Aus dem Lehrstuhl für Epidemiologie der Universität Augsburg

# Zusammenhang zwischen Ernährungsfaktoren, Blutgerinnungsparametern und Leberfettgehalt

## Kumulative Dissertation

zur Erlangung des akademischen Grades

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Medizinischen Fakultät der Universität Augsburg

von

**Michael Schepp**

Augsburg, 12.07.2024

Universität
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## Eidesstattliche Versicherung und Erklärung

Hiermit versichere ich an Eides statt, dass die vorliegende Dissertation von mir selbständig und ohne unerlaubte Hilfe angefertigt wurde. Zudem wurden keine anderen als die angegebenen Quellen verwendet. Außerdem versichere ich, dass die Dissertation keiner anderen Prüfungskommission vorgelegt wurde und ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe.

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I declare that I have authored this thesis independently, that I have not used other than the declared sources/resources. As well I declare that I have not submitted a dissertation without success and not passed the oral exam. The present dissertation (neither the entire dissertation nor parts) has not been presented to another examination board.

Augsburg, 12.07.2024

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Erstgutachter (Hauptbetreuerin/Hauptbetreuer): Prof. Dr. Jakob Linseisen

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# Inhaltsverzeichnis

# 1. Einleitung

## 1.1 Übergeordnete Fragestellung

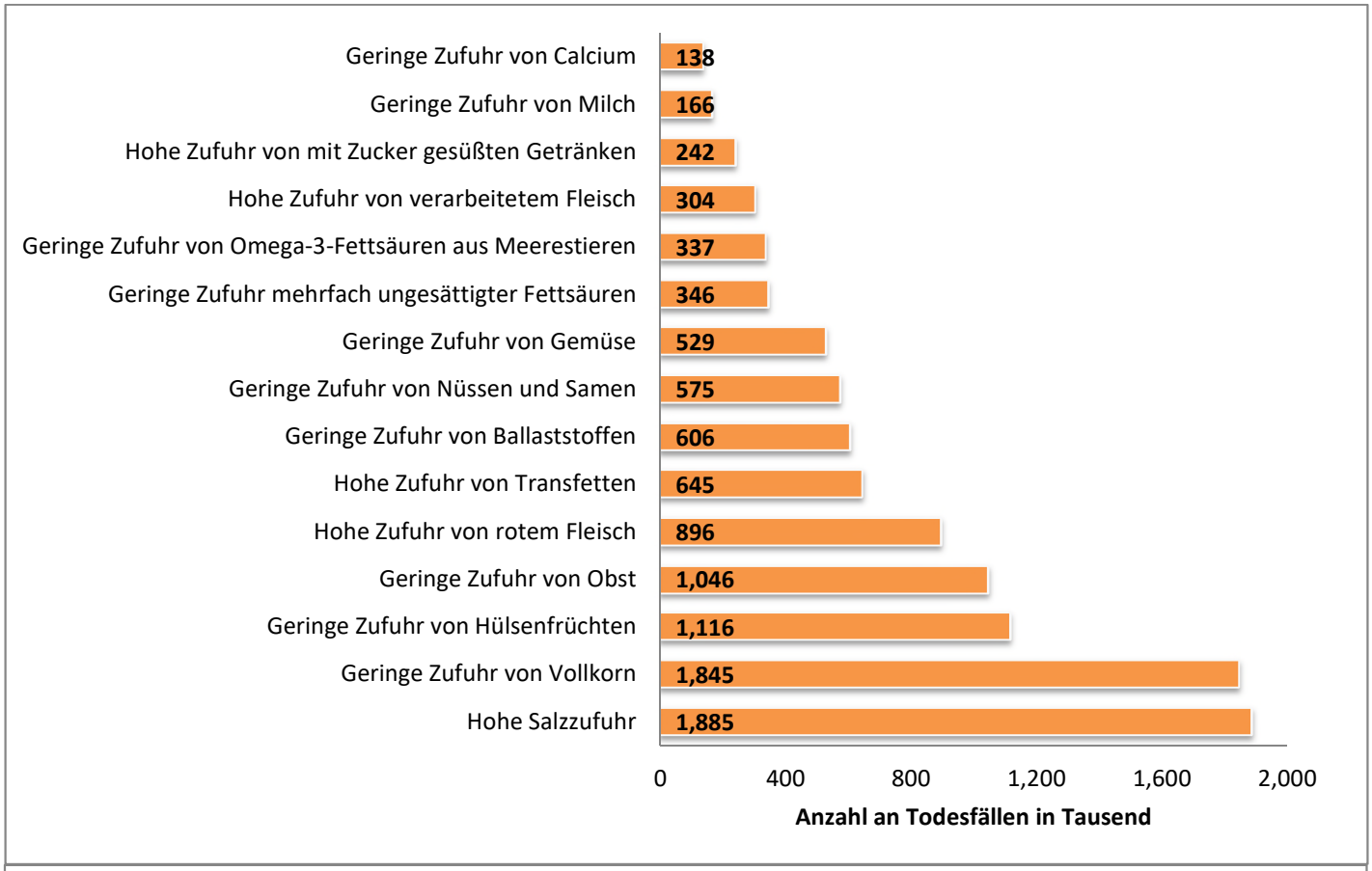
### 1.1.1 Bedeutung von Ernährung in der Prävention nicht-übertragbarer Erkrankungen

„Wir leben nicht, um zu essen; wir essen, um zu leben." – Sokrates [1]. Bereits seit der Antike ist die zentrale Bedeutung der Ernährung für eine gesunde Lebensführung bekannt und ist von prägenden zeitgenössischen Gelehrten beschrieben worden. Während in der Vergangenheit auf die medizinische Rolle von Nahrungsmitteln neben der reinen Deckung des täglichen Energiebedarfs und Befriedigung des Geschmacksbedürfnisses ein wesentlicher Schwerpunkt gelegt wurde, ist in der Neuzeit die Stellung von Ernährung in der ärztlichen Behandlung und Vorbeugung von Krankheiten eher in den Hintergrund gerückt. Im Studium der Humanmedizin wird der Ernährungsbildung nur eine geringe Aufmerksamkeit zuteil [2] und laut einer Querschnittsstudie der Universität Erlangen-Nürnberg werden unter ärztlichen Berufseinsteigern insbesondere im Fachbereich der Ernährung von den meisten Befragten empfundene Defizite gesehen [3]. Im Kontrast dazu steht der alarmierende Anstieg der Prävalenz ernährungsbedingter Krankheiten, insbesondere der sog. nicht-übertragbaren Erkrankungen (engl. non-communicable diseases, NCDs). Hierbei handelt es sich um meist chronische Krankheiten, die laut WHO geschätzt für 74% der weltweiten Todesfälle verantwortlich sind [4]. In diese Kategorie gehören unter anderem Krankheiten des Herz-Kreislauf-Systems (engl. cardiovascular diseases, CVD), Erkrankungen der Atmungsorgane, Diabetes mellitus, sowie Krebserkrankungen. Die sog. Global Burden of Disease study (GBD) beschäftigt sich schwerpunktmäßig mit den Risikofaktoren für NCDs und sammelt in regelmäßigen Zeitabständen Daten aus 195 Ländern weltweit und wertet diese aus, um gesundheitspolitische Empfehlungen und die Erstellung neuer Leitlinien anzuregen [5]. Der GBD 2019 zufolge betrug die Prävalenz von NCDs 7.104.354.703 Fälle und die Mortalität 42.034.124 [5]. Ein Auszug der Daten für die Prävalenz, Mortalität sowie für Krankheit adjustierte Lebensjahre (engl. disability-adjusted life-years, DALYs) ist für verschiedene NCDs ist in Tabelle 1 ersichtlich.

**Tabelle 1**. Prävalenz, Mortalität und für Krankheit adjustierte Lebensjahre (DALYs) verschiedener nicht-übertragbarer Krankheiten in Tausend, nach GBD 2019

| Erkrankungen | Prävalenz | Mortalität | DALYs |
|---|---|---|---|
| Herz-Kreislauf-Erkrankungen | 523.199 | 18.563 | 393.107 |
| davon ischämische Herzerkrankungen | 197.219 | 9.138 | 182.030 |
| davon Schlaganfälle | 101.475 | 6.553 | 143.232 |
| Krebserkrankungen | 85.831 | 10.023 | 250.173 |
| Erkrankungen des Verdauungssystems | 2.276.271 | 2.558 | 88.992 |
| davon Nicht-alkoholische Fettleber-Erkrankung | 1.235.700 | 169 | 4.417 |
| Diabetes mellitus | 459.875 | 1.551 | 70.880 |
| *Alle Nicht-übertragbaren Krankheiten* | *7.104.355* | *42.034* | *1.620.166* |

In der GBD-Studie sind mittels einer systematischen Analyse die 15 ernährungsbezogenen Hauptrisikofaktoren für eine erhöhte Sterblichkeit sowie DALYs identifiziert worden [5]. Ernährungsrisikofaktoren werden für weltweit ca. 7,9 Millionen Todesfälle verantwortlich gemacht, das entspricht ca. 28% aller an NCDs Verstorbenen [5]. Zu den fünf ausgeprägtesten Risikofaktoren zählen ein hoher Salzkonsum, eine unzureichende Zufuhr von Vollkornprodukten, Hülsenfrüchten und Obst sowie ein zu hoher Konsum von rotem Fleisch (Abbildung 1). Neben einer ausreichenden Zufuhr pflanzlicher Produkte scheinen auch Lebensmittel tierischen Ursprungs zu einem gewissen Grad ebenfalls zur Senkung des Mortalitäts- und Morbiditätsrisikos von Bedeutung zu sein wie am Beispiel von Omega-3-Fettsäuren aus Meeresfrüchten oder Milch zu sehen ist [5]. Laut der Analyse waren mit einer Anzahl von ca. 6,9 Millionen die meisten ernährungsbezogenen Todesfälle die Folge von Herz-Kreislauf-Erkrankungen, an zweiter Stelle standen Krebserkrankungen mit ca. 605.427 ernährungsbezogenen Todesfällen und auf Diabetes mellitus entfielen geschätzt 383.870 ernährungsbezogene Todesfälle. Geschätzt 42% aller ernährungsbezogenen Todesfälle wurden Erwachsenen zugeordnet, die noch nicht das Alter von 70 Jahren erreicht haben [5].



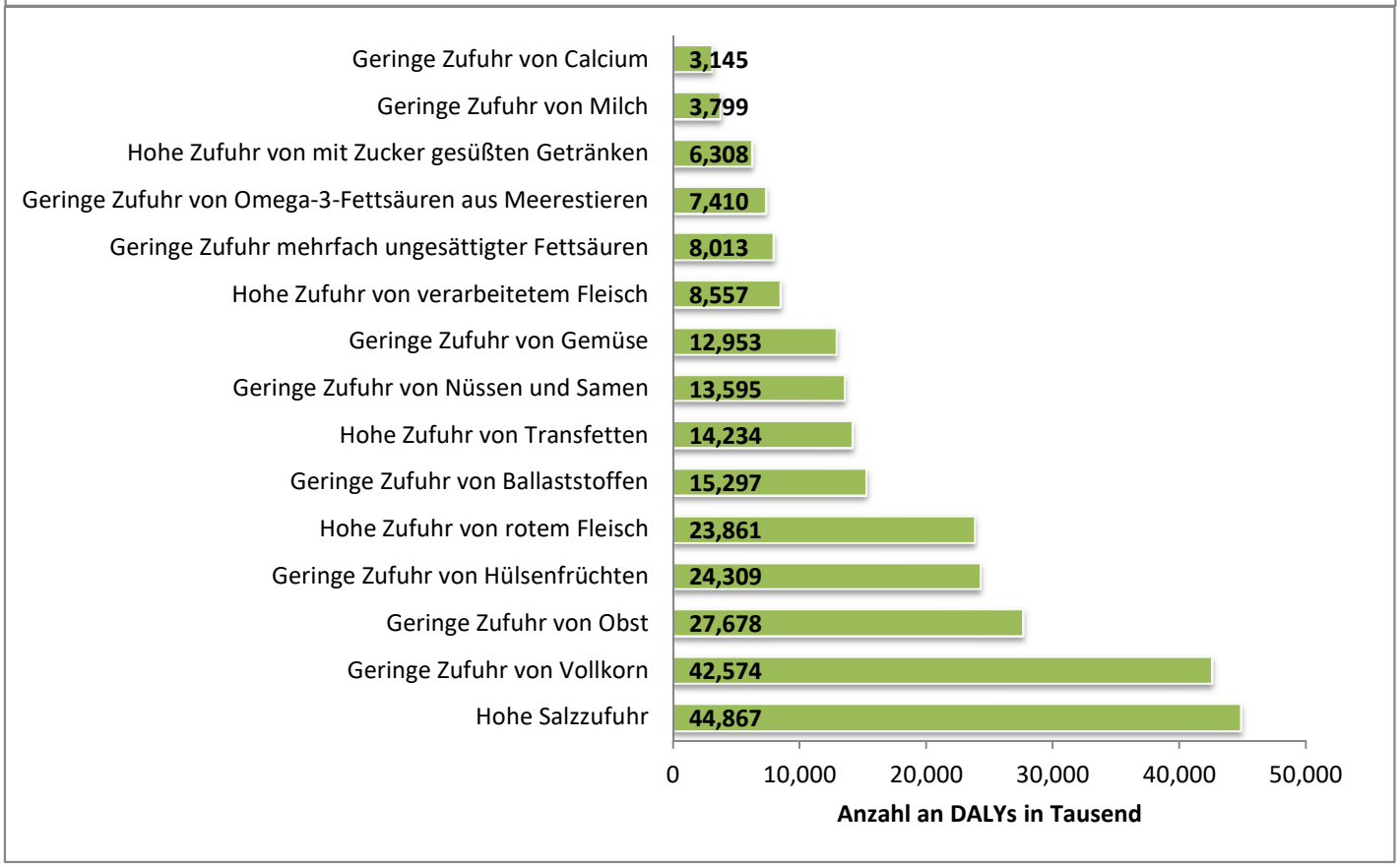




**Abbildung 1**. Ernährungsrisikofaktoren und die zugeordnete Anzahl an Todesfällen und DALYs, nach GBD 2019 [5]

### 1.1.2 Blutgerinnungsstörungen als Einflussfaktor für CVDs

Blutgerinnung beschreibt blutstillende Prozesse im Körper, die durch eine Interaktion zwischen Thrombozyten, den Blutgefäßen und Plasmakomponenten wie den Gerinnungsfaktoren entstehen [6]. Blutgerinnungsfördernde und –hemmende Wirkstoffe stehen hier in einem Gleichgewicht, einer Homöostase, sodass sowohl eine unerwünschte Gerinnung als auch eine Blutungsneigung vermieden wird [6].

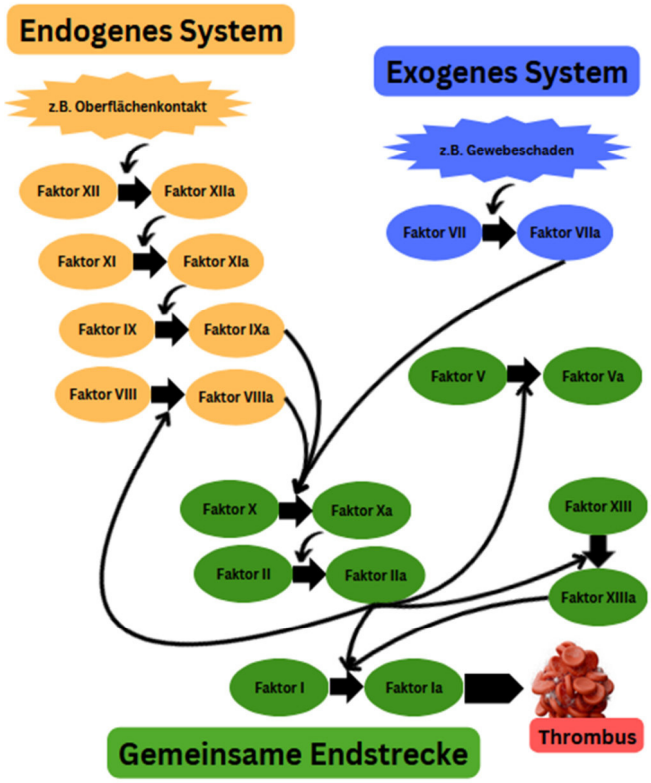



**Abbildung 2**. Vereinfachtes Schema der Blutgerinnung, modifiziert nach [8]

Über diesen Weg sind daher auch zahlreiche CVDs mit der Blutgerinnung eng verbunden, da durch eine übermäßige Blutgerinnung Blutgerinnsel, sog. Thromben, entstehen können, welche die Blutgefäße verstopfen und den Blutfluss behindern. Mögliche Folgen sind Erkrankungen wie die ischämische Herzkrankheit oder Schlaganfälle [7]. Störungen der Blutgerinnung können angeboren oder erworben sein. Während angeborene Erkrankungen wie der Antithrombin III-Mangel oder das Faktor V-Leiden eher seltener die Ursache für Thrombosen darstellen, ist der Großteil der Blutgerinnungsstörungen auf erworbene Einflussfaktoren zurückgeführt. Dazu zählen zum Beispiel bestimmte Medikamente wie orale Kontrazeptiva oder Heparin, Traumata, Schwangerschaft, Tumorerkrankungen oder chronisch entzündliche Erkrankungen [7]. Desweiteren spie-

len bei der Entstehung von Thrombosen Lebensstilfaktoren eine Rolle, wobei Rauchen, eine geringe körperliche Aktivität sowie eine ungesunde Ernährung hierbei als besonders ungünstig identifiziert wurden [8]. Während der Einfluss bestimmter Nährstoffe wie z.B. langkettiger Omega-3-Fettsäuren auf die Blutgerinnung in der Vergangenheit bereits umfassend untersucht wurde, ist über zahlreiche weitere Ernährungsfaktoren, insbesondere der Effekt bestimmter Lebensmittel oder Ernährungsmuster auf die Blutgerinnung, noch relativ wenig bekannt, sodass diesbezüglich zukünftig weitere Studien erforderlich sind, um diesen Zusammenhang weiter zu erforschen und daraus möglicherweise effektive präventive Strategien abzuleiten [9].

### 1.1.3 Die Zunahme von nicht-alkoholischer Fettleber als Faktor im Anstieg nicht-übertragbarer Erkrankungen

Die Leber ist das Organ, in welchem die meisten Blutgerinnungsfaktoren produziert werden und als zentrales Stoffwechselorgan ebenfalls verantwortlich für die Verarbeitung von Nährstoffen sowie Entgiftungsprozesse [10]. Daher ist es naheliegend, dass eine Beeinflussung der Blutgerinnung je nach Ernährungsweise auch über eine Wirkung auf die Leber vermittelt werden könnte. Ein Zusammenhang zwischen Blutgerinnungsfaktoren und dem Fettleber-Index (FLI), einem validierten Marker für das Ausmaß einer Fettlebererkrankung, wurde bereits beschrieben [11]. Während in der Vergangenheit zumeist der Begriff der nicht-alkoholischen Fettleber zur Beschreibung dieser Krankheit verwendet wurde, hat sich heutzutage stattdessen die Bezeichnung der sog. Stoffwechsel-bedingte Fettlebererkrankung (engl. metabolic-dysfunction associated fatty liver disease, MAFLD) etabliert. Diese Bezeichnung soll der Ursache einer gestörten Stoffwechselfunktion mehr gerecht werden [12]. Die MAFLD ist eine Krankheit mit steigender Prävalenz, von welcher geschätzt ca. 32% der Weltbevölkerung betroffen sind [13]. Es wird vermutet, dass auch die Folgen dieser Erkrankung wie Leberzirrhose, Leberkrebs oder Leberversagen in den kommenden Jahren ansteigen werden, sodass eine Notwendigkeit für die Identifizierung, Etablierung und Umsetzung effektiver präventiver Maßnahmen besteht [14]. Neben Rauch- und Alkoholverzicht sowie regelmäßiger körperlicher Aktivität kommt hier der Ernährung eine besonders bedeutende Rolle zu [15,16]. Während für eine kalorienreduzierte Ernährung mit folgender Gewichtsabnahme überwiegend Konsens für eine günstige Beeinflussung der MAFLD besteht, ist die genaue Verteilung der Makronährstoffe sowie die genauen Zusammenhänge mit dem Konsum von Lebensmittel oder Ernährungsmustern noch in

Diskussion [15]. Vielmehr scheinen hierbei auch individuelle Faktoren der Patienten von großer Relevanz zu sein, um möglichst genaue, auf das Individuum angepasste Ernährungsempfehlungen geben zu können [15]. Deshalb gewinnt eine differenzierte Betrachtung der unterschiedlichen Stoffwechselprofile, der sog. Metabotypen, in der Ernährungsforschung immer mehr Anklang mit dem Ziel, genauere und individuelle Ernährungsstrategien zu entwickeln [17]. Eine Schwierigkeit stellt die genaue Diagnostik der MAFLD dar. Während sich hierbei die Leberbiopsie als Goldstandard etabliert hat und als Grundlage der genauen histo-pathologischen Klassifizierung dient, gewinnen aufgrund der Kosteneffizienz und der besseren Praktikabilität nicht-invasive Diagnosemethodiken eine immer größere Bedeutung [18]. Dazu zählen zum einen bildgebende Verfahren wie der Ultraschall, Computertomographie (CT) oder die Magnet-Resonanz-Tomographie (MRT). Im Vergleich zur Leberbiopsie sind diese Verfahren für die Fettleberdiagnostik gemäß einer umfangreichen Meta-Analyse mit einer durchschnittlichen Sensitivität von 73-91% beim Ultraschall, 82-97% bei der CT und 73–89% für die MRT valide Methoden. Die Spezifität wird beim Ultraschall mit 70-85%, beim CT mit 82-97% und beim MRT mit 92–96% angegeben [19]. Zum anderen kann das Risiko einer Fettleber auch mit einfachen Formeln wie dem Fettleberindex (eng. fatty liver index, FLI) angegeben werden, welcher aufgrund der noch einfacheren Handhabbarkeit vor allem in der klinischen Praxis im Rahmen von Routineuntersuchungen Anwendung finden kann. In die Berechnung des FLI gehen Messwerte des Body-Mass-Index (BMI), Taillenumfangs, der Serum-Triglyceride und der γ-Glutamyltransferase ein [20]. Der FLI hat sich mit einer Sensitivität von ca. 76% und Spezifität von ca. 87% bei FLI-Werten >60 für die MAFLD-Diagnostik im Vergleich zur Leberbiopsie ebenfalls als valide Messmethode herausgestellt [21]. Daher wird der FLI häufig in Studien mit einer hohen Teilnehmerzahl zur Einschätzung des Ausmaßes der Fettleber eingesetzt, auch wenn die dadurch etwas ungenaueren Ergebnisse eine Limitation darstellen.

