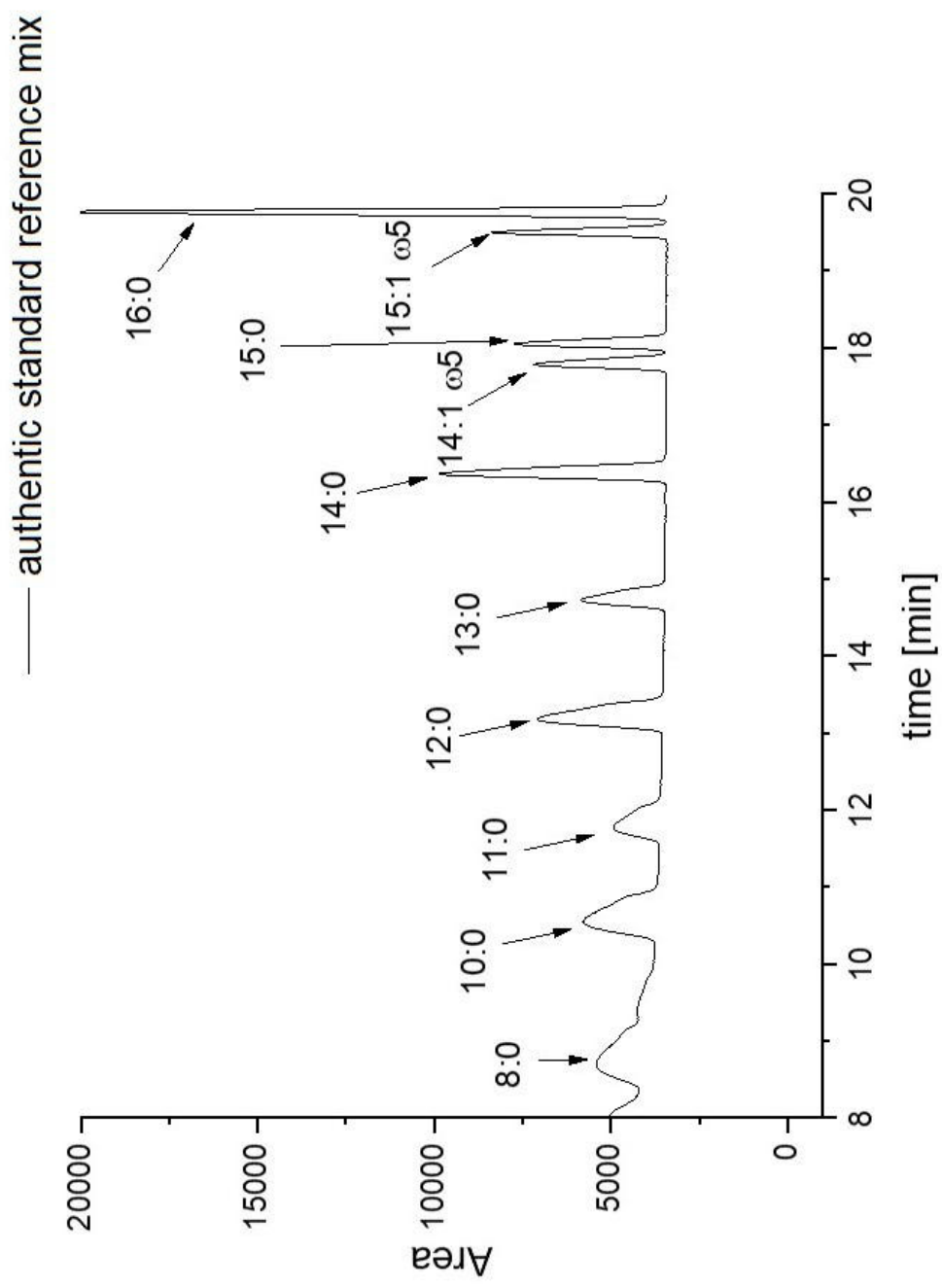
Figure A.9.: Chromatogram of the authentic standard reference mix from 8 to 20 min.
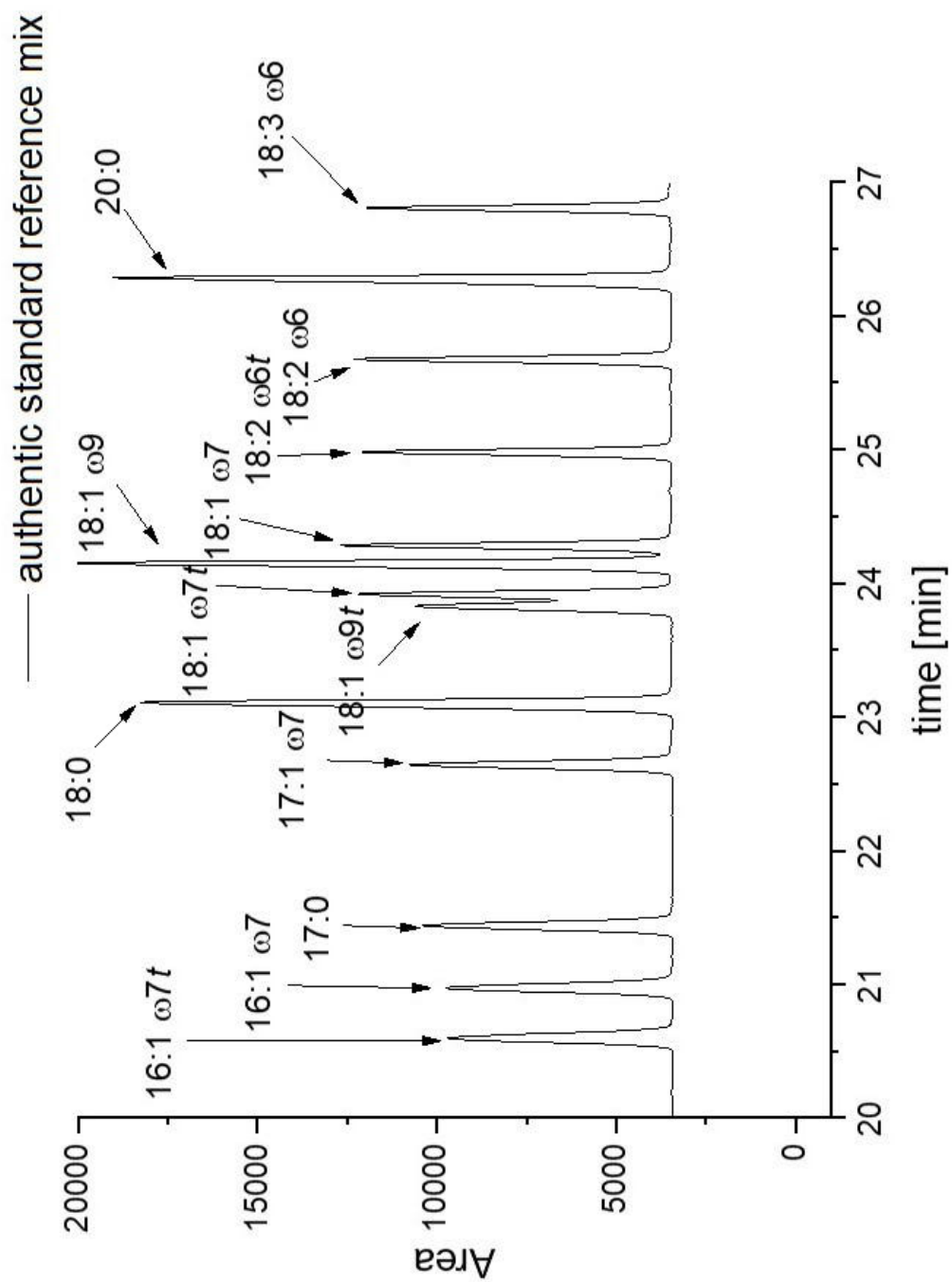
Figure A.10.: Chromatogram of the authentic standard reference mix from 20 to 27 min.
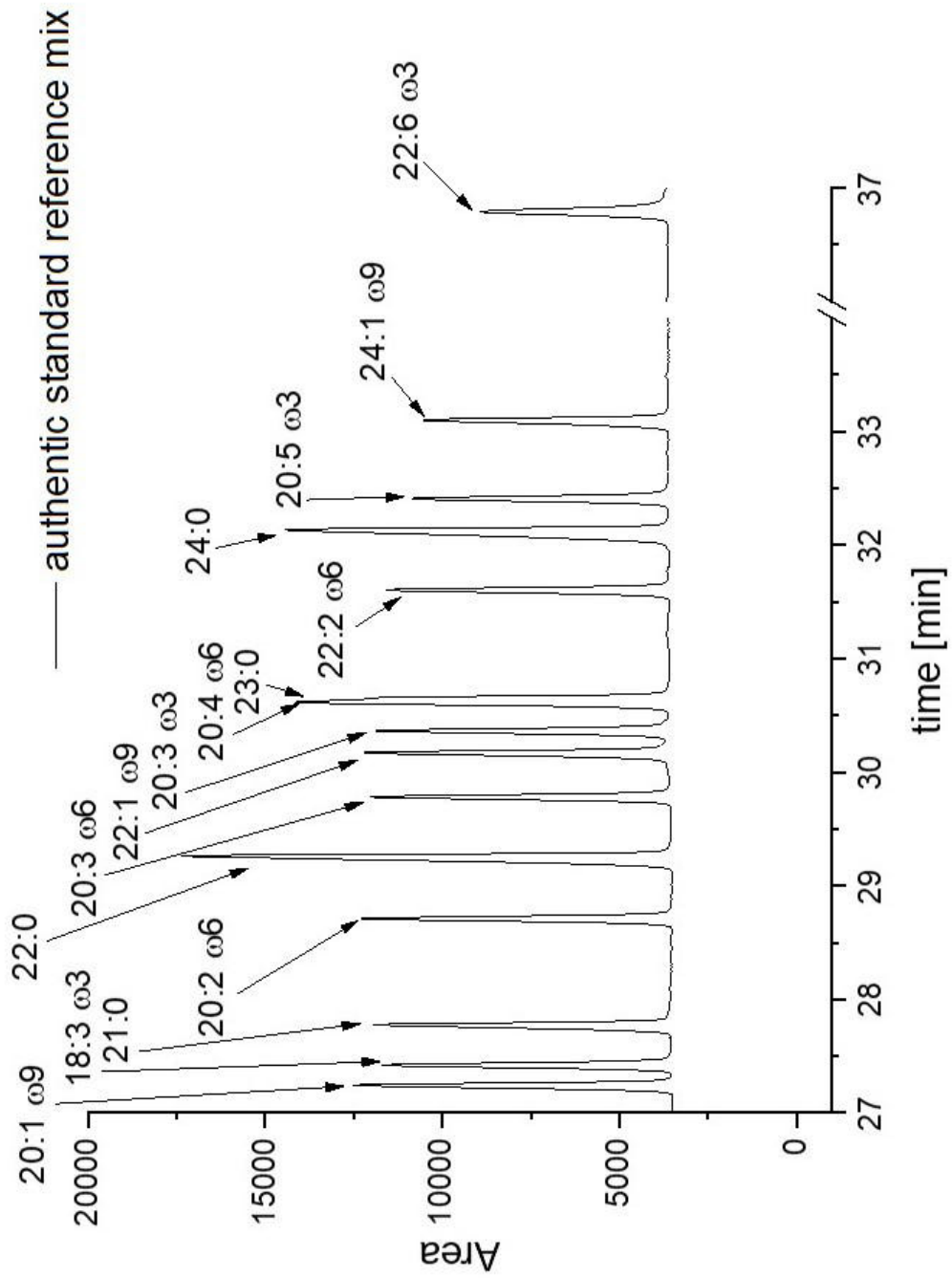
Figure A.11.: Chromatogram of the authentic standard reference mix from 27 to 37 min.