**Tabelle 2**. Sensitivität und Spezifität von Messmethoden der Fettleber im Vergleich zur Leberbiopsie [21, 23]

| Messmethode | Sensitivität [%] | Spezifität [%] |
|---|---|---|
| Ultraschall | 73-91 | 70-85 |
| Computertomographie | 82-97 | 82-97 |
| Magnet-Resonanz-Tomographie | 73–89 | 92-96 |
| Fettleber-Index >60 | 76 | 87 |
| Leberbiopsie (Referenz) | 100 | 100 |

### 1.1.4 Hintergründe und Zielsetzung der wissenschaftlichen Projekte

Die erste Studie beschäftigt sich mit dem Zusammenhang von Ernährungsfaktoren und ihrem Einfluss auf Blutgerinnungsfaktoren während im zweiten Projekt der Zusammenhang von Ernährungsfaktoren mit der Entwicklung von Fettleber untersucht. In beiden Projekten wird ein lebensmittelbasierter Ansatz bei der Analyse ernährungsbedingter Einflüsse angewendet. In der Ernährungswissenschaftskommunikation werden häufig eher Lebensmittel(-gruppen) im Vergleich zu einzelnen Nährstoffen empfohlen [22] und die Erforschung der Auswirkung von Lebensmitteln als Ganzes auf die Gesundheit scheint u.a. aufgrund der Komplexität der Wechselwirkungen unterschiedlicher in diesen Lebensmitteln enthaltenen Nährstoffe auch ein praxisrelevanter Ansatz zu sein [23]. Darüber hinaus basieren beide aus den Arbeiten entstandenen Publikationen auf der sog. KORA-Studie („Kooperative Gesundheitsforschung in der Region Augsburg“), einer prospektiven bevölkerungsbasierten Kohortenstudie im Gebiet der Stadt Augsburg und der beiden anliegenden Landkreise, in welcher u.a. die umweltbedingten Ursachen von NCDs wie Diabetes und Herz-Kreislauf-Erkrankungen bei Erwachsenen untersucht werden mit dem Ziel, daraus für die öffentliche Gesundheit relevante Präventionsstrategien abzuleiten [24]. In den Jahren 1984 bis 2001 sind vier Ausgangsstudien (S1-S4) durchgeführt worden, welchen sich mehrere Follow-Up-Studien angeschlossen haben [25]. Die für unsere Studien analysierten Daten stammen aus der sog. KORA-Fit-(S4)-Studie, welche die S4-Teilnehmer der KORA-Fit Follow-Up-Studie mit insgesamt 856 Teilnehmern einschließt.

Das Ziel der ersten Veröffentlichung lag darin zu erforschen, inwieweit die bei Teilnehmern gemessenen Blutgerinnungsparameter mit der Aufnahme spezifischer Lebensmittel assoziiert sind, um den möglichen Einfluss von Ernährungsgewohnheiten auf die Blutgerinnung zu entschlüsseln. Dadurch könnte hinsichtlich dieses Zusammenhangs die Studienlage ergänzt werden, um mögliche präventive Strategien und Ernährungsempfehlungen zu entwickeln, um blutgerinnungsbedingte Erkrankungen auf Bevölkerungsebene vorzubeugen. Ähnliche Ziele wurden ebenfalls für die zweite Veröffentlichung beschrieben. Hier lag der Schwerpunkt darin, die Effekte des Konsums bestimmter Lebensmittel, Nährstoffe und ausgewählter Ernährungsmuster auf die Fettleber, quantifiziert durch den FLI, zu analysieren (Abb. 3). Zusätzlich dazu war ein weiteres Ziel, die Rolle des Metabotypen bei dem Zusammenhang zwischen Ernährung und Fettleber aufzuzeigen. Dadurch würde ebenfalls die bisherige Studienlage hinsichtlich

dieser Thematik sinnvoll ergänzt werden und zu einem individuelleren Ansatz bei ernährungsbezogenen Präventivmaßnahmen beigetragen werden.

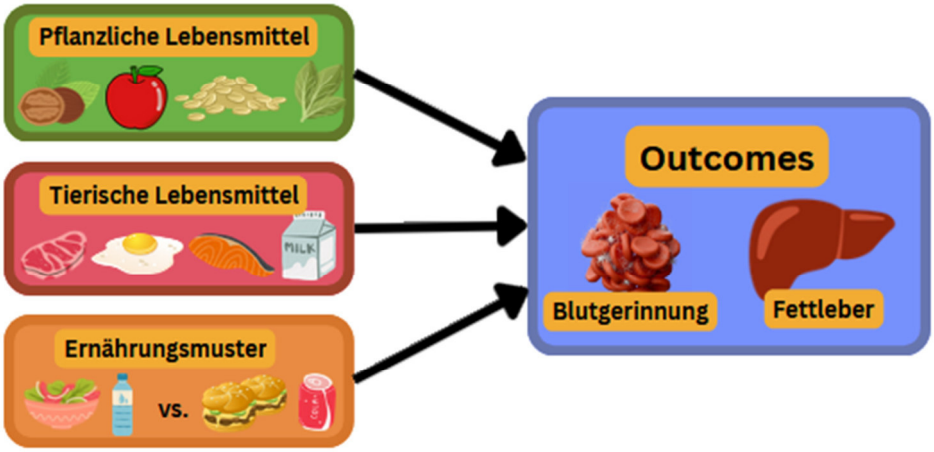



**Abbildung 3**. Untersuchung der Auswirkungen von Lebensmittelgruppen und Ernährungsmustern auf die Blutgerinnung und Fettleber als Hauptzielsetzung

## 1.2 Eigenbeitrag zu den Veröffentlichungen

In der ersten Publikation wurden auf der Basis einer umfangreichen Recherche der wissenschaftlichen Datenlage mit meinem Doktorvater für eine Publikation geeignete Arbeitshypothesen aufgestellt und ausgearbeitet. Im Anschluss habe ich anhand des vorliegenden Datensatzes der KORA-S4-Fit-Teilnehmer je nach Arbeitshypothese statistisch ausgewertet, interpretiert und auf Plausibilität geprüft. Für die Datenanalyse der Assoziationen spezifischer Lebensmittel mit verschiedenen Blutgerinnungsparametern sind multiple lineare Regressionsmodelle gebildet worden, um zusätzlich für Störvariablen wie dem Rauchverhalten, dem Alter der Studienteilnehmer oder der Gesamtkalorienaufnahme zu adjustieren. Die statistischen Analysen wurden mit Hilfe der Software R (R-Version 4.3.1) durchgeführt, unterstützt durch einen Statistiker des Lehrstuhls für Epidemiologie. Anschließend habe ich das Manuskript angefertigt und nach einem Review-Prozess durch die Co-Autoren das Manuskript bei dem Fachjournal Nutrients eingereicht. Durch meine Rolle als Erstautor und korrespondierender Autor war ich zusätzlich für die Beantwortung von Rückfragen und Anregungen für Überarbeitungen seitens des Journals und der Gutachter verantwortlich. Mein eigener Arbeitsanteil an der Publikation betrug durch die statistische Analyse, Dateninterpretation und Erstellung sowie Überarbeitung des Manuskripts ca. 65%; der Anteil von Prof. Jakob Linseisen betrug durch das Design der Studie, und als Supervisor der Arbeit ca. 15%; der

Anteil von Prof. Christine Meisinger betrug durch die Mitbeteiligung am Studiendesign, der Dateninterpretation und Überarbeitung des Manuskripts ca. 4%; der Anteil von Dr. Dennis Freuer betrug als Supervisor der statistischen Auswertung ca. 4%; die Anteile von Prof. Annette Peters und Dr. Margit Heier betrugen jeweils ca. 4% als Verantwortliche für das Design und die Durchführung der KORA-Studie; der Anteil von Prof. Daniel Teupser betrug ebenfalls ca. 4% als Hauptverantwortlicher für die Laboranalysen.

In der zweiten Publikation wurden ebenfalls auf der Basis einer umfangreichen Sichtung der Fachliteratur mit Prof. Jakob Linseisen erneut Arbeitshypothesen erarbeitet und anschließend bin ich mit der statistischen Auswertung des Datensatzes der KORA-S4-Fit-Teilnehmer fortgefahren. Im Anschluss habe ich die Daten interpretiert und auf Plausibilität geprüft. Hierbei sind multiple lineare Regressionsmodelle erstellt worden, um die Assoziationen spezifischer Lebensmittel sowie Ernährungsmuster mit dem Fettleberindex zu untersuchen. Dies geschah ebenfalls mit dem Statistik-Programm R (R-Version 4.3.1) und wurde unterstützt von Dr. Freuer. Nach der Vervollständigung des Manuskripts und dem Review-Prozess durch die Co-Autoren habe ich das Manuskript bei dem Fachjournal Lipids in Health and Disease eingereicht. Durch meine Funktion als Erstautor und korrespondierender Autor war ich erneut für die Beantwortung von Rückfragen und Anregungen für Überarbeitungen seitens des Journals oder der Peer Reviewer zuständig. Mein eigener Arbeitsanteil an der Publikation betrug durch die statistische Analyse, Dateninterpretation und Erstellung sowie Überarbeitung des Manuskripts ca. 65%; der Anteil von Prof. Jakob Linseisen betrug durch das Design der Studie sowie als Verantwortlicher für die Ernährungsanalysen und Ernährungserhebung sowie als Supervisor der Arbeit ca. 11%; der Anteil von Prof. Christine Meisinger betrug durch die Mitbeteiligung am Studiendesign, der Dateninterpretation und Überarbeitung des Manuskripts ca. 4%; der Anteil von Dr. Daniel Freuer betrug als Supervisor der statistischen Auswertung ca. 4%; der Anteil von Dr. Nina Wawro betrug ca. 4% durch die Berechnung der Daten zur Nahrungsaufnahme der Studienteilnehmer; die Anteile von Prof. Annette Peters und Dr. Margit Heier betrugen jeweils ca. 4% als Verantwortliche für das Design und die Durchführung der KORA-Studie; der Anteil von Prof. Daniel Teupser betrug ebenfalls ca. 4% als Hauptverantwortlicher für die Laboranalysen.

# 2. Manuskript I

Schepp, M.; Freuer, D.; Peters, A.; Heier, M.; Teupser, D.; Meisinger, C.; Linseisen, J. Is the Habitual Dietary Intake of Foods of Plant or Animal Origin Associated with Circulating Hemostatic Factors?-Results of the Population-Based KORA-Fit Study. Nutrients **2024**, 16, doi:10.3390/nu16030432.

*nutrients*

MDPI

*Article*

# Is the Habitual Dietary Intake of Foods of Plant or Animal Origin Associated with Circulating Hemostatic Factors?—Results of the Population-Based KORA-Fit Study

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**Abstract:** Blood coagulation is a complex physiological process critical for maintaining hemostasis, and disruptions in this system can lead to various health complications. Since the effects of specific food groups on a series of circulating coagulation parameters in the population are not well established, this study examines such associations in the population-based KORA-Fit study. A total of 595 subjects (263 men and 332 women) born between 1945 and 1964 and living in the study region of Augsburg were included in the study. Habitual food intake was estimated based on a combination of repeated 24-h food lists (24HFLs) and a food frequency questionnaire (FFQ). Antithrombin III, D-dimers, factor VIII, fibrinogen, protein C, protein S, aPTT, Quick value and INR were measured in citrate plasma. Multivariable linear regression models were applied to investigate associations between the consumption of specific foods of plant or animal origin and hemostatic factors. We found that the consumption of plant-based food groups, including green leafy vegetables (rich in vitamin K1), were hardly associated with coagulation parameters. Surprisingly, a high consumption of dairy products and especially butter were associated with higher D-dimer concentrations. These findings need further evaluation in prospective studies.

**Keywords:** nutrition; diet; coagulation; hemostatic factors; food groups; KORA

**Citation:** Schepp, M.; Freuer, D.; Peters, A.; Heier, M.; Teupser, D.; Meisinger, C.; Linseisen, J. Is the Habitual Dietary Intake of Foods of Plant or Animal Origin Associated with Circulating Hemostatic Factors?—Results of the Population-Based KORA-Fit Study. *Nutrients* **2024**, *16*, 432. https://doi.org/10.3390/nu16030432

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## 1. Introduction

According to the WHO, non-communicable diseases are the main cause of deaths worldwide and are responsible for the death of around 41 million people per year [1]. Eighty percent of these cases are caused by chronic conditions like cancer, diabetes, respiratory and cardiovascular diseases (CVD) which are often affected by lifestyle factors such as smoking, physical inactivity or an unhealthy diet [2]. Especially the importance of a healthy eating behavior has emerged in the past years as an effective primary prevention strategy for several of the mentioned diseases. For example, a comprehensive systematic review and meta-analysis of 95 prospective studies has reported an inverse dose-response relationship between fruit and vegetable consumption and cardiovascular

disease, total cancer and all-cause mortality [3]. Also, adherence to favorable dietary patterns, such as the Mediterranean Diet Score (MDS), which is characterized by a high intake of plant-based food items like fruit and vegetables, whole grains, and nuts but also a moderate number of animal-derived foods such as fish, has been shown to yield protective effects regarding CVD, cancer and the metabolic syndrome in several trials [4].

The reduction of CVD risk by following a Mediterranean-style diet is thought to be mainly mediated by its bioactive compounds which have antioxidant, anti-inflammatory and, interestingly, also anti-thrombotic properties [5,6]. There is also evidence that the risk for thrombosis is mediated by pro-inflammatory processes in the human body [7] but less is known about the specific effects on coagulation parameters which play a critical role in maintaining hemostasis and preventing excessive bleeding or coagulation (thrombosis), [8]. Therefore, the influence of habitual food consumption on these parameters is an area that warrants more investigation.

Indeed, positive effects of some specific nutrients like long-chain omega-3-fatty acids derived mainly from fish and seafood, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), on different hemostatic parameters have been observed in large cross-sectional studies [9,10] as well as in controlled intervention studies [11,12] that also may explain some of the beneficial effects of the MDS on thrombosis risk. On the other hand, the effect on coagulation parameters of other antioxidant-rich, plant-based diets such as a vegetarian diet pattern showed more mixed results [13,14] and it seems that the effects on hemostasis are more influenced by specific food choices rather than the specific dietary pattern [14]. While some studies examined the outcomes of different dietary patterns or single nutrients on coagulation parameters [14,15], to our knowledge, consumption of foods, differentiating between plant- and animal-derived food items, has not been investigated comprehensively yet at the population-based level.

Therefore, the present study aims to uncover associations between habitual food consumption with several coagulation factors.

## 2. Materials and Methods

### *2.1. Study Sample*

The study sample of our project is part of the KORA S4 Fit survey. KORA (Cooperative Health Research in the Region of Augsburg) is the continuation of the WHO MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) project which was conducted by the WHO from 1984 to 1995 [16]. In 1996, the KORA study was established by the Helmholtz Center Munich—National Research Center for Environment and Health and is a prospective population-based adult cohort study in the region of Augsburg, Germany [16].

Over the time course, a total of four cross-sectional baseline surveys from 1984 to 2001 (S1 to S4) were initiated with 18,000 participants being randomly selected and follow-up studies were conducted in intervals ranging from 4 to 20 years [17]. The KORA S4 survey included a sample of 4261 inhabitants of the study region (city of Augsburg and two surrounding counties) aged 25 to 74 years and was performed from 1999 to 2001. KORA-Fit is a follow-up study conducted from 2018 to 2019 with 3059 subjects from the S1–S4 cross-sectional surveys born between 1945 and 1964 [17].

The ethical release was provided by the Ethics Committee of the Bavarian Medical Association (Bayerische Landesärztekammer). All study participants gave written informed consent, and the study was conducted in accordance with the Declaration of Helsinki [16].

Our study sample originally consisted of 624 subjects of the S4 part of KORA-Fit who had available food intake data and blood plasma measurements of hemostatic parameters. After the exclusion of participants that were currently receiving anticoagulative treatment (n = 29), a total of 595 subjects (263 men and 332 women) remained for the analysis.

*2.2. Measurements of Exposures*

For anthropometric measurements, participants were encouraged to remove heavy clothing and shoes to determine their body weight and height, according to the WHO MONICA protocol [18]. Waist circumference was measured between the distance of the lower rib margin and the iliac crest [19]. The body mass index (BMI) of the subjects was calculated by dividing the weight (in kg) by the square of the height (in m). Other exposure variables such as smoking (current/former/never), education years (more or less than 12 years), physical activity (estimated time per week spent on sports activities during leisure time in summer and winter), and medication use were assessed during a standardized face-to-face interview by instructed medical personnel. Subjects were defined as having diabetes if they got a medical diagnosis and/or were under antidiabetic medication. Similarly, to be classified in the hypertension category, participants had a baseline blood pressure of systolic ≥ 140 or diastolic ≥ 90 mmHg or received an antihypertensive medical treatment given previously diagnosed with hypertension [18].

The usual dietary intake was assessed using repeated 24-h food lists (24HFLs) and a food frequency questionnaire (FFQ) asking about the habitual diet over the past 12 months which is based on the self-administered German version of the European Food Propensity Questionnaire (EFPQ) [20,21]. By combining both data sets as described previously by Mitry et al. [20] the habitual consumption of food items was estimated in g/day. Food items were summarized into food groups and subgroups; the analysis focused on foods of plant and animal origin including total fruits, total vegetables, green leafy vegetables (as a subgroup of vegetables), total meat, total fish, total eggs, and dairy products (w/o butter), cheese (as a subgroup of dairy products) and butter. As confounding variables, we used the estimated intake of alcohol (in grams per day) and total energy (in kilocalories per day).

In our study we assessed selected hemostatic parameters that exert different functions in the pathways of the coagulation cascade. Factor VIII is part of the intrinsic pathway whereas in the common pathway, fibrinogen is converted into fibrin strands by thrombin (factor II) which then finally get cross-linked, catalyzed by factor XIII [8]. The activation of the intrinsic pathway can be overall quantified by the aPTT which gets lowered in a pro-coagulant state while the quick value measures how the extrinsic pathway is affected and is inversely correlated with clotting tendency [22]. In the scientific literature, often the prothrombin time is used instead of the quick value to analyze the extrinsic pathway. Also, the INR value is another method to determine the activation of the extrinsic pathway in a more standardized manner and gets elevated in a pro-thrombogenic environment [22]. Antithrombin III is anti-thrombogenic molecule that works as a protease inhibitor and mainly exhibits its effect by the inactivation of thrombin. Proteins C and S are vitamin K-dependent factors that also work as inhibitors of the coagulation cascade. Both are synthesized by the liver where also most of the other factors of the coagulation system were produced [22]. D-dimers are degradation products of cross-linked fibrin and are widely recognized as a biomarker for assessing the activation of the coagulation system [23,24].

Measurements of standard clinical parameters were conducted in the central laboratory of Ludwig-Maximilians-University Munich, while hemostatic variables were measured in citrate plasma in the central lab of the University Hospital Augsburg by instructed laboratory staff, and reference values were derived from the laboratory reference sheets [25].

Non-HDL-cholesterol (non-HDLc) was defined as the difference between total cholesterol and high-density lipoprotein cholesterol (HDLc). Fatty liver index (FLI) was calculated by using the FLI formula previously described by Bedogni et al. which includes laboratory measurements of triglycerides (TG) and gamma-glutamyl transferase (GGT) [26].

The variables and methods regarding the laboratory measurements are described in Table 1 [25,27].

**Table 1.** Overview of measured laboratory variables.

| Laboratory Parameter | Method | Testing Device | Reference Value |
|---|---|---|---|
| Adjustment variables | | | |
| Total cholesterol (TC) | Enzymatic method | Cobas 8000 c702 Roche chemistry analyzer, Hoffman-La Roche AG Basel | <200 mg/dL |
| High-density lipoprotein cholesterol (HDLc) | Enzymatic method | | >45 mg/dL |
| Triglycerides (TG) | Enzymatic method | | <200 mg/dL |
| Gamma-glutamyl transferase (GGT) | IFCC method [a] | | Males: <60 U/L Females: <40 U/L |
| Hemostatic variables (dependent variables) | | | |
| Antithrombin III | Chromogenic activity assay | Innovance Antithrombin, SCS cleaner, Siemens Eschborn | 83–118% |
| D-dimers | Particle-enhanced immunoturbidimetric assay | Innovance D-Dimer Kit, Siemens Eschborn | <500 µg/L |
| Factor VIII | Photometry | Coagulation factor VIII deficient plasma, Pathromtin SL, $CaCl_2$, Siemens Eschborn | 70–150% |
| Fibrinogen | Photometry and turbidimetry | Multifibern U, Siemens Eschborn | 210–400 mg/dL |
| Protein C | Photometry | Berichrom Protein C, Siemens Healthcare | 70–140% |
| Protein S | Photometry | Hemoclot Protein S, OVB buffer, $CaCl_2$, SCS cleaner | Males: 73–130% Females: 52–126% |
| Activated partial thromboplastin time (aPTT) | Photometry | Pathromtin SL, $CaCl_2$ solution, Actin FS, Siemens Eschborn | 26–36 s |
| Quick value | Photometry | Thromborel S, Siemens Eschborn | 82–125% |
| International thromboplastin time (INR) | Prothrombin ratio (calculated) [b] | | 0.9–1.15 |
| High-sensitivity C-reactive Protein (hs-CRP) | High-sensitivity latex-enhanced nephelometric assay | BN II System analyzer, Dade Behring | <3 mg/L [c] |

[a] according to [28]; [b] according to [29]; [c] values below 1.00 mg/L were rounded down to 0.35 mg/L.

### *2.3. Statistical Analyses*

Statistical analyses were performed by using the R-software (R version 4.3.1). The Shapiro–Wilk test was used to test for normal distribution. Because the continuous parameters were not normally distributed, these variables are described by its median and 25th–75th percentile range. All categorial variables were described as absolute or relative frequencies. To analyze sex differences for continuous variables the Mann–Whitney U test was applied, while for categorial variables Fisher's exact test was utilized. A $p$-value < 0.05 was defined as statistically significant.

In the linear regression models the coagulation parameters were used as the dependent variables and food consumption data as independent variables. The ß-estimate describes the effect size of each parameter by displaying the estimated change of the dependent variable when the independent variable changes by one unit. A positive or negative sign of the ß-estimate indicates the direction of the association. To control for possible confounding variables, each model was adjusted for sex, age, physical activity, education years, smoking, diabetes, hypertension, total energy intake, alcohol consumption, non-HDLc, and BMI. By using the Variance-Inflation-Factor (VIF) we observed significant multicollinearity for two of the adjustment variables, BMI and FLI; thus, in sensitivity analyses, BMI was replaced by FLI. To secure all necessary model assumptions we tested for autocorrelation, heteroscedasticity, normality, linearity and removed extreme outliers to create more precise models. We tested for interaction effects by sex. We took into account multiple testing by using the Benjamini–Hochberg False

Discovery Rate (FDR) method and calculated adjusted *p*-values. Finally, the significant models were additionally adjusted for hs-CRP to investigate if inflammatory conditions may have contributed to the observed effects.

## 3. Results

Table 2 shows the characteristics of the male and female participants. There were significant sex differences for BMI, waist circumference, total energy intake, alcohol consumption, FLI, CRP, education years, hypertension and diabetes regarding men (versus women) showing higher values in all mentioned variables, except for education years.

**Table 2.** Characteristics of the study participants.

| | **Total** | **Males** | **Females** | |
|---|---|---|---|---|
| | **n = 595** | **n = 263** | **n = 332** | |
| **Characteristics** | **Median (25th and 75th Percentiles)** | | | ***p*-Value** |
| Age [years] | 63 (58; 68) | 64 (58; 68) | 63 (59; 68) | 0.842 |
| BMI [kg/m²] | 27.12 (23.98; 30.32) | 27.78 (25.33; 30.67) | 26.4 (23.3; 29.77) | <0.001 * |
| Waist circumference [cm] | 93.1 (82.5; 102.35) | 99.4 (91.75; 107.5) | 86.05 (78; 96.32) | <0.001 * |
| Energy (kilocalories) [kcal/d] | 1723.6 (1487.4; 2052.25) | 2049.9 (1809.9; 2298.1) | 1532.85 (1380.55; 1704.88) | <0.001 * |
| Alcohol consumption [g/d] | 5.17 (2.19; 14.19) | 14.71 (6.77; 25.94) | 2.59 (1.59; 5.02) | <0.001 * |
| Non-HDL [mg/dL] | 146.2 (121; 171.84) | 145 (115; 172.59) | 147.28 (124.83; 170.56) | 0.384 |
| FLI | 51.88 (23.19; 88.33) | 79.85 (42.88; 96.59) | 34.28 (15.67; 72.6) | <0.001 * |
| hs-CRP [mg/L] | 1 (1; 3) | 1 (1; 2.5) | 1 (1; 3) | 0.25 |
| | | **n (%)** | | |
| Education [years] | | | | |
| ≤12 years | 359 (60.34%) | 144 (54.75%) | 215 (64.76%) | <0.001 * |
| >12 years | 236 (39.66%) | 119 (45.25%) | 117 (35.24%) | |
| Physical activity | | | | |
| ≥2 h/week | 229 (38.49%) | 103 (39.16%) | 126 (37.95%) | 0.867 |
| 1 h/week | 198 (33.28%) | 84 (31.94%) | 114 (34.34%) | |
| <1 h/week | 71 (11.93%) | 34 (12.93%) | 37 (11.14%) | |
| (almost) no activity | 97 (16.30%) | 42 (15.97%) | 55 (16.57%) | |
| Smoking | | | | |
| Current smoker | 72 (12.10%) | 33 (12.55%) | 39 (11.75%) | 0.066 |
| Former smoker | 259 (43.53%) | 127 (48.29%) | 132 (39.76%) | |
| Never smoker | 264 (44.37%) | 103 (39.16%) | 161 (48.49%) | |
| Hypertension | | | | |
| Yes | 272 (45.71%) | 147 (55.89%) | 125 (37.65%) | <0.001 * |
| No | 323 (54.29%) | 116 (44.11%) | 207 (62.35%) | |
| Diabetes | | | | |
| Yes | 43 (7.23%) | 20 (7.65%) | 23 (6.93%) | 0.753 |
| No | 552 (92.77%) | 243 (92.4%) | 309 (93.07%) | |
| BMI | | | | |
| <18.5 | 3 (0.50%) | 0 (0.00%) | 3 (0.90%) | <0.001 * |
| 18.5–24.9 | 186 (31.26%) | 61 (23.19%) | 125 (37.65%) | |
| 25–29.9 | 246 (41.34%) | 122 (46.39%) | 124 (37.35%) | |
| >30 | 160 (26.89%) | 80 (30.41%) | 80 (24.10%) | |

* $p < 0.05$.

Table 3 sums up the plasma concentrations of blood coagulation factors of the study participants. For male participants, significantly lower median values for antithrombin III, protein C and Quick value levels were observed as compared to women. On the opposite, in women significantly higher median levels for protein S and INR were noted. All median values were within the laboratory reference ranges. Furthermore, there was a large proportion of participants (n = 190) with D-dimer values above the reference range (≥500 µg/L). The exact numbers of participants with measurements outside the reference range of coagulation factors can be found in the Supplementary Materials (Table S1).

**Table 3.** Plasma concentrations of blood coagulation factors in all participants and by sex.

| | **Total** | **Males** | **Females** | |
|---|---|---|---|---|
| | **n = 595** | **n = 263** | **n = 332** | |
| **Coagulation Factors** | **Median (25th and 75th Percentiles)** | | | ***p*-Value** |
| Antithrombin III [mg/dL] | 102.9 (96.15; 109) | 98.8 (93.35; 105.55) | 105.2 (99.1; 110.8) | <0.001 * |
| D-dimers [µg/L] | 403 (305; 544.5) | 405 (315.5; 561) | 402 (301.25; 532.25) | 0.407 |
| Factor VIII [%] | 120.3 (96.6; 143.1) | 118.4 (93.95; 140.2) | 123.5 (99.92; 145.02) | 0.13 |
| Fibrinogen [mg/dL] | 293.5 (261.05; 328.7) | 285.7 (258.95; 322.1) | 299.15 (262.92; 332.72) | 0.054 |
| Protein C [%] | 124.2 (111.45; 139.45) | 117.5 (109.2; 131.7) | 129.1 (116.12; 142.65) | <0.001 * |
| Protein S [%] | 125.1 (105.6; 145.7) | 131.7 (112.05; 157.7) | 119.4 (101.3; 137.62) | <0.001 * |
| aPTT [s] | 30.7 (28.7; 32.9) | 31.1 (29.4; 33.35) | 30.3 (28.5; 32.7) | 0.004 |
| Quick value [%] | 108.7 (102.15; 114.9) | 106.7 (100.25; 112.4) | 110.45 (104.08; 115.82) | <0.001 * |
| INR | 0.96 (0.92; 1) | 0.97 (0.93; 1.01) | 0.94 (0.91; 0.98) | <0.001 * |

* $p < 0.05$.

For men, a higher consumption of foods of animal origin, including meat, fish and butter was observed, whereas for women a higher consumption of fruits, vegetables and dairy products was found (Table 4).

**Table 4.** Habitual food consumption in all participants and by sex.

| | **Total** | **Males** | **Females** | |
|---|---|---|---|---|
| | **n = 595** | **n = 263** | **n = 332** | |
| **Food Groups [g/d]** | **Median (25th and 75th Percentiles)** | | | ***p*-Value** |
| Total fruits | 144.8 (88.15; 213.55) | 134.2 (75; 211.6) | 150.3 (102.97; 216.5) | 0.008 |
| Total vegetables | 163.9 (134.5; 200.65) | 147.4 (122.6; 178.05) | 178.4 (148.7; 217.5) | <0.001 * |
| Green leafy vegetables | 23.9 (17.5; 32.35) | 24.4 (18.15; 32.35) | 23.8 (16.7; 32.2) | 0.085 |
| Total meat | 99 (73.5; 131.9) | 133.7 (111.05; 160.05) | 77.6 (64.57; 94.95) | <0.001 * |
| Total fish | 18.2 (12.35; 25.9) | 19 (13.65; 27) | 17.35 (11.67; 24.95) | 0.001 |

| | | | | |
|---|---|---|---|---|
| Total eggs | 15.7 (11.3; 22.2) | 15.9 (11.6; 22.7) | 14.6 (11.07; 21.63) | 0.301 |
| Dairy products (w/o butter) | 179.2 (120.85; 260) | 154.4 (103.8; 228.9) | 200.4 (135.2; 281.33) | <0.001 * |
| Cheese | 27.8 (19.3; 37.5) | 29 (19.65; 41.4) | 26.85 (19.17; 35.8) | 0.062 |
| Butter | 14.3 (8.95; 17.1) | 16.2 (10.35; 21.5) | 12.65 (7.68; 15.43) | <0.001 * |

* $p < 0.05$.

Tables 5–7 show the associations between the food group variables as independent factors and the blood coagulation parameters as dependent variables. Statistically significant positive associations for total fruit intake and aPTT ($p = 0.036$), dairy product intake and D-dimers ($p = 0.032$), butter intake and D-dimers ($p < 0.001$) as well as butter intake and protein C ($p = 0.047$) were observed. Inverse associations were shown for dairy products and antithrombin III ($p = 0.010$) and protein C ($p = 0.001$), respectively.

After correction for multiple testing only the associations between butter consumption and D-dimers ($p < 0.001$) and dairy product intake and protein C ($p = 0.040$) remained statistically significant. To identify a possible effect of inflammation on D-dimers or protein C, we additionally adjusted for hs-CRP in these two models. Still, statistical significance was observed for the butter and D-Pdimer model ($p = 0.016$) as well as for the dairy product and protein C model ($p = 0.017$). With the adjustment for FLI replacing BMI similar results were observed; however, after FDR correction, none of the associations remained statistically significant (Supplementary Materials, Tables S2–S4).

Additionally, because of a large proportion of participants with elevated D-dimer values [>500 µg/l], we stratified the study subjects into two subgroups depending on their D-dimer levels and created two new linear regression models investigating the association between D-dimers and butter intake [g/d]. After transforming the ß-estimates, for the subgroup with D-dimers within the reference value (n = 405) we observed a ß-estimate of 1.634 (95% CI 0.992; 2.694) with a *p*-value of 0.054. For the subgroup with elevated D-dimers (n = 190) the transformed ß-estimate was 0.842 (95% CI 0.246; 2.877) and the *p*-value was 0.783.

**Table 5.** Association between habitual consumption of fruits, vegetables and green leafy vegetables [100 g/d] and blood coagulation parameters (dependent variables) [a].

| | **ß-Estimate** | **95% CI** | ***p*-Value** | **FDR Adjusted *p*-Value** |
|---|---|---|---|---|
| Total fruit consumption [per 100 g/d] | | | | |
| Antithrombin III [mg/dL] | 0.125 | −0.943; 1.192 | 0.819 | 1 |
| Ln D-dimers [µg/L] | 0.033 | −0.022; 0.088 | 0.238 | 0.916 |
| Ln Factor VIII [%] | −0.001 | −0.034; 0.032 | 0.949 | 1 |
| Ln Fibrinogen D [mg/dL] | −0.004 | −0.023; 0.016 | 0.726 | 1 |
| Protein C [%] | 0.559 | −1.305; 2.424 | 0.556 | 1 |
| Ln Protein S [%] | −0.007 | −0.034; 0.019 | 0.586 | 1 |
| aPTT [s] | 0.387 | 0.024; 0.75 | 0.036 [b] | 0.583 |
| Quick value [%] | 0.171 | −0.832; 1.173 | 0.738 | 1 |
| INR | −0.001 | −0.007; 0.005 | 0.743 | 1 |
| Total vegetable consumption [per 100 g/d] | | | | |
| Antithrombin III [mg/dL] | −0.98 | −2.676; 0.717 | 0.257 | 0.916 |
| Ln D-dimers [µg/L] | 0.023 | −0.066; 0.113 | 0.609 | 1 |
| Ln Factor VIII [%] | −0.005 | −0.057; 0.047 | 0.856 | 1 |
| Ln Fibrinogen D [mg/dL] | −0.012 | −0.044; 0.02 | 0.47 | 1 |

| | | | | |
|---|---|---|---|---|
| Protein C [%] | −1.387 | −4.357; 1.583 | 0.36 | 1 |
| Ln Protein S [%] | −0.005 | −0.048; 0.038 | 0.822 | 1 |
| aPTT [s] | −0.302 | −0.889; 0.284 | 0.312 | 0.972 |
| Quick value [%] | 0.03 | −1.568; 1.628 | 0.97 | 1 |
| INR | 0 | −0.01; 0.009 | 0.996 | 1 |
| Green leafy vegetables consumption [per 100 g/d] | | | | |
| Antithrombin III [mg/dL] | −2.192 | −9.639; 5.254 | 0.563 | 1 |
| Ln D-dimers [µg/L] | −0.045 | −0.438; 0.349 | 0.823 | 1 |
| Ln Factor VIII [%] | −0.131 | −0.359; 0.097 | 0.26 | 0.916 |
| Ln Fibrinogen D [mg/dL] | −0.09 | −0.23; 0.05 | 0.209 | 0.916 |
| Protein C [%] | −0.835 | −13.861; 12.191 | 0.9 | 1 |
| Ln Protein S [%] | −0.164 | −0.352; 0.025 | 0.089 | 0.714 |
| aPTT [s] | −0.705 | −3.299; 1.888 | 0.594 | 1 |
| Quick value [%] | 0.826 | −6.255; 7.906 | 0.819 | 1 |
| INR | −0.005 | −0.047; 0.037 | 0.807 | 1 |

[a] linear regression models adjusted for sex, age, physical activity, education years, smoking status, diabetes, hypertension, calorie intake, alcohol consumption, non-HDL cholesterol and BMI. CI, confidence interval; FDR, false discovery rate; [b] $p < 0.05$.

**Table 6.** Association between habitual consumption of foods of animal origin [100 g/d] and blood coagulation parameters (dependent variables) [a].

| | **ß-Estimate** | **95% CI** | ***p*-Value** | **FDR Adjusted *p*-Value** |
|---|---|---|---|---|
| Total meat consumption [per 100 g/d] | | | | |
| Antithrombin III [mg/dL] | 2.838 | −0.466; 6.143 | 0.092 | 0.714 |
| Ln D-dimers [µg/L] | −0.074 | −0.25; 0.102 | 0.41 | 1 |
| Ln Factor VIII [%] | 0.014 | −0.086; 0.114 | 0.785 | 1 |
| Ln Fibrinogen D [mg/dL] | 0.014 | −0.048; 0.077 | 0.65 | 1 |
| Protein C [%] | 1.745 | −4.047; 7.537 | 0.554 | 1 |
| Ln Protein S [%] | −0.024 | −0.109; 0.06 | 0.568 | 1 |
| aPTT [s] | 0.075 | −1.067; 1.218 | 0.897 | 1 |
| Quick value [%] | −1.943 | −5.089; 1.203 | 0.226 | 0.916 |
| INR | 0.011 | −0.007; 0.03 | 0.235 | 0.916 |
| Total fish consumption [per 100 g/d] | | | | |
| Antithrombin III [mg/dL] | −3.373 | −8.922; 2.176 | 0.233 | 0.916 |
| Ln D-dimers [µg/L] | −0.038 | −0.337; 0.26 | 0.8 | 1 |
| Ln Factor VIII [%] | −0.011 | −0.18; 0.157 | 0.895 | 1 |
| Ln Fibrinogen D [mg/dL] | −0.061 | −0.166; 0.044 | 0.253 | 0.916 |
| Protein C [%] | −0.124 | −9.845; 9.598 | 0.98 | 1 |
| Ln Protein S [%] | −0.047 | −0.188; 0.094 | 0.514 | 1 |
| aPTT [s] | −0.251 | −2.187; 1.685 | 0.799 | 1 |
| Quick value [%] | −0.041 | −5.328; 5.246 | 0.988 | 1 |
| INR | 0 | −0.031; 0.032 | 0.976 | 1 |
| Total egg consumption [per 100 g/d] | | | | |
| Antithrombin III [mg/dL] | 5.191 | −1.551; 11.933 | 0.131 | 0.884 |
| Ln D-dimers [µg/L] | 0.018 | −0.339; 0.375 | 0.92 | 1 |
| Ln Factor VIII [%] | 0.039 | −0.168; 0.246 | 0.711 | 1 |
| Ln Fibrinogen D [mg/dL] | −0.022 | −0.151; 0.107 | 0.739 | 1 |
| Protein C [%] | 3.827 | −7.978; 15.633 | 0.525 | 1 |
| Ln Protein S [%] | −0.002 | −0.173; 0.169 | 0.985 | 1 |
| aPTT [s] | −1.265 | −3.624; 1.094 | 0.293 | 0.972 |
| Quick value [%] | 1.142 | −5.261; 7.544 | 0.726 | 1 |
| INR | −0.007 | −0.045; 0.032 | 0.734 | 1 |

[a] linear regression models adjusted for sex, age, physical activity, education years, smoking status, diabetes, hypertension, calorie intake, alcohol consumption, non-HDL cholesterol and BMI. CI, confidence interval; FDR, false discovery rate.

**Table 7.** Association between habitual consumption of dairy products (w/o butter), cheese and butter [100 g/d] and blood coagulation parameters (dependent variables) [a].

| | ß-Estimate | 95% CI | *p*-Value | FDR Adjusted *p*-Value |
|---|---|---|---|---|
| Dairy products (w/o butter) consumption [per 100 g/d] | | | | |
| Antithrombin III [mg/dL] | −1.132 | −1.99; −0.275 | 0.01 [b] | 0.27 |
| Ln D-dimers [µg/L] | 0.049 | 0.004; 0.094 | 0.032 [b] | 0.583 |
| Ln Factor VIII [%] | 0.003 | −0.023; 0.029 | 0.808 | 1 |
| Ln Fibrinogen D [mg/dL] | 0.002 | −0.014; 0.018 | 0.782 | 1 |
| Protein C [%] | −2.644 | −4.137; −1.152 | 0.001 [b] | 0.04 [b] |
| Ln Protein S [%] | 0.015 | −0.007; 0.036 | 0.19 | 0.916 |
| aPTT [s] | 0.029 | −0.265; 0.324 | 0.845 | 1 |
| Quick value [%] | −0.083 | −0.896; 0.73 | 0.841 | 1 |
| INR | 0.001 | −0.004; 0.005 | 0.791 | 1 |
| Cheese consumption [per 100 g/d] | | | | |
| Antithrombin III [mg/dL] | 1.359 | −4.932; 7.649 | 0.672 | 1 |
| Ln D-dimers [µg/L] | 0.173 | −0.16; 0.507 | 0.308 | 0.972 |
| Ln Factor VIII [%] | 0.069 | −0.122; 0.26 | 0.478 | 1 |
| Ln Fibrinogen D [mg/dL] | −0.016 | −0.135; 0.103 | 0.786 | 1 |
| Protein C [%] | 2.31 | −8.705; 13.324 | 0.681 | 1 |
| Ln Protein S [%] | −0.115 | −0.274; 0.045 | 0.159 | 0.916 |
| aPTT [s] | 0.438 | −1.758; 2.633 | 0.695 | 1 |
| Quick value [%] | 0.001 | −5.913; 5.915 | 1 | 1 |
| INR | −0.001 | −0.037; 0.034 | 0.94 | 1 |
| Butter consumption [per 100 g/d] | | | | |
| Antithrombin III [mg/dL] | 8.939 | −5.68; 23.558 | 0.23 | 0.916 |
| Ln D-dimers [µg/L] | 1.429 | 0.669; 2.19 | <0.001 [b] | <0.001 [b] |
| Ln Factor VIII [%] | 0.145 | −0.304; 0.595 | 0.526 | 1 |
| Ln Fibrinogen D [mg/dL] | 0.231 | −0.042; 0.504 | 0.097 | 0.714 |
| Protein C [%] | 25.909 | 0.367; 51.451 | 0.047 [b] | 0.59 |
| Ln Protein S [%] | −0.111 | −0.483; 0.261 | 0.559 | 1 |
| aPTT [s] | −1.503 | −6.504; 3.498 | 0.555 | 1 |
| Quick value [%] | 13.207 | −0.529; 26.943 | 0.059 | 0.597 |
| INR | −0.082 | −0.163; 0 | 0.051 | 0.59 |

[a] linear regression models adjusted for sex, age, physical activity, education years, smoking status, diabetes, hypertension, calorie intake, alcohol consumption, non-HDL cholesterol and BMI. CI, confidence interval; FDR, false discovery rate; [b] $p < 0.05$.