A.15. Method validation - recovery set up
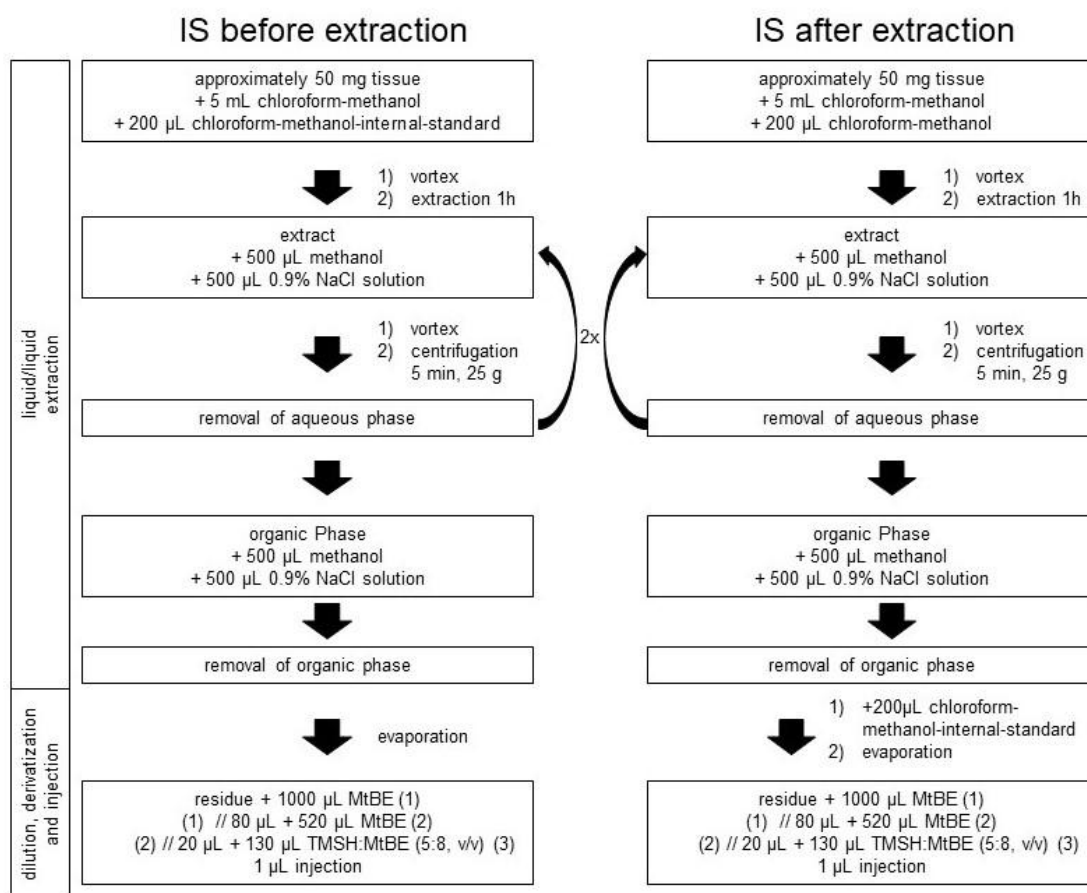
Figure A.12.: Work flow scheme of recovery analysis procedure

A.16. Comparison of methods
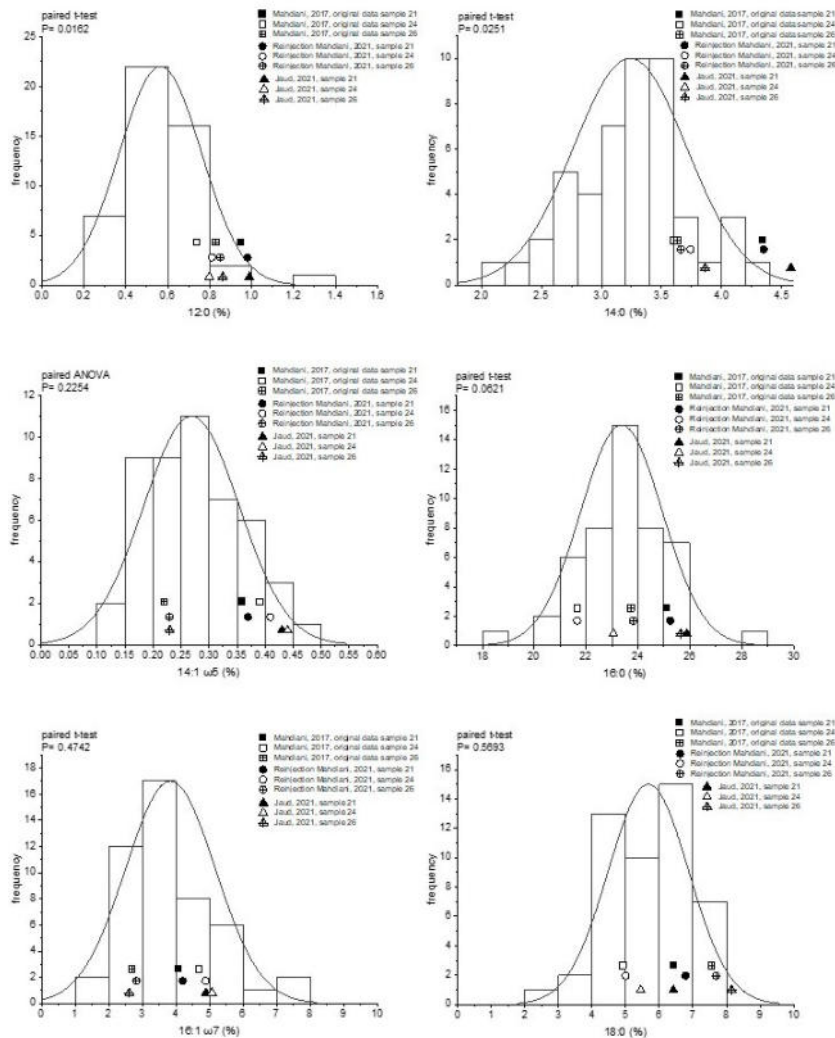
Figure A.13.: Histogram for 12:0, 14:0, 14:1 ω5, 16:0, 16:1 ω7 and 18:0. The plotted distribution was calculated from the respective FA percentages from respective composition of all ADT samples from Mahdiani (Mahdiani 2017). The location of data points from sample 21, 24 and 26 of ISOCROSS of 12:0, 14:0, 14:1 ω5, 16:0, 16:1 ω7 and 18:0, respectively, were drawn into the histograms from the original data from Mahdiani (Mahdiani 2017), the reinjection of ADT samples from Mahdiani (ch. 4.2.3.5) and the ADT samples treated according to ch. 4.2.3. Prior plotting of the location of data points, the FA composition was calculated only by all FAs Mahdiani identified (Mahdiani 2017). Paired t-tests were calculated between the reinjection of ADT samples from Mahdiani and ADT samples treated according to ch. 4.2.3.
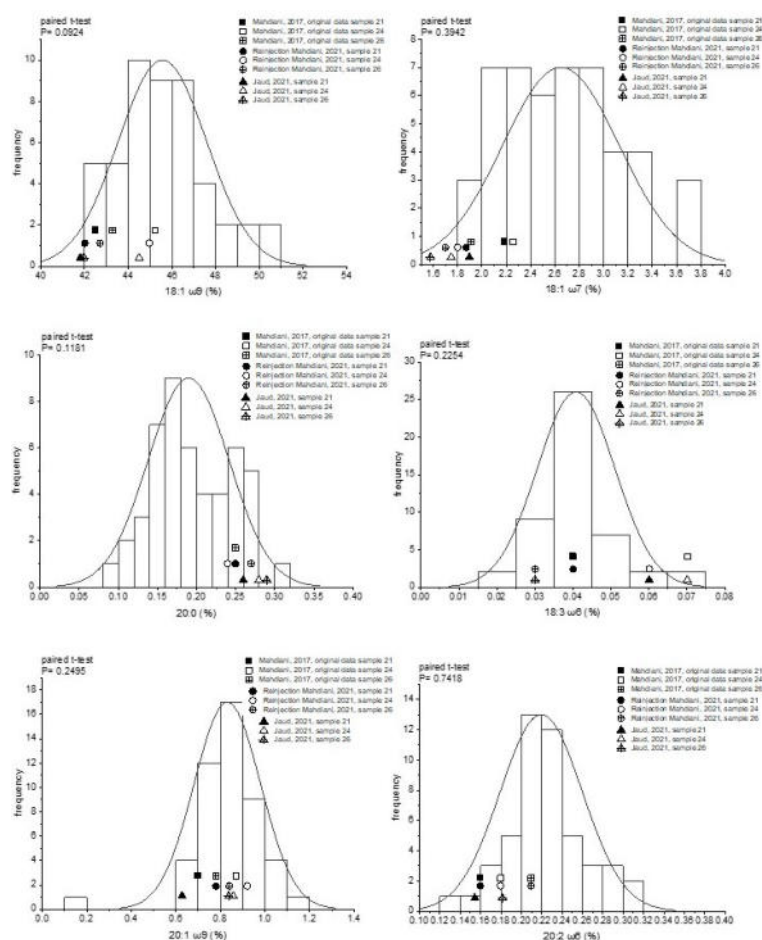
Figure A.14.: Histogram for 18:1 ω9, 18:1 ω7, 20:0, 18:3 ω6, 20:1 ω9 and 20:2 ω6. The plotted distribution was calculated from the respective FA percentages from respective composition of all ADT samples from Mahdiani (Mahdiani 2017). The location of data points from sample 21, 24 and 26 of ISOCROSS of 18:1 ω9, 18:1 ω7, 20:0, 18:3 ω6, 20:1 ω9 and 20:2 ω6, respectively, were drawn into the histograms from the original data from Mahdiani (Mahdiani 2017), the reinjection of ADT samples from Mahdiani (ch. 4.2.3.5) and the ADT samples treated according to ch. 4.2.3. Prior plotting of the location of data points, the FA composition was calculated only by all FAs Mahdiani identified (Mahdiani 2017). Paired t-tests were calculated between the reinjection of ADT samples from Mahdiani and ADT samples treated according to ch. 4.2.3.

Figure A.15.: Histogram for 20:3 ω6, 20:3 ω3 and 20:4 ω6. The plotted distribution was calculated from the respective FA percentages from respective composition of all ADT samples from Mahdiani (Mahdiani 2017). The location of data points from sample 21, 24 and 26 of ISOCROSS of 20:3 ω6, 20:3 ω3 and 20:4 ω6, respectively, were drawn into the histograms from the original data from Mahdiani (Mahdiani 2017), the reinjection of ADT samples from Mahdiani (ch. 4.2.3.5) and the ADT samples treated according to ch. 4.2.3. Prior plotting of the location of data points, the FA composition was calculated only by all FAs Mahdiani identified (Mahdiani 2017). Paired t-tests were calculated between the reinjection of ADT samples from Mahdiani and ADT samples treated according to ch. 4.2.3.

A.17. FA composition of ADT and GLT samples of ISOCROSS and respective GC-MS/FID chromatograms

In the following tables A.16 and A.17 the FA composition of GLT and ADT samples from the same study participants no. 1, 6, 45, 46 and 47 from ISOCROSS will be depicted. Respective FAs are indicated as percentage of total FA composition calculated according to ch. 4.2.3.5. The chromatograms from GC-MS/FID analysis from the respective GLT and ADT samples will be depicted in fig. A.16, A.17, A.18 and A.19.

Table A.16.: FA composition and percentages of individual FAs from GLT samples from the study population of ISOCROSS. The no. of participants indicate the number from the study protocol.