## 4. Discussion

### *4.1. Main Findings*

As main findings we observed that a higher butter intake is associated with significantly higher values for D-dimers; this association with D-dimers is also nominally significant (i.e., before correction for multiple correction) for the consumption of dairy products. Elevated D-dimer levels are typically associated with conditions such as thrombosis, disseminated intravascular coagulation or inflammation [23,24]. Interestingly, D-dimers also seem to increase with ageing and elevated values seem to be more common in middle-aged or older individuals [30]. The findings on protein C are conflicting, i.e., a significantly inverse association with the consumption of dairy products, and a (nominally) positive association with butter consumption. Among the investigated plant-derived foods, only the intake of fruits was significantly associated with higher aPTT values, and this association disappeared after FDR correction.

*4.2. Butter, Dairy Products and Cheese*

Besides the increasing effect on D-dimers, butter consumption as well seems to exert inhibitory effects on hemostasis by the association with higher protein C values while no effect for other coagulation parameters, especially for the functional tests aPTT, INR or Quick value were observed. In addition, a randomized cross-over study observed no direct effects of butter consumption on several coagulation parameters [31]. Saturated fatty acids (SFA) of different chain lengths are main nutritional components of butter [32]. And, regarding hemostasis, some pro-coagulant parameters like factor VII were previously shown to be elevated when (long-chain) SFAs were consumed instead of unsaturated fatty acids (UFA) while for other factors like antithrombin III or fibrinogen no effect was observed [33]. Another randomized cross-over study by Delgado-Lista et al. compared the effects of a diet rich in SFA mainly derived from butter with diets rich in UFA or refined carbohydrates on fasting and post-prandial hemostatic factors after 28-days intervention periods for each diet [34]. After the intervention there were no significant changes shown in measured parameters such as factor VII and D-dimers in a fasted state. However, 4 h after consuming a fat-enriched meal, for factor VII significantly lower values in the UFA diet group were observed whereas D-dimers were significantly elevated in all of the three groups with the largest increase after SFA consumption [34]. While similar effects of dietary fatty acids on fasting and postprandial factor VII measurements were also reported in previous studies as summarized in a review by Pieters et al. [15], the mechanism of these observations is still not fully established yet as well as effects of specific food items on D-dimers which were observed in our study for butter and dairy intake. Besides coagulation system activation, D-dimers are also recognized as a marker for inflammatory processes [23] which could indicate a non-favorable effect of a diet rich in SFA derived from these food items. However, even after the adjustment for CRP, the association between butter and D-dimers remained significant which indicates that other mechanisms apart from inflammation could also be a reason for this observation. While the observed association seems to be more pronounced in individuals which are still within the reference range of D dimers (versus participants above the reference range), none of the additional models were able to reach statistical significance.

On the other hand, regarding CVD risk and direct pro-coagulant effects, dairy intake generally seems to yield neutral or protective effects [35]. Besides its saturated fat content, milk is also a great source of nutrients that have been shown to be inversely associated with CVD risk like vitamin K2 [36], milk protein [37] or contain other bioactive compounds such as specific biopeptides that exhibit anti-thrombotic and antioxidant activities [38,39]. Vitamin K2, especially, which is mainly derived from ruminant meat and dairy products [40], is also directly involved in the coagulation process by the carboxylation of several hemostatic factors such as factor II, VII, IX and X [8].

It was surprising that we observed an association of dairy products consumption with higher D-dimers and lower values for protein C and antithrombin III which indicates an overall pro-coagulant effect. However, especially for cheese which is besides butter one of the dairy products richest in saturated fatty acids (per g food), we could not report similar findings. Also, in a meta-analysis of prospective studies an inverse relationship for CVD risk was observed for cheese consumption [41]. Possible explanations could be the role of beneficial nutrients that are especially found in higher concentrations in fattier dairy products such as conjugated linoleic acid which has been shown to beneficially influence inflammatory parameters and platelet aggregation markers in a cross-over intervention study [42]. Cheese also contains significant amounts of probiotics like Lactobacillus casei which has been found to reduce pro-inflammatory and pro-coagulant factors in an experimental model [43]. To our knowledge no trials on humans exist to date that comprehensively analyzed the direct effects of dairy product consumption on hemostatic factors.

Despite being linked to inflammatory processes [44], the associations of protein C with dairy products still remained significant after correction for multiple testing as well as after the additional adjustment for CRP. This observation, together with the findings on butter intake and associated increment of D-dimer values, may indicate possible unfavorable effects of these food items on thrombosis risk.

### *4.3. Fish, Eggs and Meat*

In contrast to dairy products, for total fish, total egg, and total meat consumption no significant associations with any blood coagulation parameter were observed. Especially for fish intake which goes along with a high intake of long-chain omega-3-fatty acids, an antithrombotic effect could be expected based on the results of interventional studies which showed a positive effect on aPTT [45] as well as a reduction of prothrombin time [46] which could also be applied for the Quick value. In epidemiological studies like ARIC (Atherosclerosis Risk In Communities) and CARDIA (Coronary Artery Risk Development In young Adults) more mixed findings for fish intake were reported [9,47]. In the ARIC study fish intake was around 20 g per day (1.4 servings of 3–5 ounces of fish per week) while on average 10.8 g fish per day were consumed in the CARDIA study. While no significant results were reported in the CARDIA study, the ARIC study found significant inverse associations for fibrinogen, factor VIII and Von Willebrand factor which could indicate an anticoagulant effect of an increased fish intake. However, such a high fish intake is rarely seen in the general population, and this could also be an explanation for the no significant results in the CARDIA study. In our study the mean fish intake was around 18.2 g per day which could also possibly be too low to observe any effects.

For egg consumption, to date only few studies examined the effects on blood coagulation parameters. In a prospective study by Vorster et al. no effects on coagulation factors were found when egg intake was increased from three eggs per week for 2 months to 7 or 14 eggs per week for 5 months [48]. In the ATTICA study, a large prospective observational study with 3042 subjects, especially for low SFA intakes, an inverse correlation with fibrinogen was shown for an increased egg intake suggesting an anticoagulant effect of eggs [49]. Apart from their high cholesterol content, eggs contain a variety of essential nutrients such as zinc, iodine, B-vitamins and also bioactive compounds like phospholipids or the carotenoids lutein and zeaxanthin that yield anti-oxidative and anti-inflammatory effects [50,51]. Eggs generally seem to be safe to be consumed on a regular basis and do not appear to significantly affect blood coagulation parameters as observed in our study [52].

Regarding meat intake, there is also only weak evidence for an association with hemostatic factors. The complete restriction of meat intake was analyzed in studies with vegetarians and yielded more inconclusive findings [13,14] while only few studies investigated the direct effects of increased meat intake on coagulation parameters. In a cross-sectional study from 1991 in 995 Japanese subjects, no association between total meat consumption with fibrinogen was found [53]. Also, in a randomized controlled trial which examined the effect of red meat intake on fibrinogen levels, no significant effect was observed [54]. Meat also contains bioactive compounds such as L-carnitine, coenzyme Q10 or taurine that exert antioxidant and anti-inflammatory activities [55] which may counteract some of the detrimental effects of other nutrients commonly found in meat like heme iron or saturated fatty acids and therefore also may benefit blood coagulation parameters. Therefore, the lack of association of meat intake with coagulation parameters in our study appears to be supported by the current scientific literature.

### *4.4. Fruits and Vegetables*

In our study, for total vegetable and green leafy vegetable intake, no significant association with any blood coagulation parameter could be reported. However, for total fruit intake a positive association with aPTT was observed before adjusting for multiple testing. While there is emerging evidence for the beneficial effects of fruits and vegetable

consumption on overall CVD risk [3,56], specific effects on coagulation parameters are not well established in the current literature. Possibly due to their antioxidant activity, the combined intake of fruits and vegetables has been associated with decreased fibrinogen values [57] and also, specific fruits like kiwis seem to have a fibrinogen lowering effect [58]. Therefore, positive effects on other markers of coagulation (not measured here) seem to be possible and could explain the observed anti-coagulant effect of fruit intake in our study, indicated by a positive association with aPTT. Fruits on average also appear to exert a more potent antioxidant activity, quantified by the ORAC (Oxygen Radical Absorbance Capacity) value, compared to vegetables [59,60] which could also explain the different results for those food items. Especially the lack of association of green leafy vegetables rich in vitamin K1 [61] could be due to their low intake amounts in our study with a mean value of only around 23.9 g per day.

### *4.5. Strengths and Limitations*

Our study had several strengths such as a large number of participants, and the particular analysis of food groups instead of specific nutrients which were mainly investigated in previous studies. However, also some limitations exist. While our study measured nine different hemostatic parameters, there are of course other relevant markers of the coagulation system we did not analyze such as factor VII, the Von Willebrand factor or factor X which should be examined in further studies. The cross-sectional nature of our study does preclude any causal conclusions. Also, information about other comorbidities—besides hypertension, diabetes, high blood cholesterol (non-HDLc), or obesity (BMI)—or further specific blood markers such as sex hormones that could have been influenced by the intake of specific food items was not available in our dataset. Therefore, we could not adjust for additional diseases or conditions of the participants which could possibly have affected the measurements of hemostatic parameters. Further, the results may not be completely transferable to other populations or ethnic groups due to different environmental and congenital factors that could influence the metabolism of those individuals in a different manner. Finally, the self-reported dietary intake data assessed via 24-h food lists and a food frequency questionnaire are known to be unprecise and prone to recall bias.

## 5. Conclusions

While consumption of fruits and vegetables were not clearly associated with hemostatic markers, the results for animal-derived products are more mixed. For total fish, eggs and meat, no significant associations were observed whereas dairy product and butter consumption were associated with D-dimer and protein C concentrations. These findings need to be evaluated in independent prospective studies.

**Supplementary Materials:** The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/nu16030432/s1: Table S1: Overview of participants with values outside the reference range for hemostatic parameters. Table S2: Association between habitual consumption of fruits, vegetables and green leafy vegetables and blood coagulation parameters additionally adjusted for FLI. Table S3: Association between habitual consumption of foods of animal origin and blood coagulation parameters additionally adjusted for FLI. Table S4: Association between habitual consumption of dairy products (w/o butter), cheese and butter and blood coagulation parameters additionally adjusted for FLI.

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**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Bavarian Chamber of Physicians (KORAFit EC No 17040; 14 November 2017). The investigations were conducted in accordance with the Declaration of Helsinki.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The data are subject to national data protection laws, and restrictions were imposed by the Ethics Committee of the Bavarian Chamber of Physicians to ensure data privacy of the study participants. Therefore, data cannot be made freely available in a public repository. However, data can be requested through an individual project agreement with KORA via the online portal KORA (https://www.helmholtz-munich.de/en/epi accessed on 6 December 2023).

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**Supplementary data**

Table S1.
Overview of participants with values below or above the reference range for hemostatic parameters

| | **Reference Range** | **n (%) below the reference range** | **n (%) above the reference range** |
|---|---|---|---|
| Antithrombin III | 83 - 118% | 14 (2.35%) | 42 (7.06%) |
| D-dimers | <500 µg/l | | 190 (31.93%) |
| Factor VIII | 70 - 150% | 24 (4.03%) | 114 (19.16%) |
| Fibrinogen | 210 – 400 mg/dl | 17 (2.86%) | 27 (4.54%) |
| Protein C | 70 - 140% | 4 (0.67%) | 145 (24.37%) |
| Protein S | Males: 73 - 130%<br>Females: 52 - | Males: 4 (1.52%)<br>Females: 0 | Males: 137 (52.09%)<br>Females: 138 |
| Activated partial thromboplastin time (aPTT) | 26 - 36 s | 23 (3.87%) | 39 (6.56%) |
| Quick value | 82 - 125% | 4 (0.67%) | 15 (2.52%) |
| International thromboplastin time (INR) | 0.9 - 1.15 | 61 (10.25%) | 3 (0.50%) |

**Linear regression models adjusted for Fatty Liver Index (FLI), replacing BMI**

Table S2.
Association between habitual consumption of fruits, vegetables and green leafy vegetables [100g/d] and blood coagulation parameters (dependent variables), additionally adjusted for FLI [a]

| | **ß-estimate** | **95% CI** | **p-value** | **FDR adjusted p-value** |
|---|---|---|---|---|
| Total fruit consumption [100g/d] | | | | |
| Antithrombin III [mg/dl] | -0.022 | -1.1; 1.057 | 0.969 | 0.995 |
| Ln D-dimers [µg/l] | 0.038 | -0.017; 0.093 | 0.176 | 0.881 |
| Ln Factor VIII [%] | 0 | -0.032; 0.033 | 0.983 | 0.995 |
| Ln Fibrinogen D [mg/dl] | -0.002 | -0.022; 0.018 | 0.875 | 0.995 |
| Protein C [%] | 0.571 | -1.286; 2.427 | 0.546 | 0.995 |
| Ln Protein S [%] | -0.006 | -0.033; 0.021 | 0.687 | 0.995 |
| aPTT [s] | 0.384 | 0.022; 0.746 | 0.038[b] | 0.58 |
| Quick value [%] | 0.216 | -0.779; 1.211 | 0.67 | 0.995 |
| INR | -0.001 | -0.007; 0.005 | 0.67 | 0.995 |
| Total vegetable consumption [100g/d] | | | | |
| Antithrombin III [mg/dl] | -1.085 | -2.801; 0.631 | 0.215 | 0.917 |
| Ln D-dimers [µg/l] | 0.027 | -0.063; 0.118 | 0.552 | 0.995 |
| Ln Factor VIII [%] | -0.005 | -0.057; 0.046 | 0.845 | 0.995 |
| Ln Fibrinogen D [mg/dl] | -0.01 | -0.042; 0.022 | 0.546 | 0.995 |
| Protein C [%] | -1.392 | -4.352; 1.569 | 0.356 | 0.995 |
| Ln Protein S [%] | -0.003 | -0.046; 0.04 | 0.887 | 0.995 |
| aPTT [s] | -0.314 | -0.9; 0.271 | 0.292 | 0.985 |
| Quick value [%] | 0.103 | -1.486; 1.692 | 0.899 | 0.995 |
| INR | 0 | -0.01; 0.009 | 0.923 | 0.995 |
| Green leafy vegetables consumption [100g/d] | | | | |
| Antithrombin III [mg/dl] | -2.457 | -9.986; 5.071 | 0.522 | 0.995 |
| Ln D-dimers [µg/l] | -0.032 | -0.429; 0.364 | 0.872 | 0.995 |
| Ln Factor VIII [%] | -0.144 | -0.371; 0.083 | 0.212 | 0.917 |
| Ln Fibrinogen D [mg/dl] | -0.085 | -0.227; 0.056 | 0.236 | 0.956 |
| Protein C [%] | -1.415 | -14.405; 11.574 | 0.831 | 0.995 |
| Ln Protein S [%] | -0.163 | -0.351; 0.026 | 0.091 | 0.737 |
| aPTT [s] | -0.651 | -3.24; 1.937 | 0.621 | 0.995 |
| Quick value [%] | 0.666 | -6.367; 7.699 | 0.852 | 0.995 |
| INR | -0.004 | -0.046; 0.038 | 0.839 | 0.995 |

[a]linear regression models adjusted for sex, age, physical activity, education years, smoking status, diabetes, hypertension, calorie intake, alcohol consumption, non-HDL cholesterol and FLI. CI, confidence interval; FDR false discovery rate; [b]$p<0.05$

Table S3.
Association between habitual consumption of foods of animal origin [100g/d] and blood coagulation parameters (dependent variables), additionally adjusted for FLI [a]

| | **ß-estimate** | **95% CI** | **p-value** | **FDR adjusted p-value** |
|---|---|---|---|---|
| | Total meat consumption [100g/d] | | | |
| Antithrombin III [mg/dl] | 1.396 | -1.87; 4.662 | 0.401 | 0.995 |
| Ln D-dimers [µg/l] | -0.016 | -0.19; 0.157 | 0.854 | 0.995 |
| Ln Factor VIII [%] | 0.002 | -0.096; 0.099 | 0.973 | 0.995 |
| Ln Fibrinogen D [mg/dl] | 0.042 | -0.019; 0.104 | 0.178 | 0.881 |
| Protein C [%] | 0.798 | -4.824; 6.421 | 0.78 | 0.995 |
| Ln Protein S [%] | -0.014 | -0.096; 0.068 | 0.744 | 0.995 |
| aPTT [s] | 0.298 | -0.815; 1.411 | 0.599 | 0.995 |
| Quick value [%] | -2.381 | -5.429; 0.666 | 0.125 | 0.881 |
| INR | 0.014 | -0.004; 0.032 | 0.136 | 0.881 |
| | Total fish consumption [100g/d] | | | |
| Antithrombin III [mg/dl] | -3.867 | -9.467; 1.734 | 0.176 | 0.881 |
| Ln D-dimers [µg/l] | -0.018 | -0.318; 0.282 | 0.908 | 0.995 |
| Ln Factor VIII [%] | -0.011 | -0.178; 0.156 | 0.897 | 0.995 |
| Ln Fibrinogen D [mg/dl] | -0.046 | -0.152; 0.059 | 0.39 | 0.995 |
| Protein C [%] | -0.217 | -9.88; 9.446 | 0.965 | 0.995 |
| Ln Protein S [%] | -0.043 | -0.184; 0.098 | 0.55 | 0.995 |
| aPTT [s] | -0.169 | -2.099; 1.761 | 0.863 | 0.995 |
| Quick value [%] | -0.228 | -5.473; 5.017 | 0.932 | 0.995 |
| INR | 0.002 | -0.03; 0.033 | 0.924 | 0.995 |
| | Total egg consumption [100g/d] | | | |
| Antithrombin III [mg/dl] | 3.768 | -3.014; 10.55 | 0.276 | 0.985 |
| Ln D-dimers [µg/l] | 0.08 | -0.277; 0.438 | 0.66 | 0.995 |
| Ln Factor VIII [%] | 0.032 | -0.173; 0.236 | 0.762 | 0.995 |
| Ln Fibrinogen D [mg/dl] | 0.009 | -0.121; 0.138 | 0.897 | 0.995 |
| Protein C [%] | 3.055 | -8.622; 14.731 | 0.608 | 0.995 |
| Ln Protein S [%] | 0.007 | -0.163; 0.177 | 0.934 | 0.995 |
| aPTT [s] | -1.062 | -3.403; 1.279 | 0.373 | 0.995 |
| Quick value [%] | 0.537 | -5.801; 6.875 | 0.868 | 0.995 |
| INR | -0.004 | -0.042; 0.034 | 0.841 | 0.995 |

[a]linear regression models adjusted for sex, age, physical activity, education years, smoking status, diabetes, hypertension, calorie intake, alcohol consumption, non-HDL cholesterol and FLI. CI, confidence interval; FDR false discovery rate

Table S4.
Association between habitual consumption of dairy products (w/o butter), cheese and butter [100g/d] and blood coagulation parameters (dependent variables), additionally adjusted for FLI [a]

| | **ß-estimate** | **95% CI** | **p-value** | **FDR adjusted p-value** |
|---|---|---|---|---|
| Dairy products (w/o butter) consumption [100g/d] | | | | |
| Antithrombin III [mg/dl] | -1.23 | -2.095; -0.365 | 0.005[b] | 0.135 |
| Ln D-dimers [µg/l] | 0.053 | 0.008; 0.098 | 0.022[b] | 0.445 |
| Ln Factor VIII [%] | 0.004 | -0.022; 0.03 | 0.75 | 0.995 |
| Ln Fibrinogen D [mg/dl] | 0.004 | -0.012; 0.021 | 0.599 | 0.995 |
| Protein C [%] | -2.615 | -4.1; -1.13 | 0.001[b] | 0.081 |
| Ln Protein S [%] | 0.016 | -0.006; 0.037 | 0.158 | 0.881 |
| aPTT [s] | 0.03 | -0.263; 0.324 | 0.839 | 0.995 |
| Quick value [%] | -0.066 | -0.873; 0.741 | 0.872 | 0.995 |
| INR | 0.001 | -0.004; 0.005 | 0.826 | 0.995 |
| Cheese consumption [100g/d] | | | | |
| Antithrombin III [mg/dl] | 0.714 | -5.639; 7.066 | 0.825 | 0.995 |
| Ln D-dimers [µg/l] | 0.195 | -0.14; 0.53 | 0.254 | 0.98 |
| Ln Factor VIII [%] | 0.073 | -0.117; 0.262 | 0.45 | 0.995 |
| Ln Fibrinogen D [mg/dl] | -0.001 | -0.121; 0.119 | 0.983 | 0.995 |
| Protein C [%] | 2.253 | -8.702; 13.208 | 0.686 | 0.995 |
| Ln Protein S [%] | -0.108 | -0.267; 0.052 | 0.185 | 0.881 |
| aPTT [s] | 0.497 | -1.691; 2.684 | 0.656 | 0.995 |
| Quick value [%] | -0.01 | -5.891; 5.872 | 0.997 | 0.997 |
| INR | -0.001 | -0.036; 0.034 | 0.959 | 0.995 |
| Butter consumption [100g/d] | | | | |
| Antithrombin III [mg/dl] | 13.739 | -0.804; 28.281 | 0.064 | 0.63 |
| Ln D-dimers [µg/l] | 1.198 | 0.442; 1.953 | 0.002[b] | 0.081 |
| Ln Factor VIII [%] | 0.126 | -0.315; 0.567 | 0.574 | 0.995 |
| Ln Fibrinogen D [mg/dl] | 0.147 | -0.125; 0.42 | 0.289 | 0.985 |
| Protein C [%] | 25.914 | 0.799; 51.029 | 0.043[b] | 0.58 |
| Ln Protein S [%] | -0.162 | -0.529; 0.204 | 0.385 | 0.995 |
| aPTT [s] | -1.694 | -6.61; 3.223 | 0.499 | 0.995 |
| Quick value [%] | 12.438 | -1.006; 25.881 | 0.07 | 0.63 |
| INR | -0.076 | -0.156; 0.004 | 0.062 | 0.63 |

[a]linear regression models adjusted for sex, age, physical activity, education years, smoking status, diabetes, hypertension, calorie intake, alcohol consumption, non-HDL cholesterol and FLI. CI, confidence interval; FDR false discovery rate; [b]$p<0.05$

# 3. Manuskript II

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Lipids in Health and Disease

RESEARCH

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# Association of the habitual dietary intake with the fatty liver index and effect modification by metabotypes in the population-based KORA-Fit study

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## Abstract

**Background** Non-alcoholic fatty liver disease (NAFLD) is an emerging threat for public health with diet being a major risk factor in disease development and progression. However, the effects of habitual food consumption on fatty liver are still inconclusive as well as the proposed role of the individuals' metabolic profiles. Therefore, the aim of our study is to examine the associations between diet and NAFLD with an emphasis on the influence of specific metabotypes in the general population.

**Methods** A total of 689 participants (304 men and 385 women) of the KORA-Fit (S4) survey, a follow-up study of the population-based KORA cohort study running in the Region of Augsburg, Germany, were included in this analysis. Dietary information was derived from repeated 24-h food lists and a food frequency questionnaire. The intake of energy and energy-providing nutrients were calculated using the national food composition database. The presence of fatty liver was quantified by the fatty liver index (FLI), and metabotypes were calculated using K-means clustering. Multivariable linear regression models were used for the analysis of habitual food groups and FLI; for the evaluation of macronutrients, energy substitution models were applied.

**Results** A higher consumption of nuts and whole grains, and a better diet quality (according to Alternate Healthy Eating Index and Mediterranean Diet Score) were associated with lower FLI values, while the intake of soft drinks, meat, fish and eggs were associated with a higher FLI. The isocaloric substitution of carbohydrates with polyunsaturated fatty acids was associated with a decreased FLI, while substitution with monounsaturated fatty acids and protein showed increased FLI. Statistically significant interactions with the metabotype were observed for most food groups.

**Conclusion** The consumption of plant-based food groups, including nuts and whole grains, and diet quality, were associated with lower FLI values, whereas the intake of soft drinks and products of animal origin (meat, fish, eggs) were associated with a higher FLI. The observed statistically significant interactions with the metabotype for most food groups could help to develop targeted prevention strategies on a population-based level if confirmed in independent prospective studies.

**Keywords** Diet, Fatty liver index, Food groups, Carbohydrates, Metabotype

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## Background

Non-alcoholic fatty liver disease (NAFLD) is a metabolic disorder which is characterized by an excess accumulation of fat in liver cells, and often times develops on the basis of existing obesity; this hepatic steatosis is strongly linked with insulin resistance and disturbances in glucose and lipid metabolism; thus, it is nowadays called metabolic dysfunction-associated fatty liver disease (MAFLD). If untreated, MAFLD may develop to non-alcoholic steatohepatitis (NASH) and promote the development of metabolic and cardiovascular diseases, eventually leading to increased early mortality [1–3]. During the past three decades it is estimated that the global MAFLD prevalence increased exponentially, and currently affects about one in three people; thus MAFLD became a major global health concern [3]. Despite its substantial public health impact, MAFLD is rarely addressed in national health agendas of most countries though there is a pressing need for effective prevention strategies [4]. Lifestyle modifications leading to weight loss are currently recommended to counteract the progression of MAFLD but there is still inconclusive data regarding the recommendation of a specific dietary pattern [5, 6]. Food-based dietary guidelines are commonly used in public health communication because of their simplicity and practicability for the general population compared to nutrient-based recommendations [7]. However, while the intake of specific nutrients such as fructose have shown to detrimentally affect MAFLD [8], the influence of specific food items has not been extensively researched yet, especially on a population-based level [9]. Therefore, our study aims to examine associations between the habitual consumption of different food groups in the general population and fatty liver disease as defined by the fatty liver index (FLI); this index was established and validated in 2006 by Bedogni et al. [10]. Additionally, we applied metabotyping, which is a procedure that stratifies subjects into most homogenous subgroups according to their metabolic profile [11]. Therefore, we aim to get more insights about the individual effects of the diet depending on the specific metabolic characteristics of the study participants as a more innovative and personalized approach in the analysis of dietary exposures on FLI. Recent evidence suggests that a diet low in carbohydrates may be a promising therapeutic strategy in MAFLD; its effectiveness has been shown in intervention studies in obese subjects [12]. Accordingly, the replacement of carbohydrates with other energy-providing nutrients will be explored as well using energy substitution models.

## Materials and methods

### Study sample

The KORA (Cooperative Health Research in the Region of Augsburg) is a prospective population-based study of adults in the Region of Augsburg, Germany, and aims to examine the effects of environmental and lifestyle factors on non-communicable diseases and to contribute novel strategies for primary prevention [13]. From 1984 to 1995 the WHO firstly carried out the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) project which was then continued as the KORA study in 1996 by Helmholtz Center Munich [13]. From 1984 to 2001 four cross-sectional (S1 to S4) baseline surveys with around 18,000 randomly selected participants were conducted [14]. The KORA S4 survey was carried out from 1999 to 2001 and consisted of 4,261 inhabitants of the study region (city of Augsburg and two surrounding counties) aged 25 to 74 years [14]. In the follow-up study KORA Fit, 3,059 KORA participants born between 1945 and 1964 of each baseline survey were included; the study was performed in 2018 and 2019 [14]. Our study sample includes a total of 689 subjects (304 men and 385 women) of the S4 part of KORA-Fit who had available dietary intake data and blood serum measurements for the calculation of the fatty liver index and the estimation of the metabotypes. Ethical release was provided by the Ethics Committee of the Bavarian Medical Association (Bayerische Landesärztekammer). All study participants gave informed consent, and the study was conducted in accordance with the Declaration of Helsinki [13].

### Measurements of exposures

Body weight and height were measured in light clothing and without shoes; Body Mass Index (BMI) was calculated by the division of body weight (in kg) through the square of the height (in m) [15]. For the examination of waist circumference the measuring tape was placed between the distance of the lower rib margin and iliac crest [16]. Sociodemographic and lifestyle assessments were performed in computer assisted face-to-face interviews by specifically trained and certified medical personnel. Smoking was categorized as current, former or never; education status was grouped as < 12 years and 12 years or more of scholar education; physical activity was classified into categories subdivided by the estimated time per week spent on sports activities during leisure time in summer and winter. Furthermore, information about medication use and relevant medical diagnoses such as diabetes or hypertension was gathered. Participants who previously got medically diagnosed with diabetes and/or were under antidiabetic medication were classified as having diabetes. If the subjects baseline

blood pressure exceeded values of systolic > = 140 or diastolic > = /90 mmHg, or receiving antihypertensive treatment, provided the subject is aware of hypertension, they were categorized as hypertensives [17].

Dietary data was collected via repeated 24-h food lists (24HFL) and a food frequency questionnaire (FFQ) with 246 and 148 items, respectively. The 24HFL was developed for the German National Cohort [18] and subjects were only asked to report type and frequency of food intake of the past day. The FFQ was based on the German version of the multilingual European Food Propensity Questionnaire which aims to determine dietary habits over the past 12 months [19]. The calculation of a person's dietary intake in KORA Fit is based on the estimation of consumption probability and consumption amount. Details have been published previously [20]; briefly, consumption probability is determined for each food item for each individual based on three (at least two) 24HFLs and FFQ, while usual portion size for each item is estimated based on data from the Bavarian Food Consumption Survey II (BVS II) [20]. Consumption probabilities were estimated using logistic mixed models, adjusting for covariates and the FFQ data on consumption frequency. By means of mixed linear models, the consumption amount of each food item was modeled, adjusted for age, sex, BMI, smoking, physical activity, and education level. Consumption probability multiplied by consumption amount then results in the usual intake of each food item on any given day. Food items were then categorized into 17 food groups and 21 subgroups according to the EPIC-Soft classification scheme [21].

For our study we utilized data on the consumption of total fruits, total vegetables, total nuts, total meat with the additional subgroups beef, pork and poultry, total fish, total eggs, dairy products, whole grain, and soft drinks. Furthermore, to get more insights about specific dietary patterns of the study participants, the Alternate Healthy Eating Index (AHEI) and the Mediterranean Diet Score (MDS) were calculated as previously described [22, 23] and included in our analysis. The German food composition data base (BLS, version 3.02) was linked to the individual food items to enable the estimation of the average daily intake of energy-providing macronutrients, i.e. total fat, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), protein, carbohydrates and alcohol. Daily carbohydrate, total fat, fatty acid subgroup, protein, and alcohol intake was transformed as % of total energy intake by assuming a mean energy value of 9 kcal/g for total fat and fatty acid subgroups, 4 kcal/g for carbohydrates and protein and 7 kcal/g for alcohol.

While the analysis of liver biopsies is still described as the gold standard for fatty liver diagnosis, the FLI is a far more practical tool for population-based studies because of lower costs and its non-invasiveness [24]. The formula of Bedogni et al. was utilized to determine FLI values [10]:

$$FLI = \left(e^{0.953\times\log(triglycerides)+0.139\times BMI+0.718\times\log(GGT)+0.053\times waistcircumference-15.745}\right) \div \left(1+e^{0.953\times\log(triglycerides)+0.139\times BMI+0.718\times\log(GGT)+0.053\times waistcircumference-15.745}\right)\times 100$$

The formula includes measured data of waist circumference, body mass index (BMI), serum triglycerides, and gamma glutamyl transferase (GGT). The FLI ranges from 0 to 100 with high numbers indicating a high probability of the presence of fatty liver [10]. According to Bedogni et al., FLI values < 30 seem to accurately exclude the presence of fatty liver while values > 60 indicate the presence of fatty liver [10]. However, FLI values between 30 and 60 appear to be not as accurate in the prediction of fatty liver and seem to be affected by variables such as age and sex due to recent research [25]. Thus in our study, we categorized the participants into three different FLI subgroups (< 30, normal; 30–60, indeterminate; > 60, severely increased).

Metabotypes were calculated by using a K-means clustering algorithm as described previously by Riedl et al. [26, 27]. For the creation of three different homogenous metabotype clusters we used an optimized set of parameters previously established by Dahal et al.; this set included measurements of BMI, serum triglycerides, uric acid, fasting glucose, high-density-lipoprotein cholesterol (HDLc), and Non-HDLc which was defined as the difference between total cholesterol and HDLc [28]. Regarding the three derived metabotype clusters, cluster 1 was the "healthy" metabotype, cluster 2 the "intermediate" and cluster 3 the most "unfavourable" metabotype [28]. All laboratory measurements were performed by the analysis of serum samples in the central laboratory Institute of Laboratory Medicine, LMU Munich, with a cobas c702 clinical chemistry analyser (Roche Diagnostics, Rotkreuz, Switzerland).

### Statistical analysis

Age, BMI, waist circumference, energy intake, alcohol consumption, FLI and all laboratory and dietary parameters were used as continuous variables. Due to their non-normally distributed characteristics regarding the results from the Shapiro–Wilk-test, all continuous parameters were described by medians and 25th-75th percentile

range. To analyse differences between the three FLI subgroups, the Kruskal–Wallis-test was used and for the differences between males and females the Mann–Whitney-U-test was applied. Sex, education status, physical activity, smoking status, and the diagnosis of hypertension or diabetes were defined as categorial variables. To investigate significant differences between FLI subgroups, and for differences between males and females, the Chi-squared test (categorial variables) was used.

For the analysis of the associations of food items with FLI stratified by the metabotype subgroups, multivariable linear regression models were fitted for each food item. Multicollinearity and autocorrelation were tested using the variance inflation factor and Durbin Watson test, respectively. Homoscedasticity and the normal distribution of residuals were visually assessed using the scatterplot (predicted values vs. residuals) and the Q-Q-plot, respectively. The linearity assumption between each continuous covariate and the outcome was tested by the second-degree polynomial approach. Extreme outliers were removed with regard to the Cook's D. Furthermore, we tested for interaction effects between sex and metabotypes for each food item using the Wald test. The Benjamini–Hochberg False-Discovery-Rate (FDR) method was used to additionally adjust the *p*-values of each model for multiple testing. Due to a low number of subjects in the metabotype 3 group, we summarized metabotypes 2 and 3 into one subgroup to get more robust results. All models were adjusted for age, sex, physical activity, education years, smoking status and total energy intake.

To assess the association of FLI as outcome with an isocaloric replacement of carbohydrates by fat subtypes (or total fat) and protein as exposure, a substitution model based on linear regression was calculated. The substitution model included total energy intake and all energy-providing nutrients (i.e., SFA, MUFA, PUFA (or total fat), protein and alcohol) except for carbohydrates and was additionally adjusted for potential confounding covariables. Variables for carbohydrates, fat subgroups (or total fat), protein and alcohol were adequately scaled so that the β-coefficients for energy-providing nutrients denote a change per 5% of total energy intake (5 E%). Thus, the estimated coefficient for, e.g., PUFA intake can be interpreted as the association of FLI with a 5E% increase in PUFA at the expense of carbohydrates while energy supply from other sources remains unchanged. The models were adjusted for age, sex, physical activity, education years, smoking status and total energy intake, and tested for all necessary model assumptions.

A $p$-value < 0.05 was defined as statistically significant. All analyses were performed with the R-software (R version 4.3.1).

## Results

### Study sample characteristics

Table 1 shows the characteristics of all study participants and stratified FLI subgroup. Our study sample of 689 persons (304 men and 385 women) is a subsample of the S4 part of KORA-Fit for which we could assess sufficient dietary information. Over the follow-up of the S4 cohort, deviation from a random sample of the population may have increased. The average age was 63 years and the participants had a mean BMI of 27.4 kg/m$^2$. About 12% were current smokers, and 15% reported no physical activity.

A total of 215 participants were categorized into the normal FLI subgroup, 145 subjects fell into the indeterminate FLI subgroup and 329 were attributed to the severely increased FLI subgroup. For sex, age, BMI, waist circumference, all tested laboratory parameters, FLI, education status, physical activity, hypertension and diabetes status, and metabotype significant differences between the FLI subgroups were observed. On average, participants that fell into the increased FLI subgroups were older, had more unfavourable anthropometric measurement results such as an increased BMI and waist circumference, as well as increased values for all laboratory parameters, with the exception of lower values for HDL. Moreover, participants with an increased FLI were more often male, had a lower educational status, were less physically active, were attributed to a more unfavourable metabotype, and were more frequently diagnosed with hypertension and diabetes.

### Habitual dietary consumption values

Tables 2 and 3 display the food and nutrient consumption values (in g per day) in all participants and stratified by FLI subgroups. Significant differences between subgroups were found for total vegetables, total nuts, dairy, total meat and the sub-groups beef, pork and poultry, for total fish, total eggs, soft drinks, whole grains, AHEI, MDS, energy (Kilocalories), alcohol, total fat, SFA, MUFA and total protein consumption. While on average persons with increased FLI values revealed a higher consumption of total meat, meat sub-groups, total fish, total eggs, soft drinks, as well as total energy, alcohol, total fat, SFA, MUFA, and total protein intake, they eat less total vegetables, total nuts, dairy, and whole grains. Furthermore, study participants with higher FLI values tend to have lower values of the AHEI and MDS.

Descriptive statistics stratified by sex are available in the supplementary data (Tables S1 and S2).

### Associations of diet with the FLI, stratified by metabotype clusters

The associations of the analysed food groups and subgroups with FLI were shown in Table 4. Regarding

**Table 1** Characteristics of all study participants and by FLI

| | **Total** | | **Normal FLI** | | **Indeterminate FLI** | | **Severely increased FLI** | | |
|---|---|---|---|---|---|---|---|---|---|
| | ***n* = 689** | | ***n* = 215** | | **n = 145** | | ***n* = 329** | | |
| **Characteristics** | **Median (25th—75th percentile)** | | | | | | | | ***p*-value** |
| Age [years] | 63 | (58; 68) | 61 | (57; 67) | 64 | (59; 68) | 64 | (59; 68) | ***0.001*** |
| BMI [kg/m$^2$] | 27.4 | (24.1; 30.7) | 23.2 | (21.7; 24.9) | 25.8 | (24.7; 27.8) | 30.9 | (28.7; 33.5) | ***< 0.001*** |
| Waist circumference [cm] | 93.6 | (82.8; 103.0) | 78.2 | (73.8; 84.0) | 89.5 | (85.0; 94.1) | 103.5 | (98.0; 109.5) | ***< 0.001*** |
| HDL [mg/dl] | 63.8 | (51.0; 77.0) | 77.0 | (66.5; 90.8) | 68.0 | (57.1; 78.0) | 53.0 | (45.0; 64.0) | ***< 0.001*** |
| non-HDL [mg/dl] | 146.0 | (121.0; 172.2) | 135.9 | (112.1; 158.1) | 145.1 | (121.0; 177.0) | 152.0 | (124.0; 181.0) | ***< 0.001*** |
| Triglycerides [mg/dl] | 106.0 | (77.0; 145.0) | 74.8 | (61.0; 94.0) | 100.2 | (76.2; 129.0) | 136.0 | (107.0; 179.0) | ***< 0.001*** |
| gGT [U/l] | 24.0 | (17.0; 37.0) | 16.0 | (13.0; 21.0) | 24.0 | (17.0; 32.0) | 32.0 | (23.0; 49.0) | ***< 0.001*** |
| AST [U/l] | 24.0 | (20.0; 28.0) | 22.0 | (19.0; 25.8) | 24.1 | (21.0; 27.6) | 25.0 | (21.0; 30.2) | ***< 0.001*** |
| ALT [U/l] | 24.0 | (18.7; 31.0) | 18.9 | (16.0; 24.0) | 23.4 | (19.8; 29.0) | 29.0 | (23.0; 37.0) | ***< 0.001*** |
| Uric acid [mg/dl] | 5.2 | (4.4; 6.3) | 4.5 | (3.9; 5.1) | 5.3 | (4.4; 5.9) | 6.0 | (5.0; 7.1) | ***< 0.001*** |
| Fasting glucose [mg/dl] | 97 | (92; 105) | 93 | (88; 97) | 96 | (92; 102) | 103 | (96; 112) | ***< 0.001*** |
| FLI | 54.9 | (24.4; 89.4) | 15.3 | (9.4; 22.9) | 41.9 | (35.3; 49.5) | 90.5 | (78.2; 100.2) | ***< 0.001*** |
| | **n (%)** | | | | | | | | |
| Sex | | | | | | | | | |
| Male | 304 | (44.1) | 46 | (21.4) | 67 | (46.2) | 191 | (58.1) | ***< 0.001*** |
| Female | 385 | (55.9) | 169 | (78.6) | 78 | (53.8) | 138 | (41.9) | |
| Education [years] | | | | | | | | | |
| < = 12 years | 417 | (60.5) | 114 | (53.0) | 82 | (56.6) | 221 | (67.2) | ***0.002*** |
| > 12 years | 272 | (39.5) | 101 | (47.0) | 63 | (43.4) | 108 | (32.8) | |
| Physical activity | | | | | | | | | |
| > = 2 h/week | 266 | (38.6) | 107 | (49.8) | 61 | (42.1) | 98 | (29.8) | ***< 0.001*** |
| 1 h/week | 230 | (33.4) | 65 | (30.2) | 53 | (36.6) | 112 | (34.0) | |
| < 1 h/week | 88 | (12.8) | 20 | (9.3) | 10 | (6.9) | 58 | (17.6) | |
| (almost) no activity | 105 | (15.2) | 23 | (10.7) | 21 | (14.5) | 61 | (18.5) | |
| Smoking | | | | | | | | | |
| Current smoker | 82 | (11.9) | 21 | (9.8) | 15 | (10.3) | 46 | (14.0) | 0.139 |
| Former smoker | 298 | (43.3) | 91 | (42.3) | 56 | (38.6) | 151 | (45.9) | |
| Never smoker | 309 | (44.8) | 103 | (47.9) | 74 | (51.0) | 132 | (40.1) | |
| Hypertension | | | | | | | | | |
| Yes | 313 | (45.4) | 45 | (20.9) | 54 | (37.2) | 214 | (65.0) | ***< 0.001*** |
| No | 376 | (54.6) | 170 | (79.1) | 91 | (62.8) | 115 | (35.0) | |
| Diabetes | | | | | | | | | |
| Yes | 49 | (7.1) | 5 | (2.3) | 5 | (3.4) | 39 | (11.9) | ***< 0.001*** |
| No | 640 | (92.9) | 210 | (97.7) | 140 | (96.6) | 290 | (88.1) | |
| Metabotype | | | | | | | | | |
| 1 | 140 | (20.3) | 61 | (28.4) | 24 | (16.6) | 55 | (16.7) | ***0.001*** |
| 2 | 478 | (69.4) | 154 | (71.6) | 112 | (77.2) | 212 | (64.4) | |
| 3 | 71 | (10.3) | (0.0) | (0.0) | 9 | (6.2) | 62 | (18.8) | |