No.	12:0	14:0	15:0	16:0	17:0	18:0	20:0	14:1 ω5	16:1 ω7	17:1 ω7	18:1 ω9	18:1 ω7	20:1 ω9
1	0.59	3.17	0.63	23.61	0.39	5.23	0.18	0.34	3.55	0.35	44.90	2.46	0.92
46	0.44	2.94	0.31	25.93	0.29	6.25	0.18	0.28	5.40	0.30	43.41	2.90	1.00
45	0.79	3.36	0.52	24.58	0.36	6.06	0.12	0.30	3.41	0.32	44.35	2.15	0.79
47	0.35	2.29	0.43	24.23	0.21	3.67	0.08	0.41	7.13	0.36	46.43	4.70	0.64
6	0.34	2.38	0.52	21.10	0.26	3.23	0.06	0.38	6.59	0.40	47.33	3.44	0.77

No.	18:2 ω6	18:3 ω6	18:3 ω3	20:2 ω6	20:3 ω3	20:4 ω6	22:2 ω6	20:5 ω3	22:6 ω3	20:3 ω6	16:1 ω7t	18:1 t	22:1 ω9
1	10.50	0.028	0.63	0.27	0.043	0.14	0.044	0.032	0.045	nd	0.36	1.32	0.16
46	7.79	0.050	0.55	0.20	0.015	0.18	0.075	0.033	0.041	nd	0.29	0.96	0.13
45	10.31	0.036	0.47	0.25	0.015	0.20	0.029	0.034	0.021	0.19	0.29	0.94	0.10
47	6.79	0.044	0.19	0.16	0.017	0.22	0.078	0.035	0.037	0.23	0.34	0.82	0.12
6	10.56	0.025	0.49	0.23	0.019	0.17	0.045	0.037	0.025	0.28	0.35	0.88	0.10

Table A.17.:: FA composition and percentages of individual FAs from ADT samples from the study population of ISOCROSS. The no. of participants indicate the number from the study protocol.

No.	12:0	14:0	15:0	16:0	17:0	18:0	20:0	14:1 ω5	16:1 ω7	17:1 ω7	18:1 ω9	18:1 ω7	20:1 ω9
1	0.69	3.40	0.51	24.30	0.38	5.94	0.11	0.33	3.75	0.44	45.35	2.54	0.88
46	0.53	3.47	0.34	25.92	0.28	6.75	0.21	0.33	6.02	0.34	42.43	2.83	0.68
45	0.81	3.64	0.54	24.28	0.38	6.77	0.27	0.27	3.02	0.28	44.08	2.12	0.69
47	0.05	2.23	0.30	23.13	0.22	4.12	0.13	0.32	6.91	0.33	49.72	3.24	0.59
6	0.84	3.61	0.42	26.40	0.34	7.49	0.28	0.27	3.14	0.33	44.77	1.96	0.72

No.	18:2 ω6	18:3 ω6	18:3 ω3	20:2 ω6	20:3 ω3	20:4 ω6	22:2 ω6	20:5 ω3	22:6 ω3	20:3 ω6	16:1 ω7t	18:1 t	22:1 ω9
1	8.60	0.034	0.23	0.18	nd	0.05	0.150	0.043	nd	0.10	0.31	1.48	0.18
46	6.95	0.050	0.78	0.18	0.037	0.10	0.105	0.034	nd	nd	0.25	1.16	0.25
45	9.67	0.039	0.40	0.18	0.035	0.12	0.087	0.045	0.035	nd	0.25	1.66	0.26
47	6.02	0.064	0.14	0.15	nd	0.18	0.254	0.045	0.090	nd	0.26	1.10	0.20
6	6.86	0.057	0.20	0.16	0.010	0.07	0.156	0.039	nd	nd	0.23	1.34	0.25
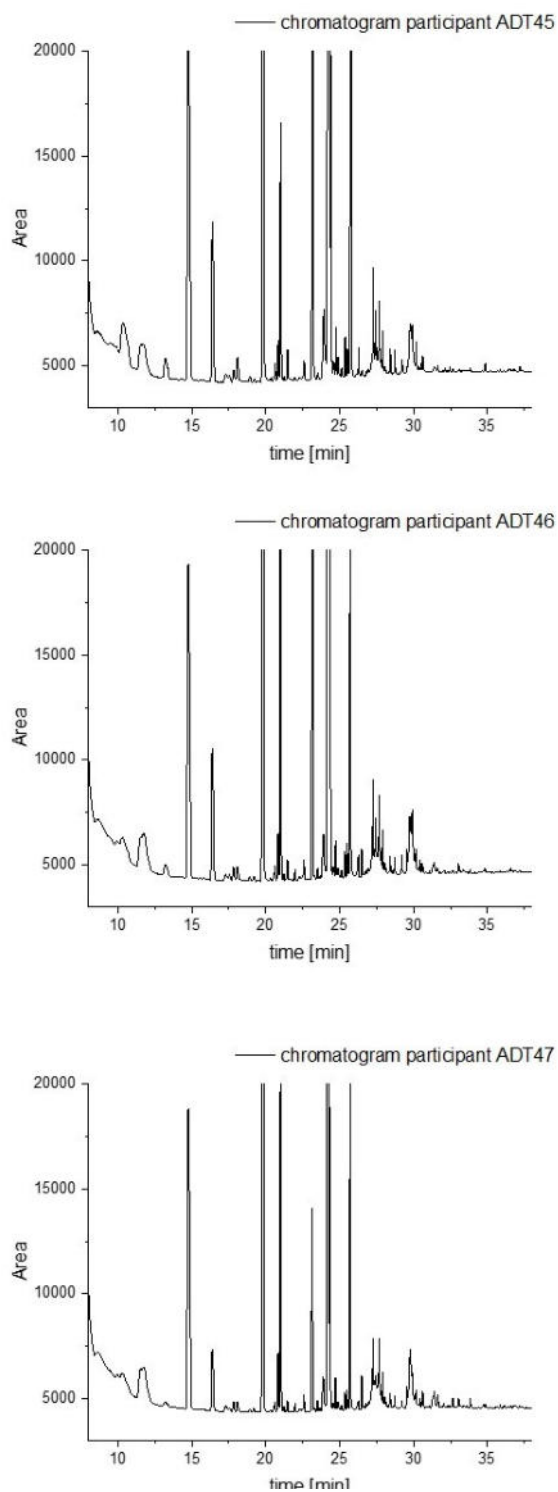
Figure A.16.: GC-MS/FID chromatograms of FAs in breast ADT of samples 45, 46 and 47.
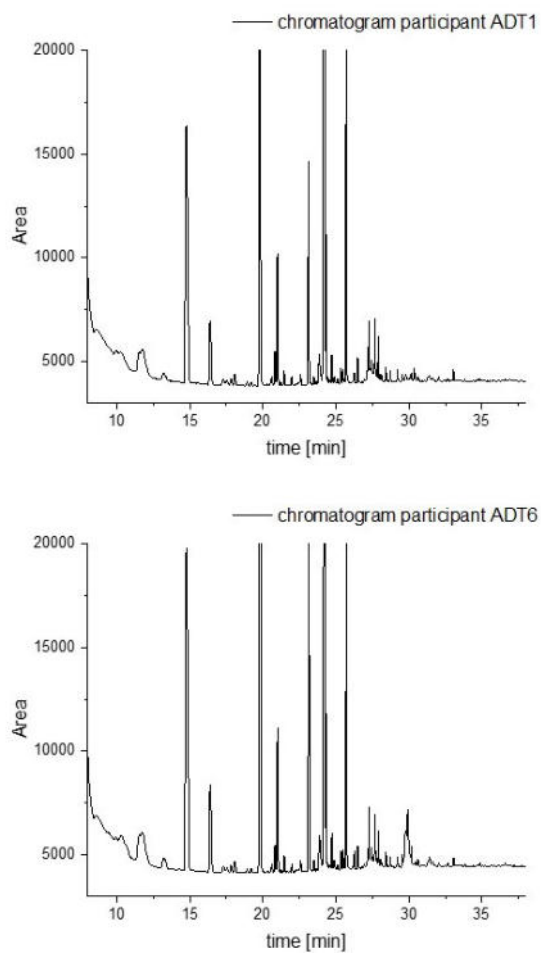
Figure A.17.: GC-MS/FID chromatograms of FAs in breast ADT of samples 1 and 6.
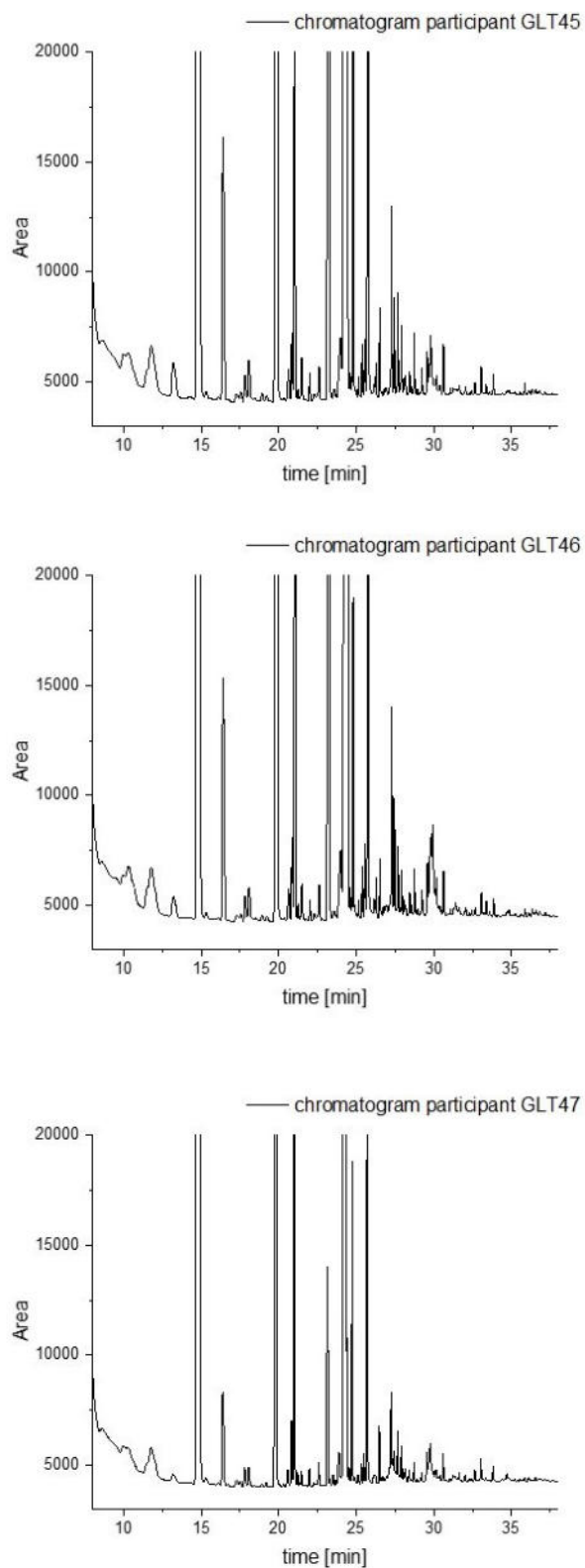
Figure A.18.: GC-MS/FID chromatograms of FAs in breast GLT of samples 45, 46 and 47.
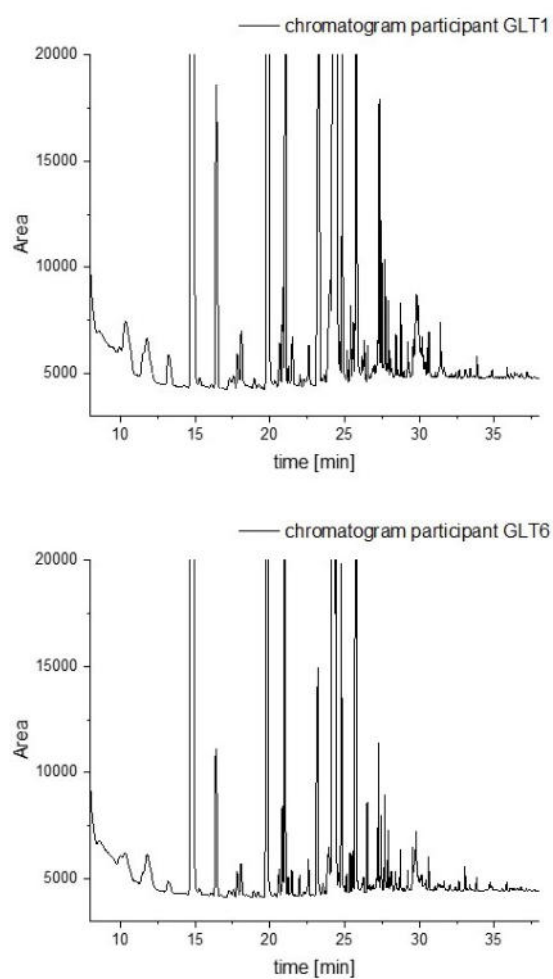
Figure A.19.: GC-MS/FID chromatograms of FAs in breast GLT of samples 1 and 6.