**Table 2** Habitual food consumption data and dietary patterns in all participants and by FLI

| | Total | | Normal FLI | | Indeterminate FLI | | Severely increased FLI | | |
|---|---|---|---|---|---|---|---|---|---|
| **Food item** | **Median (25th-75th percentile)** | | | | | | | | ***p*-value** |
| Total vegetables [g/d] | 166.2 | (136.5; 202.7) | 185.6 | (152.8; 218.9) | 160.1 | (133.3; 202) | 156.2 | (130.9; 191.4) | ***< 0.001*** |
| Total fruits [g/d] | 149.6 | (93.2; 216.2) | 159.5 | (106.2; 224.8) | 141.4 | (91.1; 212.8) | 148.7 | (87.9; 210.8) | 0.068 |
| Total nuts [g/d] | 4.4 | (2.6; 13) | 6.1 | (2.8; 18.8) | 4.2 | (2.9; 12.9) | 3.9 | (2.4; 11.2) | ***< 0.001*** |
| Dairy [g/d] | 177.5 | (121.3; 259.7) | 205.8 | (135.8; 288.7) | 177.5 | (132.4; 259.7) | 162.6 | (112.1; 229.6) | ***< 0.001*** |
| Total meat [g/d] | 101.1 | (74.5; 132.7) | 75.8 | (63; 101.1) | 95.2 | (73.6; 115.2) | 119.9 | (95.1; 152.6) | ***< 0.001*** |
| Beef [g/d] | 8.1 | (6.1; 10.7) | 6.7 | (5.6; 9.1) | 7.7 | (6.1; 9.9) | 9.2 | (6.8; 12.3) | ***< 0.001*** |
| Pork [g/d] | 15.6 | (10.7; 21.3) | 10.7 | (8.6; 15.9) | 13.5 | (10.7; 16.9) | 18.0 | (13.7; 25.8) | ***< 0.001*** |
| Poultry [g/d] | 10.4 | (9.2; 17) | 9.5 | (7.1; 13.8) | 11.8 | (9.2; 16.8) | 12.9 | (9.6; 17.7) | ***< 0.001*** |
| Total fish [g/d] | 18.7 | (12.6; 27.1) | 17.4 | (11.2; 25.6) | 17.7 | (12.9; 24.2) | 19.9 | (13.5; 30.5) | ***< 0.001*** |
| Total eggs [g/d] | 16.0 | (11.4; 22.7) | 14.1 | (10.5; 21) | 15.1 | (11.1; 21.2) | 17.6 | (13; 24.8) | ***< 0.001*** |
| Softdrinks [g/d] | 5.3 | (3.4; 13.3) | 3.4 | (2.5; 5.2) | 4.8 | (3.3; 10.7) | 7.2 | (5; 25.6) | ***< 0.001*** |
| Whole grains [g/d] | 15.3 | (7.4; 35.3) | 24.6 | (9.1; 40.5) | 18.3 | (7.3; 35.1) | 11.5 | (6.6; 26.4) | ***< 0.001*** |
| AHEI | 44.3 | (37.6; 51.2) | 48.0 | (41.3; 56.1) | 45.7 | (39.3; 50.6) | 41.4 | (35.2; 48.1) | ***< 0.001*** |
| MDS | 4 | (3; 6) | 5 | (4; 6) | 4 | (3; 6) | 4 | (3; 5) | ***0.001*** |

**Table 3** Habitual nutrient consumption data in all participants and by FLI

| | Total | | Normal FLI | | Indeterminate FLI | | Severely increased FLI | | |
|---|---|---|---|---|---|---|---|---|---|
| **Nutrient** | **Median (25th-75th percentile)** | | | | | | | | ***p*-value** |
| Energy (Kilocalories) | | | | | | | | | |
| [kcal/d] | 1735.1 | (1488.9; 2064.1) | 1674.4 | (1482.8; 2040.2) | 1729.2 | (1477.3; 2053.3) | 1794.7 | (1495.7; 2087.1) | ***0.013*** |
| Alcohol | | | | | | | | | |
| [g/d] | 5.0 | (2.3; 13.3) | 4.3 | (2.1; 8.6) | 5.9 | (3.0; 18.2) | 5.6 | (2.2; 16.6) | ***0.024*** |
| [%E] | 2.0 | (0.9; 5.4) | 1.8 | (0.9; 3.6) | 2.4 | (1.2; 7.4) | 2.2 | (0.8; 6.5) | |
| Total fat | | | | | | | | | |
| [g/d] | 75.3 | (65.0; 88.1) | 72.0 | (64.2; 87.2) | 74.3 | (63.4; 85.3) | 77.3 | (66.0; 90.0) | ***0.021*** |
| [%E] | 39.1 | (33.7; 45.7) | 38.7 | (34.5; 46.9) | 38.7 | (33.0; 44.4) | 38.8 | (33.0; 45.1) | |
| SFA | | | | | | | | | |
| [g/d] | 33.1 | (28.7; 38.6) | 32 | (28.1; 37.4) | 32.5 | (28.0; 37.9) | 34.5 | (29.5; 39.6) | ***0.026*** |
| [%E] | 17.2 | (14.9; 20.0) | 17.2 | (15.1; 20.1) | 16.9 | (14.6; 19.7) | 17.3 | (14.8; 19.9) | |
| MUFA | | | | | | | | | |
| [g/d] | 26.9 | (22.9; 32.3) | 25.6 | (22.5; 31.5) | 26.8 | (22.5; 30.8) | 28.0 | (23.9; 32.9) | ***0.008*** |
| [%E] | 14.0 | (11.9; 16.8) | 13.8 | (12.1; 16.9) | 13.9 | (11.7; 16.0) | 14.0 | (12.0; 16.5) | |
| PUFA | | | | | | | | | |
| [g/d] | 9.7 | (8.1; 11.9) | 9.6 | (7.9; 12.4) | 9.3 | (7.9; 11.5) | 10.0 | (8.5; 11.8) | 0.071 |
| [%E] | 5.0 | (4.2; 6.2) | 5.2 | (4.2; 6.7) | 4.8 | (4.1; 6.0) | 5.0 | (4.3; 5.9) | |
| Total protein | | | | | | | | | |
| [g/d] | 66.9 | (57.3; 77.4) | 63.6 | (55.1; 76.3) | 66.9 | (54.8; 75.1) | 69.4 | (60.1; 79.0) | ***< 0.001*** |
| [%E] | 15.4 | (13.2; 17.8) | 15.2 | (13.2; 18.2) | 15.5 | (12.7; 17.4) | 15.5 | (13.5; 17.6) | |
| Total carbohydrate | | | | | | | | | |
| [g/d] | 180.6 | (149.8; 214.9) | 180.4 | (150.6; 220.8) | 179.8 | (147.0; 213.0) | 181.1 | (149.8; 210.7) | 0.873 |
| [%E] | 41.6 | (34.5; 49.5) | 43.1 | (36.0; 52.7) | 41.6 | (34.0; 49.3) | 40.4 | (33.4; 47.0) | |

**Table 4** Associations of food groups and dietary patterns with FLI, overall and stratified by metabotype cluster[a]

| Food items | ß-estimate | 95% CI | *p*-value | Adjusted *p*-value** |
|---|---|---|---|---|
| Total participants (*n* = 689) | | | | |
| Total fruits [g/d] | 0.006 | (-0.026; 0.037) | 0.715 | 0.739 |
| Total vegetables [g/d] | -0.028 | (-0.079; 0.022) | 0.271 | 0.316 |
| Total nuts [g/d] | -0.221 | (-0.42; -0.022) | ***0.029*** | ***0.041*** |
| Total meat [g/d] | 0.466 | (0.389; 0.543) | ***< 0.001*** | ***< 0.001*** |
| Beef [g/d] | 0.801 | (0.229; 1.372) | ***0.006*** | ***0.011*** |
| Pork [g/d] | 1.016 | (0.694; 1.338) | ***< 0.001*** | ***< 0.001*** |
| Poultry [g/d] | 0.557 | (0.271; 0.842) | ***< 0.001*** | ***< 0.001*** |
| Total fish [g/d] | 0.325 | (0.173; 0.478) | ***< 0.001*** | ***< 0.001*** |
| Total eggs [g/d] | 0.607 | (0.42; 0.794) | ***< 0.001*** | ***< 0.001*** |
| Total dairy [g/d] | -0.004 | (-0.029; 0.021) | 0.739 | 0.739 |
| Whole grains [g/d] | -0.221 | (-0.347; -0.095) | ***0.001*** | ***0.001*** |
| Softdrinks [g/d] | 0.024 | (0.002; 0.045) | ***0.033*** | ***0.041*** |
| AHEI | -0.908 | (-1.163; -0.654) | ***< 0.001*** | ***< 0.001*** |
| MDS | -1.913 | (-3.353; -0.473) | ***0.009*** | ***0.014*** |
| **Food items** | **ß-estimate** | **95% CI** | ***p*-value** | **Adjusted *p*-value**** |
| Metabotype 1 (*n* = 140) | | | | |
| Total fruits [g/d] | -0.008 | (-0.074; 0.057) | 0.805 | 0.835 |
| Total vegetables [g/d] | -0.041 | (-0.161; 0.079) | 0.504 | 0.672 |
| Total nuts [g/d] | 0.032 | (-0.328; 0.392) | 0.861 | 0.861 |
| Total meat [g/d] | 0.273 | (0.081; 0.464) | ***0.006*** | ***0.016*** |
| Beef [g/d] | 0.207 | (-0.801; 1.215) | 0.685 | 0.781 |
| Pork [g/d] | 0.605 | (-0.269; 1.479) | 0.173 | 0.276 |
| Poultry [g/d] | 0.884 | (0.103; 1.665) | ***0.027*** | 0.058 |
| Total fish [g/d] | 0.359 | (-0.029; 0.746) | 0.069 | 0.129 |
| Total eggs [g/d] | 0.538 | (0.076; 1) | ***0.023*** | 0.053 |
| Total dairy [g/d] | 0.012 | (-0.033; 0.057) | 0.591 | 0.719 |
| Whole grains [g/d] | -0.206 | (-0.51; 0.098) | 0.182 | 0.276 |
| Softdrinks [g/d] | 0.03 | (-0.015; 0.075) | 0.187 | 0.276 |
| AHEI | -0.505 | (-1.114; 0.103) | 0.103 | 0.18 |
| MDS | -1.069 | (-4.678; 2.541) | 0.559 | 0.711 |
| **Food items** | **ß-estimate** | **95% CI** | ***p*-value** | **Adjusted *p*-value**** |
| Metabotypes 2 and 3 (summarized) (*n* = 549) | | | | |
| Total fruits [g/d] | 0.006 | (-0.029; 0.042) | 0.725 | 0.781 |
| Total vegetables [g/d] | -0.021 | (-0.076; 0.034) | 0.457 | 0.64 |
| Total nuts [g/d] | -0.32 | (-0.554; -0.085) | ***0.008*** | ***0.019*** |
| Total meat [g/d] | 0.487 | (0.404; 0.571) | ***< 0.001*** | ***< 0.001*** |
| Beef [g/d] | 1.002 | (0.322; 1.682) | ***0.004*** | ***0.012*** |
| Pork [g/d] | 1.083 | (0.734; 1.433) | ***< 0.001*** | ***< 0.001*** |
| Poultry [g/d] | 0.516 | (0.208; 0.824) | ***0.001*** | ***0.004*** |
| Total fish [g/d] | 0.3 | (0.136; 0.465) | ***< 0.001*** | ***0.002*** |
| Total eggs [g/d] | 0.629 | (0.424; 0.834) | ***< 0.001*** | ***< 0.001*** |
| Total dairy [g/d] | -0.006 | (-0.036; 0.024) | 0.711 | 0.781 |
| Whole grains [g/d] | -0.234 | (-0.371; -0.097) | ***0.001*** | ***0.004*** |
| Softdrinks [g/d] | 0.026 | (0; 0.051) | ***0.046*** | 0.091 |
| AHEI | -0.987 | (-1.264; -0.709) | ***< 0.001*** | ***< 0.001*** |
| MDS | -2.352 | (-3.911; -0.792) | ***0.003*** | ***0.011*** |

[a] linear regression models adjusted for sex, age, physical activity, education years, smoking status, energy intake, and metabotype cluster. CI, confidence interval

** False discovery rate (FDR)-adjusted

all participants, significant positive associations were observed for total meat and the sub-groups beef, pork, and poultry, for total fish, total eggs, and soft drinks whereas negative associations were obtained for nuts, whole grain products, and the diet quality indices, AHEI and MDS. No associations existed between fruits, vegetables and dairy products consumption and FLI.

For most food parameters, with the exception for total vegetables and the AHEI, a significant interaction with metabotypes (food * metabotype) was observed. These findings were supported by the results of the stratified analysis. For participants in the metabotype 1 cluster, the consumption of total meat, poultry and total eggs was significantly associated with the FLI, and only total meat remained its significance after correction for multiple testing. For the summarized metabotype cluster 2 and 3, we recognized many more significant associations which were similar to those reported for all participants; in the summarized metabotype cluster of 2 and 3, the consumption of total meat and the sub-groups beef, pork, and poultry, for total fish, total eggs, and softdrinks showed positive associations with FLI whereas negative associations were obtained for nuts, whole grain products, AHEI and MDS. However, the observed association with softdrinks lost its significance after FDR adjustment. Moreover, the ß-estimates were higher also in the metabotype 2 + 3 groups as compared to all study participants, suggesting greater effects of dietary intakes on FLI in this subgroup. The separate findings for cluster 2 and 3 are displayed in the supplementary data (Table S3).

### Effects of substituting carbohydrates with other macronutrients on the FLI

Table 5 provides the results of the carbohydrate substitution model. Replacing total carbohydrates with an isoenergetic amount of MUFA and protein was significantly positively associated with FLI. A greater effect was shown for the replacement of carbohydrates with protein which increased the FLI on average by 26.121 points compared to MUFA which increased the FLI by 11.482 points per 5 energy-% increase. On the other hand, an increase in PUFA intake at the expense of an isoenergetic amount of carbohydrate intake was significantly related with reduced FLI values by -23.332 points. Other energy substitution models (Table S4) confirm these findings.

**Table 5** Effects of substitution of carbohydrates by macronutrients (per 5 energy percent) on Fatty Liver Index[a]

| Per 5 energy-% increase | ß-estimate | 95% CI | *p*-value |
|---|---|---|---|
| SFA | -6.324 | (-14.391; 1.744) | 0.124 |
| MUFA | 11.482 | (1.129; 21.836) | ***0.03*** |
| PUFA | -23.332 | (-40.003; -6.662) | ***0.006*** |
| Protein | 26.121 | (18.825; 33.416) | ***< 0.001*** |
| Alcohol | 0.775 | (-3.384; 4.934) | 0.715 |

[a] Substitution models contained total energy intake, SFA, MUFA, PUFA, protein, and alcohol intake. Estimates are therefore interpreted as the association of FLI with a 5 E% increase in e.g. PUFA at the expense of carbohydrates while energy supply from other macronutrients remains unchanged; linear regression models adjusted for sex, age, physical activity, education years, smoking status and energy intake. CI, confidence interval

## Discussion

### Main findings

In our study we observed beneficial effects of plant-based food items and dietary patterns, especially nuts, whole grain products, the AHEI, and the MDS on FLI values in contrast to unfavourable effects of soft drinks and animal-derived products such as meat, eggs or fish. According to the results of the energy substitution models, a higher PUFA intake exerts a favourable relationship with FLI while protein and MUFA intakes are associated with higher FLI. Regarding metabotype subgroups, participants with metabotype 2 and 3 revealed stronger associations with dietary factors, as compared to the metabolically more healthy participants in cluster 1. These findings argue for a differential benefit from dietary modifications between metabotypes.

### Effects of foods of plant or animal origin on fatty liver index

The positive outcomes of plant-based diets and food items regarding fatty liver are concordant with previously conducted studies investigating the effects of diet on specific liver markers or liver imaging diagnostics. In 2023, a cohort study from North China with 14,541 participants reported a significant risk reduction for MAFLD measured by liver ultrasonography when replacing animal derived food items, i.e. meat, eggs or fish, with an equivalent serving of whole grains [29]. In the 2005–2018 NHANES study with 25,360 adult participants from the US, especially nut intake was significantly associated with lower FLI values [30]. Whole grain products and nuts are both rich sources of dietary fiber, minerals and antioxidants which are known to lower the risk of chronic diseases [31, 32], and especially fiber has been shown to yield a protective effect on the development of MAFLD [33]. Whole grains and nuts are characteristic components of the Mediterranean Diet which also has been suggested as an effective dietary approach in the management of MAFLD [34, 35]; also in our study, a high MDS was inversely associated with FLI.

High consumption of animal-derived food items, especially meat products, has been shown to detrimentally affect liver health [36]. A meta-analysis from 2020 of 24 observational studies found that red meat consumption

is associated with a higher likelihood of MAFLD but did not report unfavourable effects of other animal products, such as dairy, eggs or fish [37]. Especially the presence of heme–iron in red meat which has also been shown to unfavourably affect liver health [38] could be a possible explanation for this observation. While other studies reported conflicting results for egg consumption [39, 40], regarding fish and dairy products even inverse relationships with fatty liver measurements were reported; these findings may be attributable to the content of long-chain omega-3-fatty acids in fish or probiotics and minerals like calcium present in dairy products [41, 42]. These reports are partly in contrast to the findings of our study which showed unfavourable associations for all animal-based food items, except for dairy products. A possible explanation could be the low amount and differences in the processing of such foods. The last report from the 2023 European Market Observatory for Fisheries and Aquaculture products (EUMOFA) described the majority of the fish in Germany were sold in a processed form (e.g. preserved in brine, salt water or with a sauce) [43], possibly outweighing health-favourable aspects of sea food, especially long-chain n3-PUFA. Processed fish consumption has already been associated with a higher all-cause mortality risk in previous studies [44].

We also found a positive association between soft drink consumption and FLI values, which may be due to their high sugar, and especially high fructose content [45]. This observation is in concordance with the previously mentioned meta-analysis which came to the same conclusion [37]. Fructose is known as a main mediator in the development of fatty liver [8] and clinical guidelines from the European Society for Clinical Nutrition and Metabolism (ESPEN) generally advise to limit its consumption [35].

#### Effects of different macronutrients on fatty liver

While the beneficial effects and mechanisms of the isocaloric replacement of dietary carbohydrates with total fat on liver fat content has been demonstrated in intervention studies in obese subjects (recently reviewed by Lundsgaard et al. [46]), less is known about the impact of the different types of fatty acids and the related food sources.

A potential mechanism of the liver health-promoting effects of plant-derived foods, especially nuts and seeds, is their high PUFA content. In randomized controlled trials, PUFAs have been shown to improve metabolic health by lowering liver fat accumulation compared to an isocaloric amount of SFAs [47, 48]. Conversely, the effects of protein and MUFA consumption on the development of fatty liver are still inconclusive. Interventional studies that directly assessed the effects of MUFAs or protein reported beneficial outcomes on indicators of fatty liver [49–51] while observational studies did not find any or even unfavourable associations [52, 53]. Moreover, the dietary source seems to play a major role in the effects of both macronutrients on liver health which could explain the diverging findings in the mentioned studies. Proteins from plant sources have been shown to have beneficial effects on MAFLD risk while the opposite seems to be the case for animal-based protein [29]. Also, MUFA administration in interventional studies is often derived from plant products such as olive oil [49]. In contrast, the main sources of protein and MUFAs in countries like Germany are typically animal products, especially meat [54, 55], which may also explain the observed associations of protein and MUFA consumption with FLI values in our study. Surprisingly, for SFA intake we did not observe a significant association with FLI which may be due to its presence (partly as medium-chain SFA) in dairy products which are a main contributor to total SFA consumption in Europe [55]. Moreover, recent evidence suggests that the likelihood of the development of MAFLD is indeed inversely associated with dairy consumption [42].

However, if consumed in excess, dietary fat appears to promote overall energy consumption and thus, contribute to the development of metabolic diseases including obesity [56]. Potential mechanisms for this observation include a detrimental modulation of hormones such as ghrelin, glucagon-like peptide-1, and cholecystokinin (involved in the regulation of appetite) as well as a potential dysregulation of gastrointestinal motor function which has been comprehensively reviewed by Little et al. [56]. The importance of gut hormones on MAFLD development is also supported by evidence from clinical intervention studies involving bariatric surgery which, besides effectively promoting long-term body weight reduction, also could ameliorate obesity-related MAFLD by the modulation of hormones associated with appetite regulation [57]. Therefore, dietary fat could be consumed in exchange, and not on top of dietary carbohydrates to avoid potential detrimental effects on gut hormones and excessive energy intake.

#### Influence of metabotypes on the relationship between diet and MAFLD

Besides the higher prevalence of MAFLD in obese populations, it has been shown that obese subjects with MAFLD additionally show a more unfavourable metabolic profile compared to lean MAFLD patients [58]. Therefore, the individual metabotype (as used here) may be a potential effect modifier by influencing the individual response to environmental factors such as diet. In fact, in our study we got clear indication of effect modification by metabotype clusters for the association of dietary factors and the prevalence of FLI.

Stratifying study participants into metabotype subgroups is an emerging approach in nutrition research to identify groups that benefit most from specific dietary modifications [11]. Previous studies investigated the effects of food items or nutrients on specific disease markers in different metabotype subgroups. For example, it has been shown that the effect of bread consumption on postprandial insulin response [59] or the influence of vitamin D on markers of the metabolic syndrome [60] differ depending on the individual metabotype. However, studies with more detailed dietary data to analyse the associations of several food groups on health outcomes while also considering effect modification by metabotypes are still rare. In an earlier study, Riedl et al. used metabotype subgrouping to investigate the effects of the consumption of different food items on type 2 diabetes mellitus (T2DM) [27]. Three metabotype clusters were created which were based on BMI and 15 other parameters derived from blood serum measurements. Cluster 1 was defined as the more favourable metabolic profile while cluster 3 was described as the most unfavourable. While in cluster 1 significant positive associations with T2DM were reported only for increased intakes of total meat and processed meat, in the cluster 3, however, a significant positive association with T2DM was found for the increased consumption of sugar-sweetened beverages and an inverse association was observed for increased fruit intake. The authors concluded that depending on the individual metabotype, dietary factors may exert differential effects on the risk of T2DM [27]. Our study complements these results by using a similar metabotyping approach in the analysis of the consumption of different food items with the FLI. We observed significant associations of meat consumption with the FLI in all metabotype subgroups which is in line with the clear negative impact of meat intake on fatty liver as reported frequently in previous studies [36, 37]. The conflicting results for other food items in the literature, especially of foods of animal origin, may be partly explained by possible heterogenous metabolic profiles of the subjects included in the studies, a point which should be addressed in further investigations.

#### Practical implications and potential applications

The knowledge of the differential associations between dietary factors and fatty liver depending on the individuals' metabotype may contribute to a more personalized approach in dietary counselling. In 2015, a study by O'Donovan et al. firstly suggested a targeted method based on individual metabotypes to individualize dietary advices in clinical settings [61] which could be expanded on a population-based level to provide innovative primary prevention strategies for public health. In fact, Hillesheim et al. could demonstrate the effectiveness of such a concept in a randomized trial [62].