A.18. Comparison of GLT FAs with ADT samples from the same women from the study population of ISOCROSS
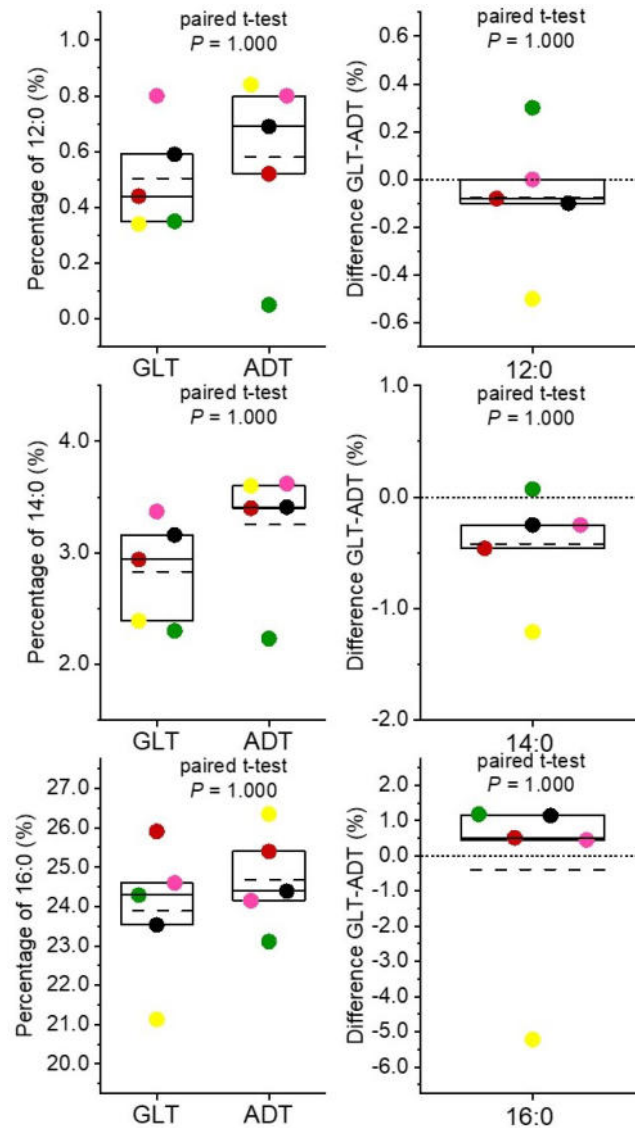
Figure A.20.: Boxplots of the the proportion of 12:0, 14:0 and 16:0 to total FA found in GLT and ADT (left plots) and boxplots of percentage differences of 12:0, 14:0 and 16:0 between GLT and ADT samples. Percentage differences between GLT and ADT were plotted for GLT-ADT-pairs from the same female donor from the ISOCROSS study (different colours) and statistical significance from 0 was tested with paired t-tests and adjusted P-values are given.
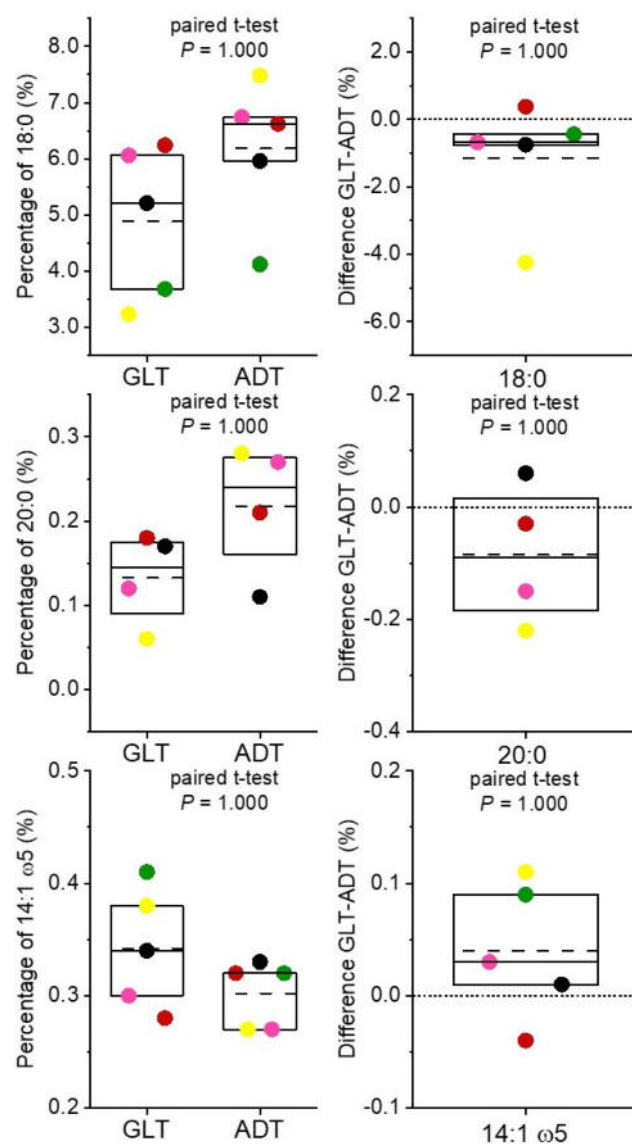
Figure A.21.: Boxplots of the the proportion of 18:0, 20:0 and 14:1 ω5 to total FA found in GLT and ADT (left plots) and boxplots of percentage differences of 18:0, 20:0 and 14:1 ω5 between GLT and ADT samples. Percentage differences between GLT and ADT were plotted for GLT-ADT-pairs from the same female donor from the ISOCROSS study (different colours) and statistical significance from 0 was tested with paired t-tests and adjusted P-values are given.
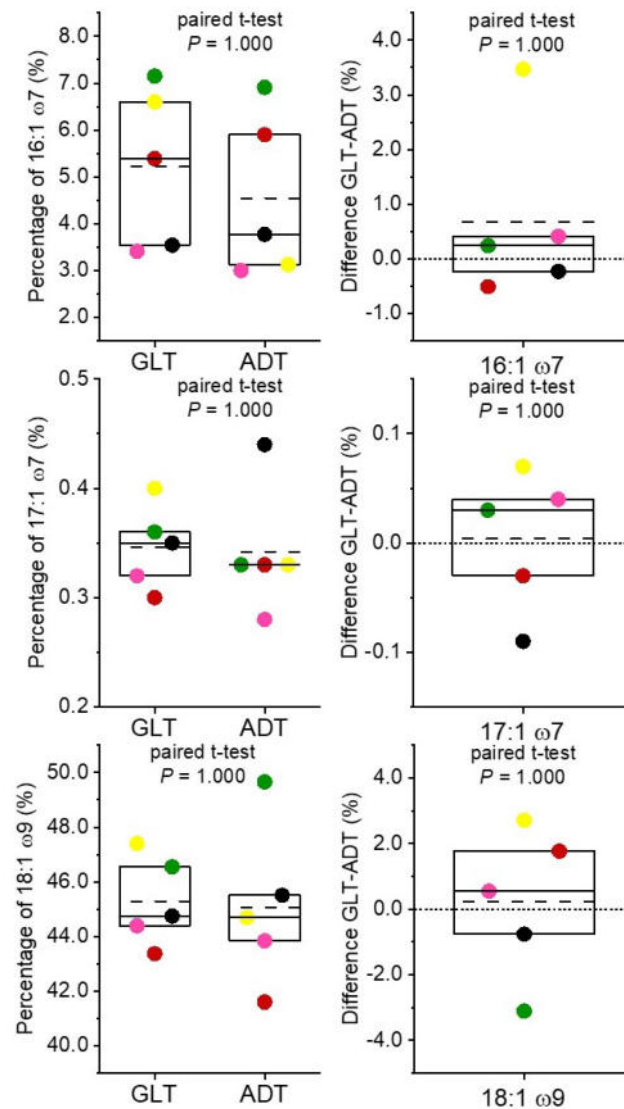
Figure A.22.: Boxplots of the the proportion of 16:1 ω7, 17:1 ω7 and 18:1 ω9 to total FA found in GLT and ADT (left plots) and boxplots of percentage differences of 16:1 ω7, 17:1 ω7 and 18:1 ω9 between GLT and ADT samples. Percentage differences between GLT and ADT were plotted for GLT-ADT-pairs from the same female donor from the ISOCROSS study (different colours) and statistical significance from 0 was tested with paired t-tests and adjusted P-values are given.
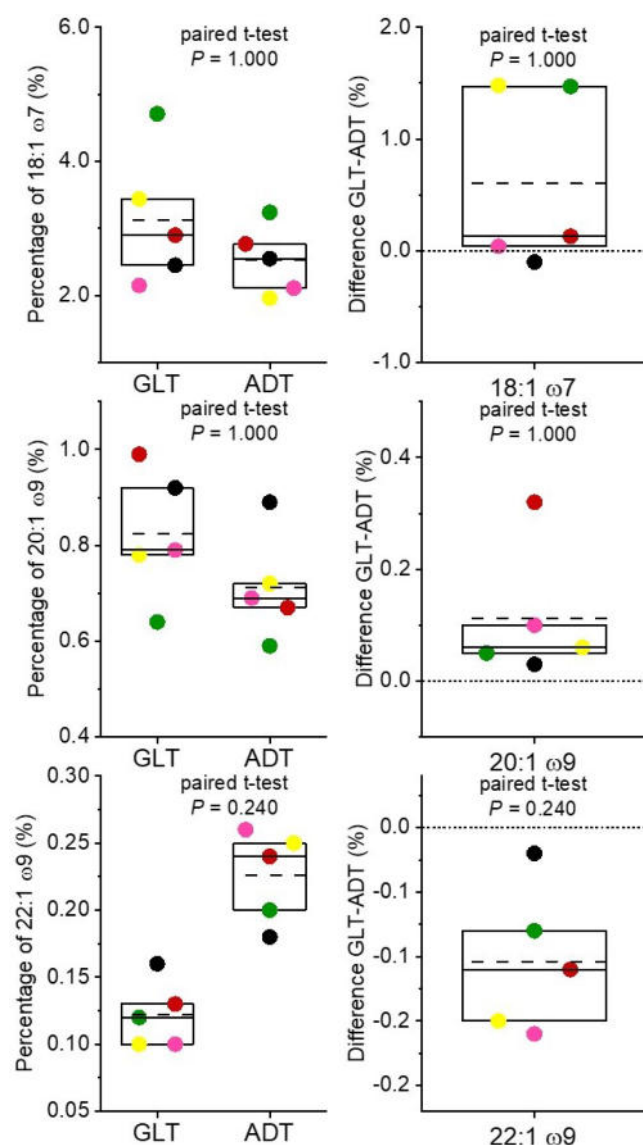
Figure A.23.: Boxplots of the the proportion of 18:1 ω7, 20:1 ω9 and 22:1 ω9 to total FA found in GLT and ADT (left plots) and boxplots of percentage differences of 18:1 ω7, 20:1 ω9 and 22:1 ω9 between GLT and ADT samples. Percentage differences between GLT and ADT were plotted for GLT-ADT-pairs from the same female donor from the ISOCROSS study (different colours) and statistical significance from 0 was tested with paired t-tests and adjusted P-values are given.
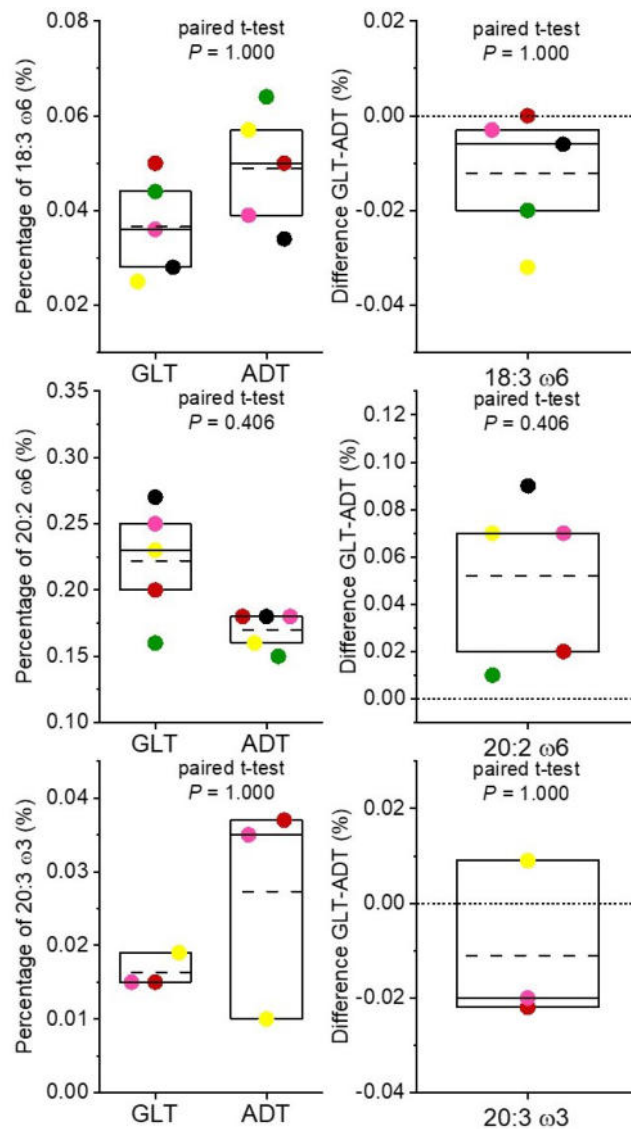
Figure A.24.: Boxplots of the the proportion of 18:3 ω6, 20:2 ω6 and 20:3 ω3 to total FA found in GLT and ADT (left plots) and boxplots of percentage differences of 18:3 ω6, 20:2 ω6 and 20:3 ω3 between GLT and ADT samples. Percentage differences between GLT and ADT were plotted for GLT-ADT-pairs from the same female donor from the ISOCROSS study (different colours) and statistical significance from 0 was tested with paired t-tests and adjusted P-values are given.
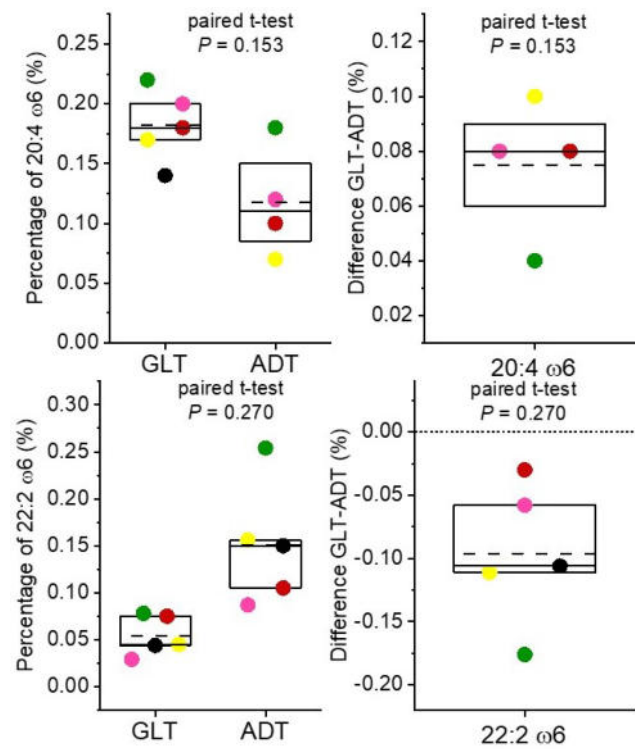
Figure A.25.: Boxplots of the the proportion of 20:4 ω6 and 22:2 ω6 to total FA found in GLT and ADT (left plots) and boxplots of percentage differences of 20:4 ω6 and 22:2 ω6 between GLT and ADT samples. Percentage differences between GLT and ADT were plotted for GLT-ADT-pairs from the same female donor from the ISOCROSS study (different colours) and statistical significance from 0 was tested with paired t-tests and adjusted P-values are given.

A.19. FA compositions of WASP samples and respective GC-MS/FID chromatograms

In the following tables A.18, A.19, A.20 and A.21 the FA composition from all study participants (n=27) from WASP will be depicted. Respective FAs are indicated as percentage of total FA composition calculated according to ch. 4.2.3.5. The chromatograms from GC-MS/FID analysis from every study participant of WASP will be depicted in fig. A.26, A.27, A.28, A.29, A.30, A.31, A.32, A.33 and A.34.

Table A.18.: FA composition and percentages of individual FAs from the study population (n=27; participant no. 1001-1034) of WASP. The no. of participants indicate the number from the study protocol. The percentages of the FAs 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 14:1 ω5, 16:1 ω7, 17:1 ω7, 18:1 ω9, 18:1 ω7, 20:1 ω9 and 22:1 ω9 are depicted in this table.

No.	12:0	14:0	15:0	16:0	17:0	18:0	20:0	14:1 ω5	16:1 ω7	17:1 ω7	18:1 ω9	18:1 ω7	20:1 ω9	22:1 ω9
1001	0.64	2,98	0,43	21,61	0,29	4,44	0,14	0,41	5,47	0,36	48,92	2,32	0,62	0,17
1002	0.62	3,09	0,42	22,34	0,27	4,47	0,21	0,32	4,97	0,31	48,24	2,1	0,78	0,22
1003	0.63	3,64	0,46	24,65	0,32	4,48	0,25	0,57	7,54	0,4	42,73	2,19	0,65	0,26
1004	1.04	4,06	0,6	21,92	0,39	6,04	0,15	0,46	4,63	0,34	46,29	1,88	0,73	0,19
1005	0.43	3,88	0,59	25,05	0,4	4,84	0,12	0,3	4,1	0,41	47,42	2,61	0,88	0,1
1006	0.61	4,21	0,53	24,72	0,33	3,68	0,12	0,62	7,43	0,43	42,17	2,2	0,61	0,09
1007	0.73	3,66	0,42	24,2	0,31	5,14	0,15	0,29	3,23	0,3	44,52	2,11	0,68	0,15
1008	0.91	4,21	0,4	25,24	0,36	6,6	0,24	0,35	3,67	0,28	41,19	1,92	0,61	0,15
1009	0.69	3,76	0,56	24,53	0,34	4,02	0,09	0,48	5,01	0,4	45,44	2,37	0,65	0,14
1011	0.83	3,94	0,49	22,27	0,34	4,2	0,22	0,33	3,81	0,33	46,29	2,01	0,95	0,11
1012	1.19	4,08	0,41	23,47	0,36	7,08	0,35	0,24	3,06	0,27	41,6	1,81	0,83	0,24
1013	0.28	2,57	0,41	21,07	0,29	3,58	0,09	0,41	7,12	0,44	46,95	2,48	0,55	0,11
1016	0,55	3,51	0,54	21,17	0,31	3,43	0,13	0,53	7,06	0,43	45,3	2,14	0,53	0,11
1034	0,59	4,35	0,55	23,63	0,41	5,33	0,2	0,43	4,66	0,34	43,81	2,1	0,99	0,11

Table A.19.: FA composition and percentages of individual FAs from the study population (n=27; participant no. 1041-1058) of WASP. The no. of participants indicate the number from the study protocol. The percentages of the FAs 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 14:1 ω5, 16:1 ω7, 17:1 ω7, 18:1 ω9, 18:1 ω7, 20:1 ω9 and 22:1 ω9 are depicted in this table.

No.	12:0	14:0	15:0	16:0	17:0	18:0	20:0	14:1 ω5	16:1 ω7	17:1 ω7	18:1 ω9	18:1 ω7	20:1 ω9	22:1 ω9
1041	0,91	4,7	0,5	24,67	0,35	5,83	0,39	0,4	3,69	0,32	44,3	1,96	0,69	0,19
1044	0,7	3,75	0,48	23,68	0,37	6,52	0,23	0,25	2,83	0,33	47,34	1,95	0,82	0,18
1045	0,67	3,72	0,41	24,23	0,32	4,84	0,16	0,5	5,71	0,37	46,22	2,12	0,61	0,15
1046	0,87	4,36	0,54	25,86	0,4	6	0,16	0,34	3,75	0,38	43,34	2,07	0,77	0,15
1047	0,7	3,71	0,46	24,57	0,37	5,81	0,21	0,27	3,21	0,33	43,94	2,22	1,02	0,16
1048	0,59	2,98	0,3	22,63	0,24	5	0,17	0,36	4,62	0,29	50,72	2,04	0,65	0,09
1050	0,62	2,98	0,4	22,31	0,32	5,92	0,21	0,23	3,23	0,29	48,18	1,87	0,75	0,08
1051	0,85	4,63	0,6	23,94	0,41	5,42	0,33	0,42	3,75	0,42	44,89	2,13	0,85	0,33
1052	0,81	3,56	0,42	20,3	0,26	3,94	0,22	0,37	3,86	0,28	48,46	1,76	0,73	0,14
1053	0,83	3,6	0,43	25,56	0,38	7,46	0,3	0,21	2,33	0,3	44,71	1,98	1	0,2
1054	0,55	3,66	0,37	22,95	0,32	5,56	0,25	0,34	5,03	0,3	46,57	2,33	0,77	0,19
1055	0,9	4,41	0,5	23,27	0,39	6,96	0,24	0,35	4,43	0,32	42,87	1,87	1,05	0,22
1058	0,63	3,66	0,5	23,86	0,4	5,82	0,17	0,21	3,19	0,28	47,89	2,31	0,93	0,12

Table A.20.: FA composition and percentages of individual FAs from the study population (n=27; participant no. 1001-1034) of WASP. The percentages of the FAs 18:2 ω6, 18:3 ω6, 18:3 ω3, 20:2 ω6, 20:3 ω6, 20:3 ω3, 20:4 ω6, 22:2 ω6, 20:5 ω3, 22:6 ω3, 16:1 ω7 *trans* and 18:1 *trans* are depicted in this table. nd not detected, <LOD below respective limit of detection.