In the study by Riedl et al., the authors also suggested that the prevention of diabetes could especially benefit from a change in the dietary behaviour of individuals with an unfavourable metabotype [27]. Thus, this implication may be also applicable to the primary prevention of NAFLD according to the findings of our study where we so more distinct relationships between dietary factors on FLI in clusters 2 and 3. Explicitly, the results of our study may suggest a reduction in the consumption of animal-based food items and soft drinks in favour of PUFA-rich foods, nuts as well as whole grain products in subjects with a more unfavourable metabolic profile as a working hypothesis for primary prevention of MAFLD. Moreover, for those individuals, it may be also advisable to follow a healthy dietary pattern as indicated by the AHEI to further reduce the likelihood of fatty liver development. Besides the current ESPEN guidelines for NAFLD which mainly recommend adherence to an energy-reduced Mediterranean diet [35], the findings of our study may add new possibilities for the dietary prevention strategies of NAFLD by suggesting specific food item choices and respecting the individual metabolic profile. However, future, large, prospective studies and finally intervention studies are needed to confirm our results and the delineated hypotheses.

#### Strengths and limitations

Strengths of our study are a food-based approach compared to previously nutrient-based analyses as a more practical method for generating public health recommendations, and the investigation on a population-based level with a high number of study subjects. Also, our study is among the first to stratify the analysis of food items and FLI by metabolic characteristics (i.e., metabotypes) of the study participants which may allow more individualized and effective dietary recommendations regarding fatty liver prevention. However, some limitations have to be addressed. Compared to metabotype cluster 2, cluster 1 and, especially cluster 3 contained remarkably fewer participants which led to a more uneven distribution of the study participants across the metabotype clusters. Thus, we combined the persons in clusters 2 and 3 for the statistical analysis, but the relatively small sample size of cluster 1 remains as a potential limitation. Another weakness of population-based studies is the measurement error adherent to dietary assessment data. As a consequence of recall bias, study participants may not be able to remember the exact food items and portion sizes which were required to precisely answer the questions about their habitual diet. However, with the combination of repeated 24-h recalls and an FFQ we tried to improve the validity and precision

of the dietary intake data. Moreover, the derivation of macronutrient intake values is based on average values for the individual food items (as taken from the German food composition database), and the true macronutrient content in food actually consumed may deviate from the average. Also, the fatty liver index is not as precise as other methods to classify subjects according to their liver fat content (MR imaging; liver biopsy) and to classify fatty liver disease. Moreover, further data on other laboratory parameters for insulin resistance such as the HOMA index were not available in our dataset for a more precise description of the metabolic health of the study sample. Furthermore, the KORA S4 Fit survey was performed in a specific population and the results may not be fully applicable to other age or ethnic groups.

## Conclusion

In our population-based study, the consumption of nuts, whole grains, and PUFAs or following a healthy dietary pattern, represented as AHEI and MDS, was inversely associated with FLI, whereas for the intake of soft drinks and animal products such as meat, fish and eggs, we reported positive associations with FLI. The impact of diet on fatty liver may be modified by the individual metabotype of the study participants which needs further investigation in future prospective studies. By offering new insights about the interplay of diet and fatty liver at the population level, our study may promote the development of novel primary prevention strategies for MAFLD.

### Abbreviations

| | |
|---|---|
| 24HFLs | 24-H food lists |
| FFQ | Food frequency questionnaire |
| KORA | Cooperative Health Research in the region of Augsburg |
| MDS | Mediterranean Diet Score |
| AHEI | Alternate Healthy Eating Index |
| MONICA | Monitoring trends and determinants in cardiovascular disease |
| WHO | World Health Organization |
| EPFQ | European Food Propensity Questionnaire |
| VIF | Variance-Inflation-Factor |
| BMI | Body-Mass-Index |
| FDR | False-Discovery-Rate |
| SFA | Saturated fatty acids |
| PUFA | Polyunsaturated fatty acids |
| MUFA | Monounsaturated fatty acids |
| ESPEN | European Society for Clinical Nutrition and Metabolism |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12944-024-02094-0.

**Additional file 1: Table S1.** Characteristics of all study participants and by sex. **Table S2.** Habitual food and nutrient consumption data and dietary patterns in all participants and by metabotype cluster. **Table S3.** Associations of food groups and subgroups and dietary patterns with FLI, overall and stratified by metabotype cluster. **Table S4.** Effects of substitution of macronutrients by saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), protein and alcohol (per 5 energy percent) on Fatty Liver Index (FLI).

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### Informed consent statement

Informed consent was obtained from all subjects involved in the study.

### Authors' contributions

M.S. conducted the statistical analysis, interpreted the data, and drafted and revised the manuscript; J.L. designed the study, was responsible for the dietary assessment and intake calculation, and supervised the work; C.M. contributed to the design of the study, the data interpretation, and the revision of the draft manuscript; D.F. supervised the statistical analysis; N.W. calculated the dietary intake data; A.P. and M.H. were responsible for the design and conduct of the KORA FIT study; D.T. was in charge of the laboratory analyses; All authors have read and agreed to the submitted version of the manuscript.

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### Availability of data and materials

The data are subject to national data protection laws, and restrictions were imposed by the Ethics Committee of the Bavarian Chamber of Physicians to ensure data privacy of the study participants. Therefore, data cannot be made freely available in a public repository. However, data can be requested through an individual project agreement with KORA via the online portal KORA (https://www.helmholtz-munich.de/en/epi. Accessed 06 Dec 2023).

## Declarations

### Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the Bavarian Chamber of Physicians (KORAFit EC No 17040; 14 Nov 2017). The investigations were conducted in accordance with the Declaration of Helsinki.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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**Supplementary data for the article "Association of the habitual dietary intake with the fatty liver index and effect modification by metabotypes in the population-based KORA-Fit study"**

**Table S1.** Characteristics of the study participants and by sex

| | **Total n = 689** | | **Males n = 304** | | **Females n = 385** | | |
|---|---|---|---|---|---|---|---|
| **Characteristics** | **Median (25th-75th percentile)** | | | | | | **p-value** |
| Age [years] | 63 | (58; 68) | 64 | (58; 68) | 63 | (59; 68) | 0.659 |
| BMI [kg/m²] | 27.4 | (24.1; 30.7) | 27.9 | (25.3; 31.1) | 26.64 | (23.4; 30.4) | ***0.001*** |
| Waist circumference [cm] | 93.6 | (82.8; 103.0) | 99.6 | (91.8; 107.9) | 86.5 | (78.1; 97.4) | ***< 0.001*** |
| FLI | 54.9 | (24.4; 89.4) | 79.1 | (41.4; 96.5) | 36.2 | (16.1; 76.9) | ***< 0.001*** |
| HDL [mg/dl] | 61.0 | (49.0; 75.4) | 53.0 | (43.5; 64.0) | 71.0 | (57.0; 83.5) | ***< 0.001*** |
| non-HDL [mg/dl] | 146.0 | (120.1; 174.0) | 144.3 | (115.0; 174.9) | 147.0 | (122.3; 173.9) | 0.200 |
| Triglycerides [mg/dl] | 107.0 | (77.0; 150.3) | 114.0 | (81.3; 166.0) | 100.0 | (73.6; 139.0) | ***< 0.001*** |
| gGT [U/l] | 25.0 | (17.0; 39.0) | 32.0 | (22.0; 48.0) | 20.0 | (14.0; 30.0) | ***< 0.001*** |
| AST [U/l] | 24.0 | (20.3; 28.1) | 26.0 | (22.0; 30.3) | 22.5 | (19.1; 26.7) | ***< 0.001*** |
| ALT [U/l] | 24.8 | (19.0; 32.0) | 29.0 | (23.0; 36.2) | 21.5 | (17.0; 27.0) | ***< 0.001*** |
| Uric acid [mg/dl] | 5.3 | (4.4; 6.4) | 6.28 | (5.4; 7.2) | 4.7 | (4.1; 5.4) | ***< 0.001*** |
| Fasting glucose [mg/dl] | 97.0 | (92; 105) | 101.0 | (94; 110) | 95.0 | (90; 102) | ***< 0.001*** |
| | **n (%)** | | | | | | |
| Education [years] | | | | | | | |
| <=12 years | 417 | (60.5) | 166 | (54.6) | 251 | (65.2) | ***< 0.001*** |
| >12 years | 272 | (39.5) | 138 | (45.4) | 134 | (34.8) | |
| Physical activity | | | | | | | |
| >=2h / week | 266 | (38.6) | 127 | (41.8) | 139 | (36.1) | 0.402 |
| 1h / week | 230 | (33.4) | 95 | (31.3) | 135 | (35.1) | |
| <1h / week | 88 | (12.8) | 40 | (13.2) | 48 | (12.5) | |
| (almost) no activity | 105 | (15.2) | 42 | (13.8) | 63 | (16.4) | |
| Smoking | | | | | | | |
| Current smoker | 82 | (11.9) | 40 | (13.2) | 42 | (10.9) | ***< 0.001*** |
| Former smoker | 298 | (43.3) | 149 | (49.0) | 149 | (38.7) | |
| Never smoker | 309 | (44.9) | 115 | (37.8) | 194 | (50.4) | |
| Hypertension | | | | | | | |
| Yes | 313 | (45.4) | 165 | (54.3) | 148 | (38.4) | ***< 0.001*** |
| No | 376 | (54.6) | 139 | (45.7) | 237 | (61.6) | |
| Diabetes | | | | | | | |
| Yes | 49 | (7.1) | 25 | (8.2) | 24 | (6.2) | 0.371 |
| No | 640 | (92.9) | 279 | (91.8) | 361 | (93.8) | |
| Metabotype | | | | | | | |
| 1 | 140 | (20.3) | 72 | (23.7) | 68 | (17.7) | ***< 0.001*** |
| 2 | 478 | (69.4) | 185 | (60.9) | 293 | (76.1) | |
| 3 | 71 | (10.3) | 47 | (15.5) | 24 | (6.2) | |

**Table S2**. Habitual food and nutrient consumption data and dietary patterns in all participants and by metabotype cluster

| | **Total** | | **Males** | | **Females** | | |
|---|---|---|---|---|---|---|---|
| **Food item** | **Median (25th-75th percentile)** | | | | | | **p-value** |
| Total fruits [g/d] | 149.6 | (93.2; 216.2) | 141.9 | (79.3; 214.6) | 151.9 | (103.5; 216.3) | ***0.041*** |
| Total vegetables [g/d] | 166.2 | (136.5; 202.7) | 150.9 | (125.7; 183.8) | 180.8 | (149.9; 218.3) | ***< 0.001*** |
| Total nuts [g/d] | 4.4 | (2.6; 13.0) | 5.4 | (3.2; 14.0) | 3.8 | (2.4; 11.9) | ***< 0.001*** |
| Total meat [g/d] | 101.1 | (74.5; 132.7) | 133.9 | (111.4; 160.0) | 79.3 | (65.0; 96.2) | ***< 0.001*** |
| Beef [g/d] | 8.1 | (6.1; 10.7) | 10.2 | (8.8; 14.7) | 6.2 | (5.2; 7.8) | ***< 0.001*** |
| Pork [g/d] | 15.6 | (10.7; 21.3) | 20.8 | (16.3; 26.6) | 11 | (9.3; 15.3) | ***< 0.001*** |
| Poultry [g/d] | 10.4 | (9.2; 17) | 13.5 | (9.97; 23.1) | 9.5 | (7.1; 13) | ***< 0.001*** |
| Total fish [g/d] | 18.7 | (12.6; 27.1) | 19.7 | (14.2; 30.1) | 17.6 | (11.9; 25.6) | ***< 0.001*** |
| Total eggs [g/d] | 16.0 | (11.4; 22.7) | 16.0 | (12.1; 23.2) | 15.9 | (11.2; 22.3) | 0.371 |
| Dairy [g/d] | 177.5 | (121.3; 259.7) | 156.9 | (108.3; 237.4) | 193.5 | (133.5; 268.7) | ***< 0.001*** |
| Whole grains [g/d] | 15.3 | (7.4; 35.3) | 14.8 | (7.3; 33.5) | 15.8 | (7.6; 36.1) | 0.936 |
| Sugar sweetened beverages [g/d] | 5.3 | (3.4; 13.3) | 7.8 | (5.3; 21.7) | 3.8 | (2.8; 5.9) | ***< 0.001*** |
| AHEI | 44.3 | (37.6; 51.2) | 42.8 | (36.5; 48.9) | 45.6 | (38.8; 52.5) | ***< 0.001*** |
| MDS | 4 | (3; 6) | 5 | (3; 6) | 4 | (3; 6) | ***0.015*** |
| Energy (Kilocalories) [kcal/d] | 1735.1 | (1488.9; 2064.1) | 2051.1 | (1839.2; 2291.6) | 1533.8 | (1383.3; 1711.4) | ***< 0.001*** |
| Alcohol consumption [g/d] | 5.0 | (2.3; 13.3) | 13.3 | (6.3; 25.7) | 2.7 | (1.6; 5.2) | ***< 0.001*** |
| Total fat consumption [g/d] | 75.3 | (65.0; 88.1) | 86.4 | (76.6; 97.5) | 67.1 | (60.3; 76.2) | ***< 0.001*** |
| SFA consumption [g/d] | 33.1 | (28.7; 38.6) | 38.0 | (33.9; 42.6) | 30.2 | (26.9; 33.9) | ***< 0.001*** |
| MUFA consumption [g/d] | 26.9 | (22.9; 32.3) | 31.2 | (27.6; 36.0) | 24.0 | (21.0; 27.5) | ***< 0.001*** |
| PUFA consumption [g/d] | 9.7 | (8.1; 11.9) | 11.1 | (9.5; 13.4) | 8.6 | (7.5; 10.4) | ***< 0.001*** |
| Total protein consumption [g/d] | 66.9 | (57.3; 77.4) | 76.0 | (68.0; 87.2) | 59.5 | (53.2; 68.1) | ***< 0.001*** |
| Total carbohydrate consumption [g/d] | 180.6 | (149.8; 214.9) | 206.7 | (179.9; 240.8) | 159.7 | (139.7; 189.1) | ***< 0.001*** |

**Table S3.** Associations of food groups and subgroups and dietary patterns with FLI, overall and stratified by metabotype cluster*

| Food items | ß-estimate | 95% CI | p-value | Adjusted p-value** |
|---|---|---|---|---|
| Metabotype 1 (n = 140) | | | | |
| Total fruits [g/d] | -0.008 | (-0.074; 0.057) | 0.805 | 0.835 |
| Total vegetables [g/d] | -0.041 | (-0.161; 0.079) | 0.504 | 0.672 |
| Total nuts [g/d] | 0.032 | (-0.328; 0.392) | 0.861 | 0.861 |
| Total meat [g/d] | 0.273 | (0.081; 0.464) | ***0.006*** | ***0.016*** |
| Beef [g/d] | 0.207 | (-0.801; 1.215) | 0.685 | 0.781 |
| Pork [g/d] | 0.605 | (-0.269; 1.479) | 0.173 | 0.276 |
| Poultry [g/d] | 0.884 | (0.103; 1.665) | ***0.027*** | 0.058 |
| Total fish [g/d] | 0.359 | (-0.029; 0.746) | 0.069 | 0.129 |
| Total eggs [g/d] | 0.538 | (0.076; 1) | ***0.023*** | 0.053 |
| Total dairy [g/d] | 0.012 | (-0.033; 0.057) | 0.591 | 0.719 |
| Whole grains [g/d] | -0.206 | (-0.51; 0.098) | 0.182 | 0.276 |
| Softdrinks [g/d] | 0.030 | (-0.015; 0.075) | 0.187 | 0.276 |
| AHEI | -0.505 | (-1.114; 0.103) | 0.103 | 0.180 |
| MDS | -1.069 | (-4.678; 2.541) | 0.559 | 0.711 |
| **Food items** | **ß-estimate** | **95% CI** | **p-value** | **Adjusted p-value**** |
| Metabotype 2 (n = 478) | | | | |
| Total fruits [g/d] | 0.009 | (-0.028; 0.047) | 0.617 | 0.700 |
| Total vegetables [g/d] | -0.034 | (-0.092; 0.025) | 0.257 | 0.392 |
| Total nuts [g/d] | -0.312 | (-0.559; -0.064) | ***0.014*** | ***0.048*** |
| Total meat [g/d] | 0.527 | (0.438; 0.616) | ***<0.001*** | ***<0.001*** |
| Beef [g/d] | 1.092 | (0.382; 1.801) | ***0.003*** | ***0.016*** |
| Pork [g/d] | 1.295 | (0.902; 1.687) | ***<0.001*** | ***<0.001*** |
| Poultry [g/d] | 0.492 | (0.155; 0.829) | ***0.004*** | ***0.023*** |
| Total fish [g/d] | 0.282 | (0.108; 0.456) | ***0.002*** | ***0.011*** |
| Total eggs [g/d] | 0.595 | (0.368; 0.821) | ***<0.001*** | ***<0.001*** |
| Total dairy [g/d] | -0.013 | (-0.045; 0.019) | 0.433 | 0.593 |
| Whole grains [g/d] | -0.286 | (-0.432; -0.141) | ***<0.001*** | ***0.001*** |
| Softdrinks [g/d] | 0.040 | (0.009; 0.071) | ***0.013*** | ***0.048*** |
| AHEI | -1.011 | (-1.304; -0.717) | ***<0.001*** | ***<0.001*** |
| MDS | -2.369 | (-4.025; -0.712) | ***0.005*** | ***0.023*** |
| **Food items** | **ß-estimate** | **95% CI** | **p-value** | **Adjusted p-value**** |
| Metabotype 3 (n = 71) | | | | |
| Total fruits [g/d] | 0.027 | (-0.025; 0.079) | 0.306 | 0.442 |
| Total vegetables [g/d] | 0.043 | (-0.033; 0.119) | 0.261 | 0.392 |
| Total nuts [g/d] | -0.272 | (-0.56; 0.015) | 0.063 | 0.145 |
| Total meat [g/d] | 0.100 | (-0.006; 0.205) | 0.063 | 0.145 |
| Beef [g/d] | 0.326 | (-0.598; 1.251) | 0.482 | 0.632 |
| Pork [g/d] | -0.130 | (-0.463; 0.204) | 0.438 | 0.593 |
| Poultry [g/d] | 0.272 | (-0.031; 0.574) | 0.077 | 0.154 |
| Total fish [g/d] | 0.191 | (-0.014; 0.396) | 0.067 | 0.145 |
| Total eggs [g/d] | 0.214 | (-0.05; 0.479) | 0.110 | 0.201 |
| Total dairy [g/d] | 0.037 | (0.004; 0.07) | ***0.029*** | 0.080 |
| Whole grains [g/d] | 0.196 | (0.016; 0.377) | ***0.034*** | 0.088 |
| Softdrinks [g/d] | 0.006 | (-0.021; 0.032) | 0.673 | 0.719 |
| AHEI | -0.107 | (-0.491; 0.278) | 0.580 | 0.689 |
| MDS | -0.420 | (-2.434; 1.595) | 0.677 | 0.719 |

* linear regression models adjusted for sex, age, physical activity, education years, smoking status, energy intake, and metabotype cluster. CI, confidence interval; ** False discovery rate (FDR)-adjusted

9

10 **Table S4.** Effects of substitution of macronutrients by saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), protein and alcohol (per 5 energy percent) on Fatty Liver Index (FLI) (dependent variables)*

| Per 5% increased nutrient | ß-estimate | 95% CI | p-value |
|---|---|---|---|
| w/o SFA | | | |
| Carbohydrates | 5.546 | (-2.361; 13.453) | 0.169 |
| MUFA | 16.654 | (0.156; 33.152) | ***0.048*** |
| PUFA | -17.263 | (-31.524; -3.002) | ***0.018*** |
| Protein | 31.921 | (20.566; 43.276) | ***<0.001*** |
| Alcohol | 6.255 | (-1.379; 13.889) | 0.108 |
| w/o MUFA | | | |
| Carbohydrates | -11.395 | (-21.146; -1.645) | ***0.022*** |
| SFA | -17.919 | (-33.774; -2.065) | ***0.027*** |
| PUFA | -34.462 | (-57.886; -11.039) | ***0.004*** |
| Protein | 14.099 | (0.805; 27.394) | ***0.038*** |
| Alcohol | -10.270 | (-20.844; 0.305) | 0.057 |
| w/o PUFA | | | |
| Carbohydrates | 18.942 | (2.553; 35.331) | ***0.024*** |
| SFA | 14.065 | (-0.241; 28.37) | 0.054 |
| PUFA | 28.981 | (4.53; 53.432) | ***0.020*** |
| Protein | 45.626 | (25.787; 65.465) | ***<0.001*** |
| Alcohol | 19.409 | (3.837; 34.982) | ***0.015*** |
| w/o Carbohydrates | | | |
| SFA | -6.324 | (-14.391; 1.744) | 0.124 |
| MUFA | 11.482 | (1.129; 21.836) | ***0.030*** |
| PUFA | -23.332 | (-40.003; -6.662) | ***0.006*** |
| Protein | 26.121 | (18.825; 33.416) | ***<0.001*** |
| Alcohol | 0.775 | (-3.384; 4.934) | 0.715 |
| w/o Protein | | | |
| Carbohydrates | -24.648 | (-31.495; -17.801) | ***<0.001*** |
| SFA | -31.074 | (-41.949; -20.199) | ***<0.001*** |
| MUFA | -14.014 | (-27.263; -0.765) | ***0.038*** |
| PUFA | -46.623 | (-65.562; -27.683) | ***<0.001*** |
| Alcohol | -23.113 | (-30.319; -15.906) | ***<0.001*** |
| w/o Alcohol | | | |
| Carbohydrates | -0.954 | (-5.229; 3.321) | 0.661 |
| SFA | -7.201 | (-15.207; 0.805) | 0.078 |
| MUFA | 10.468 | (-1.072; 22.008) | 0.075 |
| PUFA | -24.107 | (-40.388; -7.826) | ***0.004*** |
| Protein | 25.161 | (17.269; 33.054) | ***<0.001*** |
| w/o Total fat | | | |
| Carbohydrates | 0.128 | (-3.57; 3.826) | 0.946 |
| Protein | 25.148 | (16.014; 34.282) | ***<0.001*** |
| Alcohol | 1.955 | (-2.637; 6.548) | 0.403 |

* Substitution models contained total energy intake, SFA, MUFA, PUFA, protein, and alcohol intake. Estimates are therefore interpreted as the association of FLI with a 5 E% increase in e.g. PUFA at the expense of carbohydrates while energy supply from other macronutrients remains unchanged; linear regression models adjusted for sex, age, physical activity, education years, smoking status and energy intake. CI, confidence interval

# 4. Diskussion

## 4.1 Hauptergebnisse der Veröffentlichungen

In beiden Publikationen konnte gezeigt werden, dass sich der Konsum pflanzlicher Lebensmittel oder gesunder Ernährungsmuster förderlich auf die untersuchten gesundheitlichen Parameter auswirkt; dagegen war ein vermehrter Verzehr von Lebensmitteln tierischen Ursprungs oder Softdrinks insbesondere für die Entwicklung der Fettleber ungünstig zu bewerten. Über eine Erhöhung der D-Dimere könnten Butter oder Milchprodukte möglicherweise zu einer Blutgerinnungsneigung und damit zu einem erhöhten Risiko für Herz-Kreislauf-Erkrankungen beitragen, während der Konsum von Fleisch, Fisch und Eiern mit einer vermehrten Ausprägung der Fettleber assoziiert war; dagegen konnten für Nüsse, Vollkornprodukte oder eine Mediterrane Ernährungsweise gegenteilige Zusammenhänge gezeigt werden.