No.	18:2 ω6	18:3 ω6	18:3 ω3	20:2 ω6	20:3 ω6	20:3 ω3	20:4 ω6	22:2 ω6	20:5 ω3	22:6 ω3	16:1 ω7 *trans*	18:1 *trans*
1001	8,63	0,052	0,42	0,17	NA	0,046	0,12	0,105	0,03	0,085	0,25	1,2
1002	9,42	0,047	0,44	0,17	NA	0,017	0,11	0,112	0,03	0,04	0,22	0,98
1003	8,75	0,084	0,22	0,16	0,1	0,032	0,1	0,16	0,05	0,064	0,33	1,16
1004	9,3	0,031	0,44	0,14	0,09	nd	0,06	0,059	0,043	0,029	0,25	0,86
1005	6,36	0,045	0,2	0,19	0,25	0,017	0,07	0,11	0,031	<LOD	0,29	1,33
1006	9,68	0,066	0,76	0,16	0,16	0,044	0,23	0,033	0,033	0,045	0,3	0,74
1007	12,1	0,036	0,45	0,14	nd	0,014	0,05	0,062	0,029	<LOD	0,22	0,96
1008	11,74	0,034	0,44	0,17	nd	nd	0,06	0,085	0,021	<LOD	0,22	1,02
1009	9,28	0,028	0,33	0,17	0,14	0,04	0,09	0,109	0,044	<LOD	0,28	1,02
1011	11,19	0,048	0,66	0,27	0,21	0,036	0,26	0,035	0,032	0,035	0,24	0,87
1012	12,54	0,052	0,35	0,26	nd	0,035	0,13	0,055	0,029	0,059	0,24	1,25
1013	10,45	0,054	1,03	0,19	0,29	0,1	0,47	0,063	0,085	0,109	0,24	0,58
1016	11,96	0,033	0,49	0,16	0,15	0,063	0,17	0,034	0,032	0,053	0,31	0,8
1034	9,25	0,035	0,98	0,22	0,26	0,075	0,24	0,033	0,064	0,14	0,3	0,88

Table A.21.: FA composition and percentages of individual FAs from the study population (n=27; participant no. 1041-1058) of WASP. The no. of participants indicate the number from the study protocol. The percentages of the FAs 18:2 ω6, 18:3 ω6, 18:3 ω3, 20:2 ω6, 20:3 ω6, 20:3 ω3, 20:4 ω6, 22:2 ω6, 20:5 ω3, 22:6 ω3, 16:1 ω7 *trans* and 18:1 *trans* are depicted in this table. nd not detected, <LOD below respective limit of detection.

| No.	18:2 ω6	18:3 ω6	18:3 ω3	20:2 ω6	20:3 ω6	20:3 ω3	20:4 ω6	22:2 ω6	20:5 ω3	22:6 ω3	16:1 ω7 *trans*	18:1 *trans*
1041	8,89	0,034	0,39	0,16	0,11	nd	0,15	nd	0,018	nd	0,27	1,08
1044	8,28	0,049	0,4	0,15	nd	0,02	0,05	0,09	0,03	<LOD	0,24	1,22
1045	8,01	0,042	0,42	0,16	nd	nd	0,23	nd	0,025	nd	0,26	0,81
1046	8,79	0,027	0,26	0,17	nd	0,021	0,06	0,097	0,029	<LOD	0,28	1,22
1047	10,59	0,042	0,6	0,24	nd	0,038	0,17	0,049	0,025	0,04	0,23	0,85
1048	7,55	0,028	0,31	0,11	nd	nd	0,1	0,039	0,024	<LOD	0,18	0,89
1050	10,53	0,051	0,51	0,18	0,11	0,022	0,13	0,029	0,024	0,044	0,2	0,78
1051	8,4	0,047	0,36	0,14	nd	0,016	0,07	0,149	0,038	<LOD	0,34	1,47
1052	12,94	0,037	0,47	0,16	nd	0,008	0,05	0,038	0,024	<LOD	0,21	0,87
1053	8,29	0,031	0,59	0,23	nd	0,053	0,14	0,061	0,033	nd	0,22	1,09
1054	8,58	0,027	0,54	0,17	0,12	0,048	0,15	0,029	0,027	0,039	0,25	0,83
1055	9,86	nd	0,55	0,19	0,14	nd	0,26	nd	0,039	0,097	0,26	0,85
1058	7,51	0,063	0,36	0,25	0,31	0,042	0,27	0,05	0,025	0,032	0,25	0,85
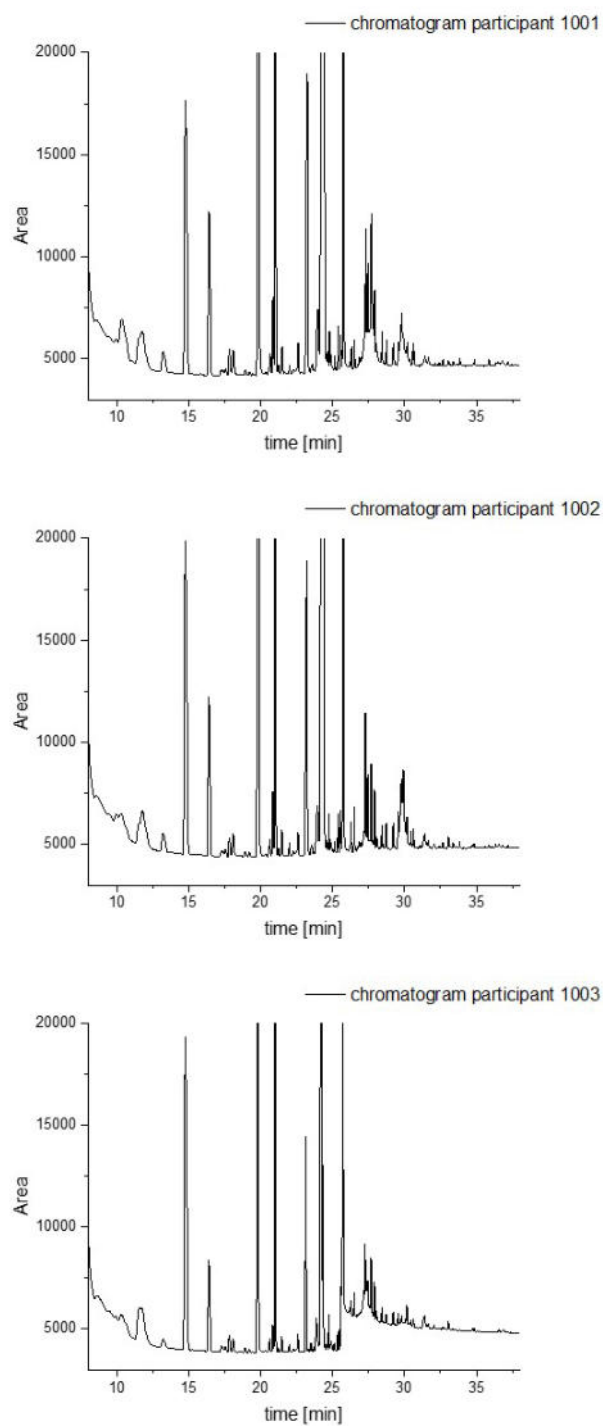
Figure A.26.: GC-MS/FID chromatograms of FAs in ADT of samples 1001, 1002 and 1003 from the study population of WASP.
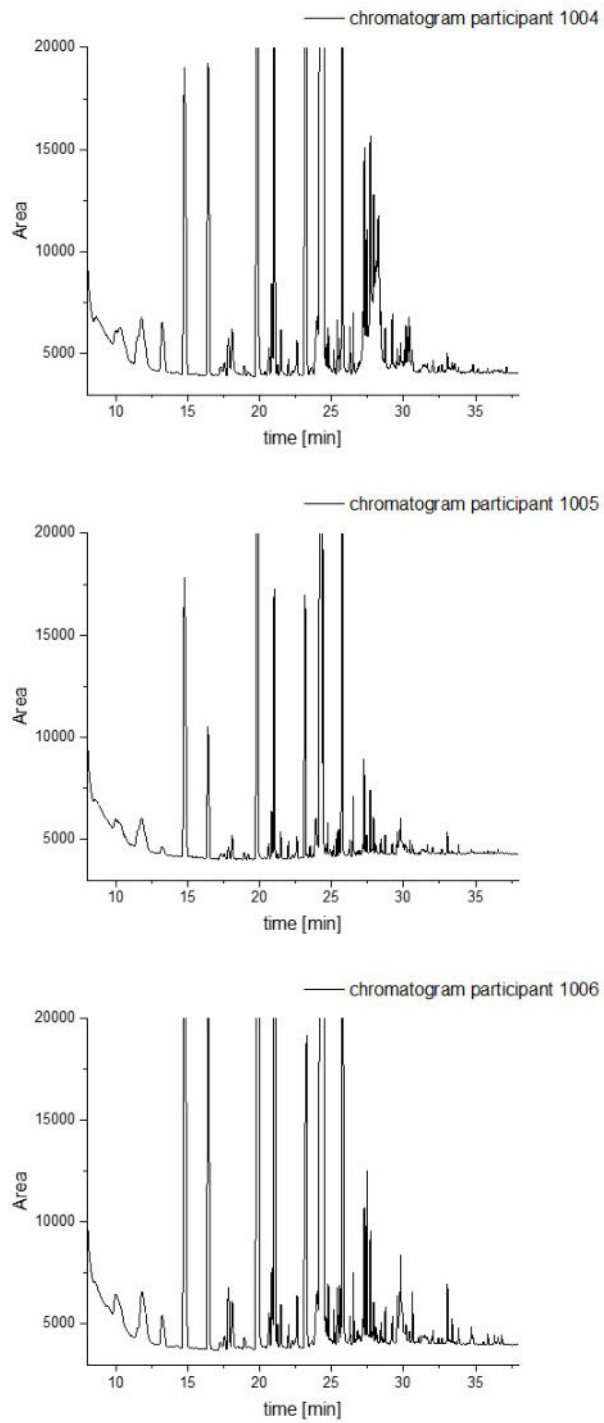
Figure A.27.: GC-MS/FID chromatograms of FAs in ADT of samples 1004, 1005 and 1006 from the study population of WASP.
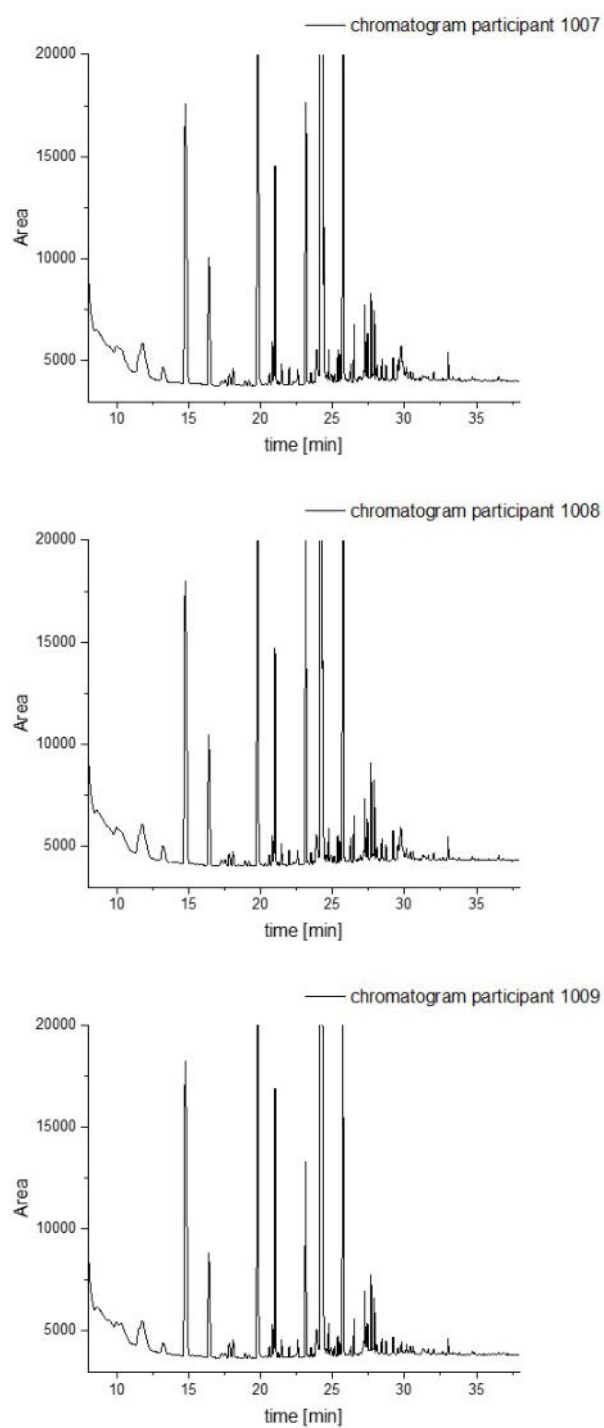
Figure A.28.: GC-MS/FID chromatograms of FAs in ADT of samples 1007, 1008 and 1008 from the study population of WASP.

Figure A.29.: GC-MS/FID chromatograms of FAs in ADT of samples 1011, 1012 and 1013 from the study population of WASP.

Figure A.30.: GC-MS/FID chromatograms of FAs in ADT of samples 1016, 1034 and 1041 from the study population of WASP.n

Figure A.31.: GC-MS/FID chromatograms of FAs in ADT of samples 1044, 1045 and 1046 from the study population of WASP.

Figure A.32.: GC-MS/FID chromatograms of FAs in ADT of samples 1047, 1048 and 1050 from the study population of WASP.
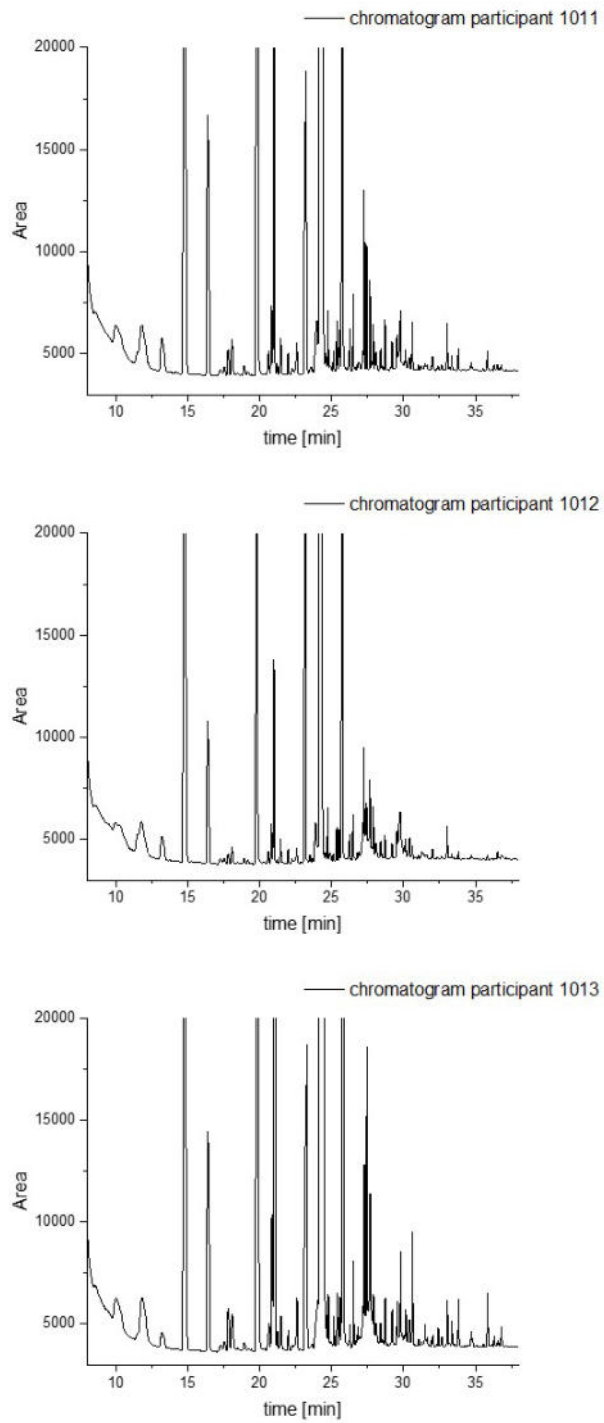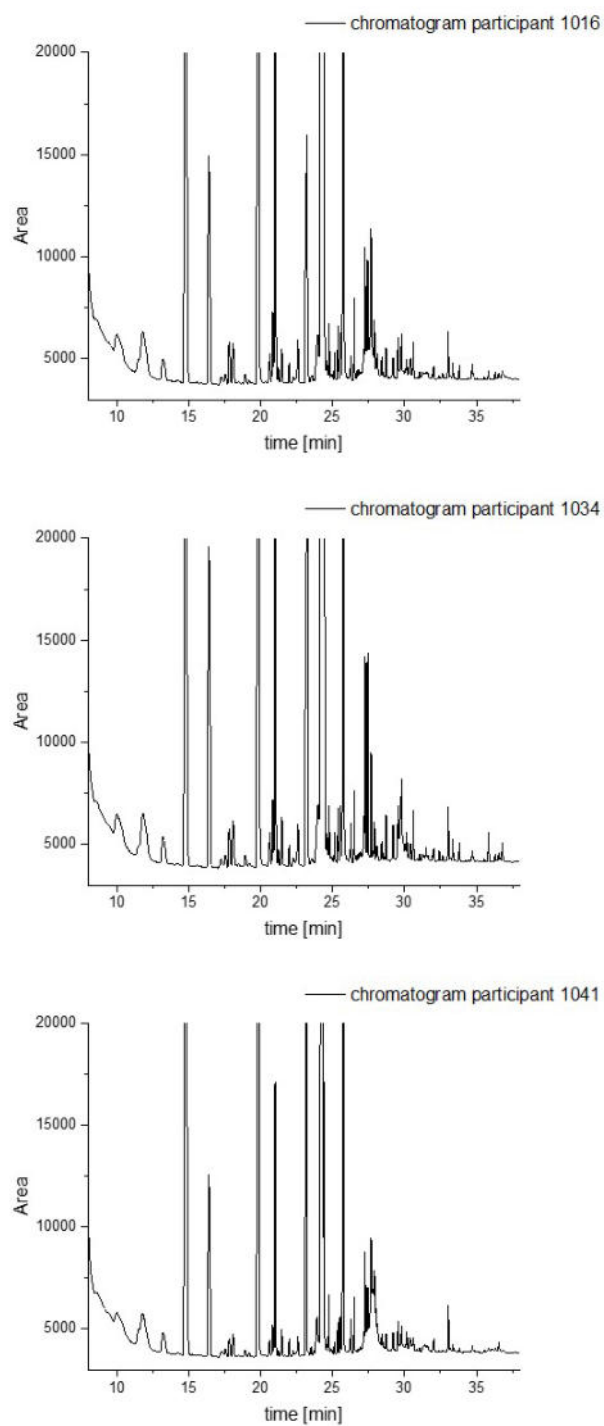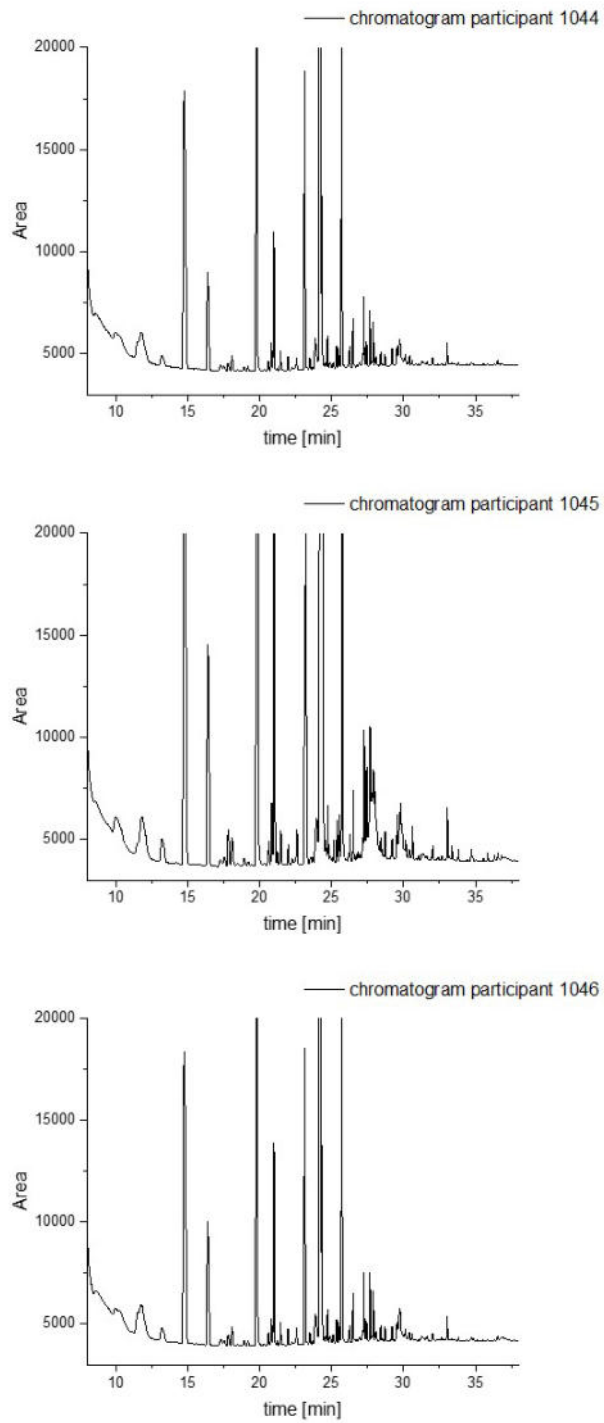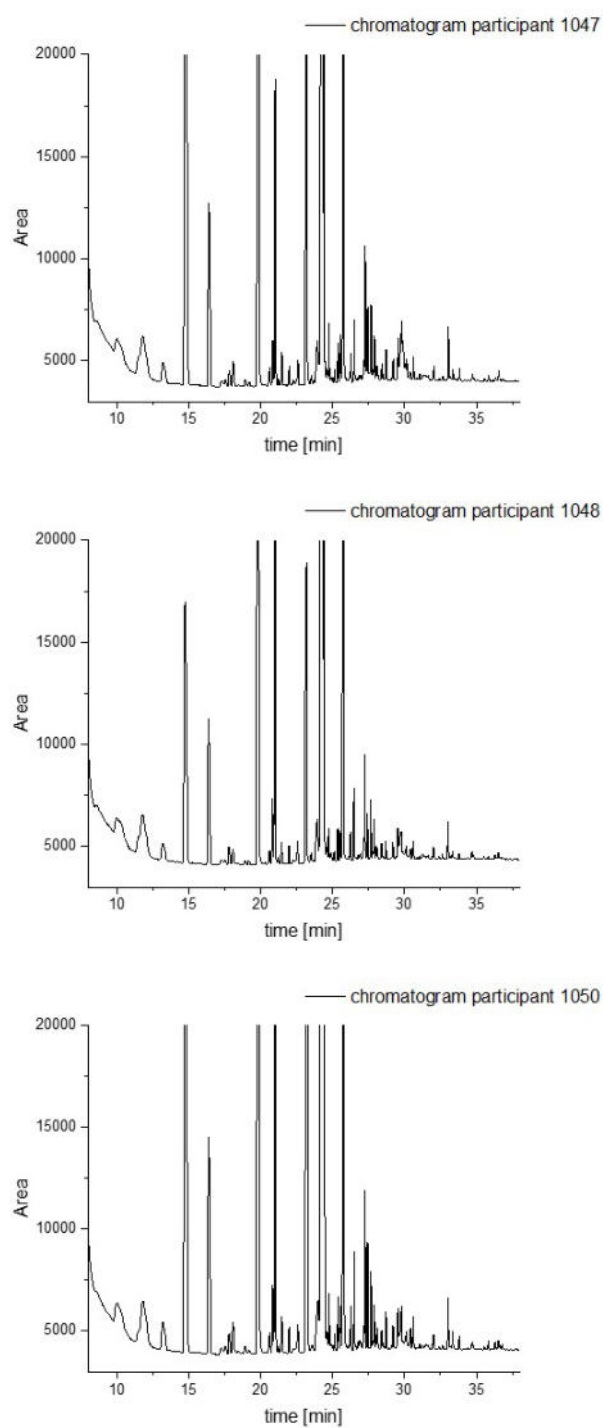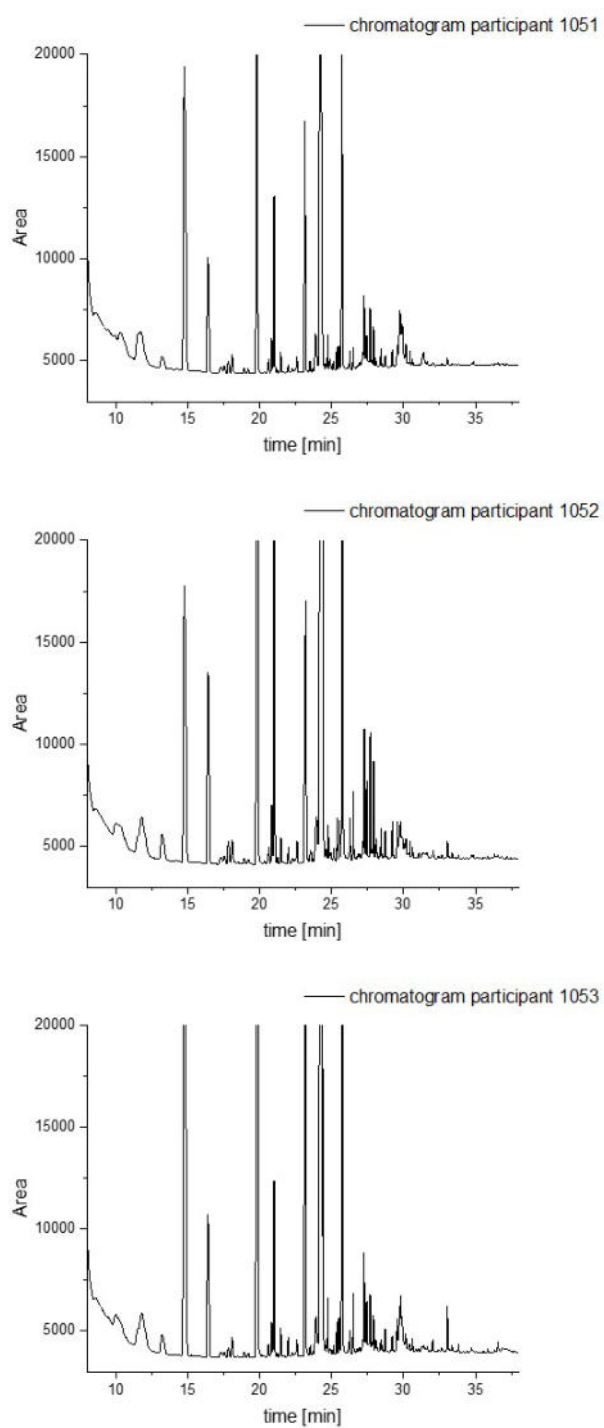
Figure A.33.: GC-MS/FID chromatograms of FAs in ADT of samples 1051, 1052 and 1053 from the study population of WASP.
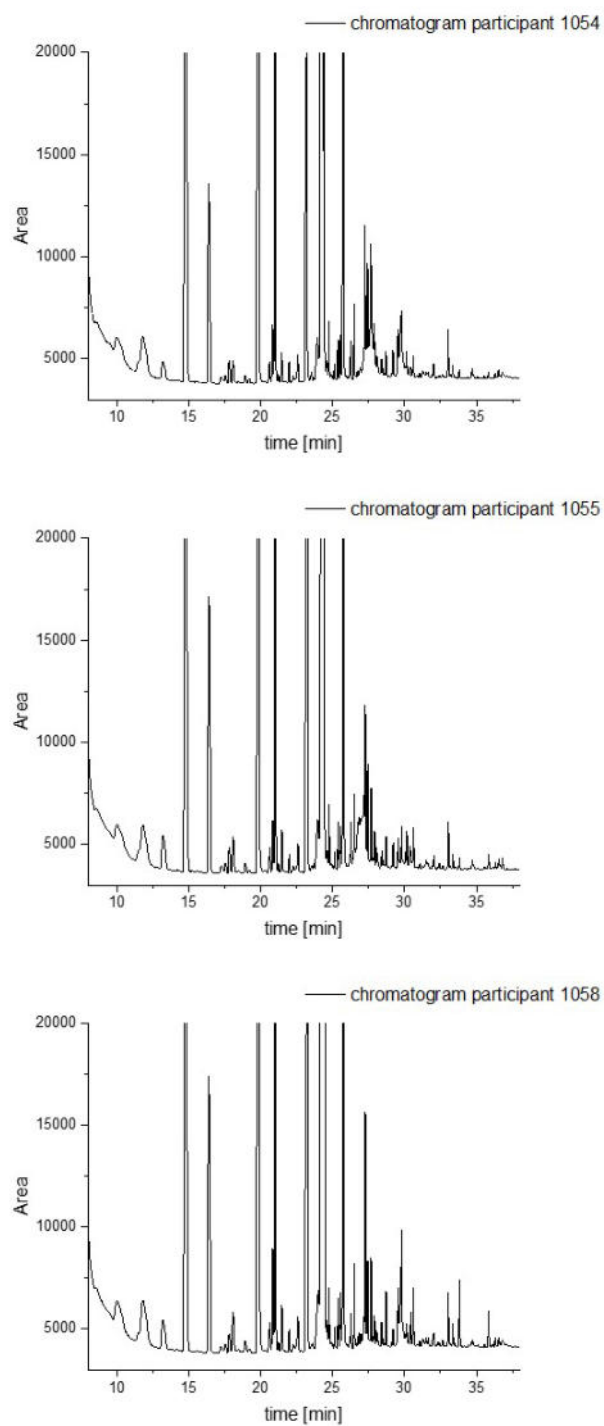
Figure A.34.: GC-MS/FID chromatograms of FAs in ADT of samples 1054, 1055 and 1058 from the study population of WASP.

A.20. Correlation coefficients of correlation analysis of dietary FA biomarker in the study population WASP

Variable 1	R	Variable 2
15:0	0.64	17:0
15:0	0.73	16:1 ω7 *trans*
15:0	0.54	20:5 ω3
17:0	0.44	16:1 ω7 *trans*
16:1 ω7 *trans*	0.53	20:5 ω3
18:2 ω6	0.46	18:3 ω3
18:3 ω3	-0.68	20:5 ω3

A.21. Factor loadings of selected FA biomarkers in the study population WASP without the sum of 18:1 *trans*

Composition of PCs calculated in R using 15:0, 17:0, 16:1 ω7 *trans*, 18:2 ω6, 18:3 ω3 and 20:5 ω3 in human ADT from different compartments derived from 27 study participants and resulting values of PC ($PC_{diet}1$, $PC_{diet}2$ and $PC_{diet}3$, respectively). Percentages of variation explained by the PCs are provided and eigenvectors of prominent variables influencing the PCs are indicated with bold characters. ADT levels of 15:0, 17:0, 16:1 ω7 *trans*, 18:2 ω6, 18:3 ω3 and 20:5 ω3 derived from 27 study participants of the WASP study were calculated as the relative concentration of each FA using the FID signal of the FA in relation to the sum of all FA-related signals (n=26).

*PCs, which could be used as objective markers of dietary intake.

Table A.22.: Results of PCA of selected FA biomarkers 15:0, 17:0, 16:1 ω7 *trans*, 18:2 ω6, 18:3 ω3 and 20:5 ω3. Eigenvectors of prominent variables influencing the PCs are indicated with bold characters and percentages of variation explained by the PCs are provided. *PC indicates, that PC could be used as objective markers of dietary intake.

Variable	Eigenvectors					
	$PC_{diet}1$*	$PC_{diet}2$*	$PC_{diet}3$*	$PC_{diet}4$	$PC_{diet}5$	$PC_{diet}6$
15:0	**0.58**	-0.01	0.29	-0.05	-0.03	-0.76
17:0	**0.50**	-0.10	0.23	0.68	0.24	0.41
16:1 ω7 *trans*	**0.54**	0.03	0.05	-0.57	-0.38	0.48
18:2 ω6	-0.27	0.32	**0.86**	-0.19	0.19	0.12
18:3 ω3	0.02	**0.70**	-0.05	0.38	-0.6	-0.05
20:5 ω3	0.23	**0.62**	-0.35	-0.19	0.63	0.02
variance (%)	41.23	27.77	13.76	9.99	4.16	3.08

Table A.23.: Factorloadings of $PC_{diet}1$, $PC_{diet}2$ and $PC_{diet}3$ of individual study participants (n=27) from the study population of WASP calculated without the sum of 18:1 *trans.*

No.	$PC_{diet}1$	No.	$PC_{diet}1$	No.	$PC_{diet}2$	No.	$PC_{diet}2$	No.	$PC_{diet}3$	No.	$PC_{diet}3$
1	-0.83	15	-0.40	1	-0.46	15	0.86	1	-0.80	15	0.86
2	3.13	16	-1.04	2	-0.57	16	-0.14	2	0.26	16	1.50
3	-1.66	17	-0.58	3	-0.21	17	4.50	3	-0.58	17	-1.47
4	1.14	18	0.52	4	-0.26	18	0.52	4	-0.81	18	1.45
5	1.61	19	-1.02	5	0.08	19	-0.23	5	0.35	19	-0.87
6	-2.58	20	0.96	6	0.33	20	-1.36	6	1.31	20	-0.44
7	2.50	21	2.42	7	-1.87	21	2.93	7	-0.74	21	-0.35
8	0.31	22	0.82	8	-0.78	22	0.43	8	-0.41	22	0.35
9	1.54	23	0.37	9	-1.27	23	-1.17	9	0.34	23	0.22
10	-1.40	24	-0.51	10	-0.35	24	-0.85	10	1.20	24	-0.92
11	1.22	25	-0.21	11	-0.13	25	0.00	11	-0.01	25	-0.70
12	-1.70	26	0.88	12	0.22	26	1.03	12	1.02	26	0.23
13	-3.21	27	-0.46	13	-1.25	27	0.15	13	-2.02	27	0.76
14	-1.85			14	-0.13			14	0.26		

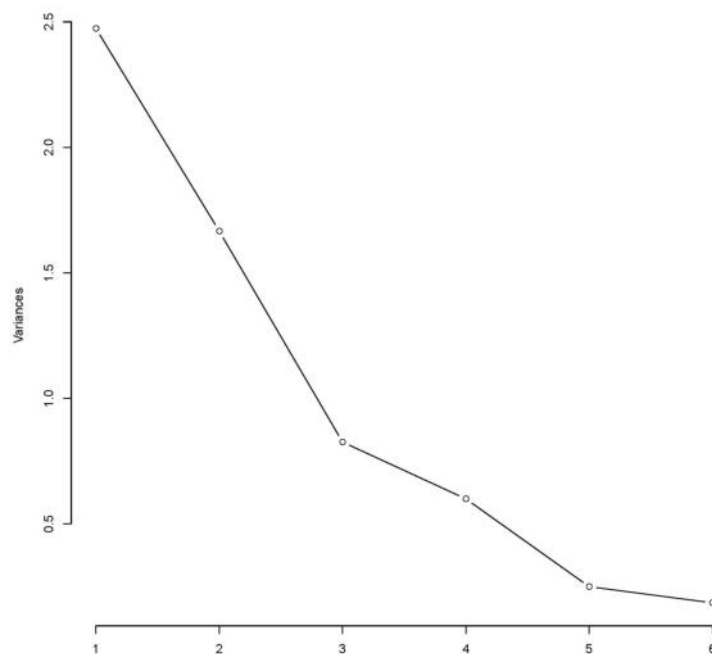## A.22. Screeplot of PCAs of selected FA biomarkers in the study population WASP without the sum of 18:1 *trans*

Figure A.35.: Scree plot of PCA of selected FA biomarker (15:0, 17:0, 16:1 ω7 *trans*, 18:2 ω6, 18:3 ω3 and 20:5 ω3, respectively). The "Elbow point" is located at 3 PCs. The Kaiser-Criterion is in accordance with the scree plot. X-axes, numbers of PCs.

B. Publication list

Jaud T., Pemp P., Mahdiani M., Esch H., Lehmann L. Identification of variables influencing desaturase and elongase indexes in human breast adipose tisse - *in preparation.*

Affidavit

I hereby confirm that my thesis entitled *Application based personalized food choices and health sustainment: scientific background and investigation of biomarkers in human tissue specimens* is the result of my own work. I did not receive any help or support from commercial consultants. All sources and / or materials applied are listed and specified in the thesis.

Furthermore, I confirm that this thesis has not yet been submitted as part of another examination process neither in identical nor in similar form.

Place, Date   Signature

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die Dissertation *Gesundheitserhaltende Ernährung: Wissenschaftlicher Hintergrund einer App zur personalisierten Lebensmittelauswahl und Identifizierung von Biomakern für die Ernährungsweise im Humangewebe* eigenständig, d.h. insbesondere selbständig und ohne Hilfe eines kommerziellen Promotionsberaters, angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

Ich erkläre außerdem, dass die Dissertation weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen hat.

Ort, Datum   Unterschrift