## 4.2 Einfluss pflanzlicher Lebensmittel oder einer pflanzenbetonten Ernährungsweise (Ernährungsmuster)

In der eingangs erwähnten GBD-Studie konnte sowohl auf die Lebenserwartung als auch auf DALYs gezeigt werden, dass sich besonders eine zu geringe Zufuhr pflanzlicher Lebensmittel wie Hülsenfrüchten, Obst, Gemüse, Nüsse und Samen sowie besonders ein unzureichender Konsum von Vollkornprodukten ungünstig auf die Gesundheit auswirken könnte [5]. Alleine dem letzteren Parameter waren im Jahr 2019 geschätzt 1,8 Millionen Todesfälle und 42,6 Millionen DALYs zuzuschreiben [5].

Pflanzliche Lebensmittel unterscheiden sich von vielen tierischen Produkten nennenswert im Gehalt an Antioxidantien, Mikronährstoffen und Ballaststoffen. Während bei einer Analyse von 3100 Lebensmitteln nur in ausgewählten tierischen Produkten Antioxidationsmengen von über 0,5 mmol/100 g nachgewiesen werden konnten, sind beispielsweise für die analysierte Kategorie „Pflanzen-basierte Lebensmittel“ durchschnittlich 11,57 mmol Antioxidantien pro 100 g festgestellt worden. Allgemein war das für pflanzliche Produkte bestimmte arithmetische Mittel in Bezug auf die absoluten Antioxidationsmengen um mehr als den Faktor 60 erhöht im Vergleich zu tierischen Produkten [26]. Bedeutende antioxidativ wirkende Nährstoffe, die besonders in pflanzlich-basierten Lebensmitteln vorkommen, sind die Vitamine C und E, Carotenoide sowie

Polyphenole [27]. Im menschlichen Körper können diese die bei Entzündungsreaktionen produzierten sog. reaktiven Sauerstoffspezies eindämmen, wodurch eine kontinuierliche, gering-gradige systemische Inflammation beschränkt und so der Entwicklung von NCDs wie Herz-Kreislauf-Erkrankungen oder Diabetes entgegengewirkt werden könnte [28]. In der Tat sind die Blutkonzentrationen von Carotenoiden und der Vitamine C und E stark mit einer geringeren Gesamt-Sterblichkeit sowie einem geringeren Risiko für Herz-Kreislauf-Erkrankungen assoziiert [29]. Vor allem für Polyphenole wird auch eine gerinnungshemmende Wirkung vermutet, die sich zusätzlich günstig auf Thrombose-assoziierte Erkrankungen auswirken könnte [30]. Zudem konnte für das Risiko der MAFLD eine inverse Beziehung mit der Zufuhr der die Vitamine C und E festgestellt werden [31].

Ballaststoffe sind darüber hinaus (bis auf die Ausnahme des Chitins in Insekten) ausschließlich in pflanzlichen Lebensmitteln enthalten [32,33]. Im menschlichen Körper werden Ballaststoffe von den Darmbakterien in sog. kurzkettige Fettsäuren umgewandelt, die sich vor allem förderlich auf das Epithel der Darmschleimhaut und letztlich die Prävention von Stoffwechselerkrankungen auswirken [34]; kurzkettige Fettsäuren weisen auch einen günstigen Zusammenhang mit Blutgerinnungs- und Entzündungsmarkern auf [35]. Mögliche zugrundeliegende Mechanismen könnten u.a. eine Bindung der kurzkettigen Fettsäuren an Free Fatty Acid-Rezeptoren auf Immunzellen sein, das die Produktion entzündungshemmender Zytokine modulieren könnte, sowie in Makrophagen eine Hemmung von Histondeacetylasen, welche die Bildung pro-entzündlicher Substanzen auf Zellebene beeinflussen [36]. Darüber hinaus hat ein hoher Konsum von Ballaststoffen auf weitere NCDs wie Herz-Kreislauf-Erkrankungen oder Darmkrebs nachweislich einen schützenden Effekt [32]. Eine geringe Zufuhr von Ballaststoffen wird in der GBD-Studie als unabhängiger ernährungsbedingter Risikofaktor hinsichtlich der Gesamtsterblichkeit an siebter Stelle gelistet [5].

Dennoch ist der gesundheitsförderliche Einfluss einer vegetarischen oder rein veganen Ernährung, bei welcher der Konsum tierischer Produkte vollständig vermieden wird, umstritten. Während in Meta-Analysen und umfangreichen Kohortenstudien überwiegend gezeigt werden konnte, dass vor allem eine vegetarische Ernährungsweise mit einem geringeren Risiko für kardiovaskuläre Erkrankungen assoziiert ist [37-40], wird hingegen häufig kein signifikanter Effekt auf die Gesamtsterblichkeit beobachtet [37,39,41]. Eine der wenigen Studien, in welcher für die sich vegan ernährende Untergruppe ein signifikant geringeres Sterblichkeitsrisiko im Vergleich zur Mischköstler-

Gruppe gezeigt werden konnte, ist die Adventist Health Study 2, einer umfangreichen Kohortenstudie mit insgesamt 73.308 Teilnehmern aus den USA. Während jedoch hier eine Risikoreduktion bei -15% für die vegane Gruppe gezeigt werden konnte, konnte hingegen bei den pesco-vegetarischen Studienteilnehmern, die noch zusätzlich Fischprodukte in ihre Ernährung inkludierten, die größte Risikoreduktion von -19% im Vergleich zur Mischköstler-Gruppe beobachtet werden [38].

In Bezug auf die Beeinflussung der Blutgerinnung durch eine vegetarische Ernährung wurden unterschiedliche Ergebnisse berichtet, sodass hierfür letztendlich keine klare Aussage getroffen werden kann [42,43]. Hinsichtlich der Entwicklung der Fettleber schien sich in bisherigen Studien eine vegetarische Ernährung überwiegend positiv auf das Erkrankungsrisiko auszuwirken, jedoch verschwindet der signifikante Effekt häufig nach einer Adjustierung für den BMI, sodass vermutlich eine im Schnitt geringere Energiezufuhr in vegetarischen Ernährungsformen für die Ergebnisse verantwortlich ist [44,45]. Eine ungesündere Form der vegetarischen Ernährung, reich an Zucker und verarbeiteten Produkten, wirkt sich zudem nachweislich abträglich auf das MAFLD-Risiko aus [46]. Vor allem für den Konsum gezuckerter Getränke sind sowohl in bisherigen Studien als auch in der zweiten Publikation dieser Dissertation signifikante Effekte auf die Fettleberentwicklung beschrieben worden [47,48]. Besonders Zucker in der isolierten Form von Fructose wird hierfür mechanistisch verantwortlich gemacht, während für einen Konsum von Fructose über den Verzehr von Lebensmitteln natürlichen Ursprungs wie z.B. Obst keine derartigen Auswirkungen beobachtet werden [49,50].

Neben der Lebensmittelqualität ist für die gesundheitlichen Auswirkungen einer pflanzlichen Ernährung auch die absolute Zufuhrmenge entscheidend. Bis zu einer Menge von 800 g/Tag scheint sich beispielsweise der Obst- und Gemüsekonsum in einer Dosis-Wirkungs-Beziehung günstig auf das Risiko von mit Thrombose-assoziierten NCDs wie die koronare Herzkrankheit und Schlaganfälle sowie auch die Lebenserwartung allgemein auszuwirken [51]. Für die Ballaststoff-Zufuhr aus Getreide, Obst und Gemüse konnte zudem auch für die MAFLD eine lineare inverse Beziehung gezeigt werden [52]. Die deutsche Gesellschaft für Ernährung (DGE) empfiehlt daher für Erwachsene täglich 2 Portionen Obst sowie 3 Portionen Gemüse; bei einer Portionsmenge von ca. 110 g ergibt sich daraus die Empfehlung, 550 g Obst und Gemüse pro Tag zu verzehren [53]. Laut der letzten durchgeführten Nationalen Verzehrsstudie II wird dies jedoch von über 80% der Befragten in Deutschland nicht erreicht [54]. Auch in den beiden vorgelegten Publikationen sind im Schnitt bei den Studienteilnehmenden deutlich geringere

Zufuhrmengen beobachtet worden. Im ersten Projekt betrug die durchschnittliche Zufuhrmenge von Obst ca. 145 g und für Gemüse 164 g; im zweiten Projekt für Obst ca. 150 g und für Gemüse 166 g. Auch wenn in den Publikationen kein signifikanter Effekt für den Obst- und Gemüse-Konsum auf die untersuchten Parameter gezeigt werden konnte, konnten für eine Ernährungsweise (dietary pattern), wie die Mediterrane Ernährung, im Falle des FLI nachweislich günstige Zusammenhänge beobachtet werden. Dies deckt sich auch mit Daten aus weiteren Studien, die für die Mediterrane Ernährung, aber auch für ein allgemein Gemüse-reiches Ernährungsmuster gesundheitsförderliche Effekte hinsichtlich einer MAFLD-Entwicklung aufgezeigt haben [55,56]. Zudem wird auch bezüglich der Blutgerinnung ein Zusammenhang mit anti-thrombotischen Effekten einer Mediterranen Ernährung berichtet, das wiederum mit günstigen kardiovaskulären Auswirkungen einer solchen Ernährungsweise in Verbindung gebracht wird [57].

## 4.3 Einfluss von Lebensmitteln tierischen Ursprungs

Der Konsum von Fisch ebenso wie die Zufuhr der langkettigen Omega-3-Fettsäuren Eicosapentaen- und Docosahexaensäure zeigten in randomisierten, kontrollierten Studien eine protektive Wirkung auf die Ausprägung einer Fettleber bei den Studienteilnehmenden [58-60]. Auch hinsichtlich der Herz-Kreislauf-Gesundheit besteht eine überzeugende Datenlage für eine krankheitspräventive Wirkung der Fischöl-Fettsäuren [61]. Im Allgemeinen ist auch der GBD-Studie zufolge ein Konsum an tierischen Lebensmitteln wie Milch oder Omega-3-Fettsäure-reiche Meerestiere für eine Senkung der Sterblichkeit und DALYs durch NCDs von Bedeutung [5]. Eicosapentaensäure und Docosahexaensäure sind vor allem in fettigem Fisch (auch in Algen) enthalten und scheinen sich im menschlichen Körper über die Bildung von entzündungshemmenden und anti-thrombotischen Metaboliten (z.B. spezifische Eicosanoide) positiv auf Stoffwechsel- und Herz-Kreislauf-Erkrankungen auszuwirken [62-64]. Daher plädieren auch Leitlinien internationaler Fachgesellschaften sowohl bei Herz-Kreislauf-Erkrankungen als auch bei der MAFLD für eine sog. Mediterrane Ernährung, die zwar pflanzlich basiert ist, jedoch auch tierische Produkte wie z.B. fettigen Fisch in einem gewissen Maß als festen Bestandteil mit einschließt [65,66]. Bereits in mehreren Meta-Analysen und systematischen Reviews konnte gezeigt werden, dass sich eine derartige Ernährungsweise sehr günstig auf die Herz-Kreislauf-Gesundheit [67,68], Leberfettparameter [69,70] und letztendlich auch auf die Gesamt-Sterblichkeit [71,72] auswirkt. Jedoch ist wie bei der vegetarischen Ernährung auch bei dem Konsum von Lebensmitteln im

Rahmen einer Mediterranen Ernährung der Grad an industrieller Verarbeitung zu beachten. Industriell verarbeiteter Fisch, wie z.B. in frittierter Form, scheint in einigen Kohortenstudien mit einer erhöhten Herz-Kreislauf- und Gesamt-Mortalität assoziiert zu sein [73], was eine mögliche Erklärung für die nicht signifikanten bzw. im Falle der Assoziation mit der Fettleber sogar gesundheitlich ungünstigen Zusammenhängen mit dem Fischkonsum in den beiden Publikationen darstellen könnte. Eine Mediterrane Ernährung war auch hinsichtlich des Risikos für die Entwicklung von Fettleber in der vorgelegten Studie günstig zu bewerten, was auch den beschriebenen aktuellen Stand der Datenlage wiederspiegelt [56].

Für den regelmäßigen hohen Konsum von rotem Fleisch wurde ein ungünstiger Einfluss auf das Risiko für NCDs beobachtet [5]. Dies könnte unter anderem am sogenannten Häm-Eisen liegen, einer oxidierten Form des Eisens, das ausschließlich in tierischen Lebensmitteln natürlich vorkommt und unabhängig von der Gesamteisenaufnahme mit einer erhöhten Prävalenz von Herz-Kreislauf-Erkrankungen assoziiert ist [74,75]. Häm-Eisen wird eine entzündungsförderliche Wirkung durch eine Begünstigung der Produktion von ROS zugeschrieben [75], die auch für andere Krankheitsbilder gezeigt werden konnte, einschließlich der Entwicklung von MAFLD [76]. In der zweiten Publikation konnte von uns für einen zunehmenden Fleischkonsum eine signifikante Assoziation mit der Fettleber sowohl in der metabolisch günstigeren als auch in der ungünstigeren Untergruppe gezeigt werden. Auch die vor allem in tierischen Produkten enthaltenen gesättigten Fettsäuren scheinen sich besonders bei einem Austausch gegen mehrfach ungesättigte Fettsäuren abträglich auf das Herz-Kreislauf-Risiko sowie die Lebenserwartung auszuwirken [77]. Dies geschieht vermutlich hauptsächlich durch eine ungünstige Beeinflussung des Lipidprofils wie etwa einer Erhöhung der LDL-Cholesterin-Konzentrationen; blutgerinnungsfördernde Effekte werden jedoch ebenfalls als weiterer möglicher Faktor diskutiert [78].

Auch wenn Lebensmittel tierischen Ursprungs bei einem vermehrten Konsum häufig mit ungünstigen Auswirkungen, als erhöhten Krankheitsrisiken assoziiert sind, sollten diese jedoch nicht vollständig in der täglichen Ernährungsweise exkludiert werden. Laut DGE sollte eine ausgewogene Ernährung beispielsweise auch eine tägliche Zufuhr von Milchprodukten sowie den wöchentlichen Konsum von fettreichem Fisch beinhalten [53]. Unverarbeitete tierische Produkte sind Quellen von essentiellen Nährstoffen, die in einer solchen Konzentration teilweise gering oder nur in bestimmten pflanzlichen Lebensmitteln auffindbar sind und sich möglicherweise ebenfalls positiv auf Herz-

Kreislauf- und Leber-Parameter auswirken könnten. Neben den bereits erwähnten langkettigen Omega-3-Fettsäuren aus Fisch wären diesbezüglich beispielhaft das Vitamin B12 und Cholin zu nennen. Insbesondere letzterer Nährstoff ist teilweise nicht in nationalen Lebensmitteldatenbanken gelistet [79] und wird in der Auswertung von Ernährungs-Fragebögen nicht berücksichtigt, wie es auch bei unserem Datensatz der KORA-Studie der Fall war. Neben seiner Rolle bei der DNA-Synthese [80] ist Vitamin B12 auch am Abbau von Homocystein beteiligt; einem Stoff, der als unabhängiger Risikofaktor das Herz-Kreislauf-Risiko erhöht, was teilweise auch über eine gerinnungsfördernde Wirkung erklärt werden könnte [81]. Ein Vitamin-B12-Mangel geht zudem möglicherweise mit einer erhöhten Prävalenz von Herz-Kreislauf-Erkrankungen einher [82]. Cholin ist ein essentieller Nährstoff, der besonders in höheren Konzentrationen in tierischen Lebensmitteln wie Leber oder Eiern enthalten ist [83] und ähnlich wie Vitamin B12 unter anderem auch am Abbau von Homocystein beteiligt ist. Daher führt ein Cholin-Mangel ebenfalls zu erhöhten Homocysteinspiegeln, was das Herz-Kreislauf-Risiko wiederum steigern könnte [84]. Zudem ist eine ausreichende Cholinzufuhr bedeutsam für eine adäquate Leberfunktion [85] und in Kohortenstudien sowohl mit einer verringerten Prävalenz als auch einer Senkung der Progression der MAFLD assoziiert worden [86,87]. Auch für weitere hauptsächlich in tierischen Lebensmitteln vorkommende semiessentielle Nährstoffe, welche in der Fachliteratur als auf Fleisch basierende bioaktive Stoffe (engl. meat-based bioactive compounds) bezeichnet werden, werden gesundheitsförderliche Effekte diskutiert. Hierzu zählen u.a. Taurin, L-Carnitin, Kreatin oder Carnosin [88]. Ähnlich wie im Fall von Cholin sind diese Stoffe ebenfalls nicht in der KORA-Studie berechnet worden und sollten Bestandteil der Analyse zukünftiger Studien sein.

Allgemein scheint zudem für den Konsum tierischer Lebensmittel häufig hinsichtlich NCDs eine U-förmige Beziehung zu bestehen, d.h. ein moderater Konsum wirkt sich gesundheitsförderlich aus im Vergleich zu keiner oder einer vermehrten Aufnahme. In einer Meta-Analyse zum Fischkonsum konnte beispielsweise in Bezug auf das Herz-Kreislauf-Risiko sowie die Gesamt-Sterblichkeit für eine moderate Menge von 20 g pro Tag in westlichen Industriestaaten die größte Risikoreduktion gezeigt werden [89]. Auch konkret für Butter konnte hinsichtlich der Prävalenz von Herzinfarkten mit dem Scheitelpunkt der U-Kurve bei ca. 3 g pro 1000 kcal pro Tag bei Patienten mit stabiler Angina pectoris [90]. Jedoch muss hierbei ähnlich wie auch bei den pflanzlichen Lebensmitteln die Lebensmittelqualität berücksichtigt werden. Besonders für verarbeitetes rotes Fleisch (z.B. durch Räuchern oder Salzen) konnten in mehreren Meta-

Analysen sowohl für Herz-Kreislauf- als auch Stoffwechsel-Erkrankungen sehr ungünstige Auswirkungen gezeigt werden [91]. Auch die DGE empfiehlt daher allgemein, den Konsum verarbeiteter Lebensmittel eher zu meiden [53]. Neben den bereits erwähnten eher abträglich wirkendenden gesättigten Fettsäuren und Häm-Eisen in rotem Fleisch, könnte hier vor allem das zusätzliche Salzen bei Verarbeitungsprozessen mechanistisch gesehen eine weitere wichtige Rolle spielen [92]. Vor allem auf das kardiovaskuläre Risiko wirkt sich ein vermehrter Salz-Konsum nachweislich ungünstig aus und ist laut der GBD 2019 der größte Risikofaktor für eine erhöhte Sterblichkeit [5]. Hierbei wird vor allem die durch Salzkonsum bedingte Erhöhung des Blutdrucks als Ursache vermutet [93]. Zudem gibt es aus Tiermodellen Hinweise, dass Salz über entzündungsfördernde Mechanismen Lebererkrankungen fördern könnte [94] und dadurch zum ebenfalls in epidemiologischen Studien am Menschen beobachteten ungünstigen Effekt des Salzkonsums auf das MAFLD-Risiko beiträgt [95]. Ein Effekt auf Blutgerinnungsparameter konnte jedoch bisher über eine Zeitspanne von 6 Wochen beim Menschen nicht nachgewiesen werden [96].

Zusammenfassend sind sowohl bei pflanzlichen als auch tierischen Produkten die Art und Qualität der Lebensmittel sowie die absolute Zufuhrmenge daher bedeutende Faktoren, die bei der Diskussion von Ernährungsempfehlungen zur Prävention von NCDs – und hier bei NAFLD und Blutgerinnungsparameter - eine entscheidende Rolle spielen.

## 4.4 Effektmodifikation durch die Stoffwechselsituation (Metabotypen)

Die zweite Publikation hat sich zusätzlich mit dem Einfluss der Stoffwechselsituation der Studienteilnehmer auf die Zusammenhänge zwischen verschiedenen Lebensmittelgruppen und dem FLI beschäftigt. Hier wurden drei Metabotyp-Cluster unterschieden, die auf Basis von fünf Parametern erstellt wurden. Im Detail geht es um die Frage, ob für Personen mit einem bestimmten Stoffwechselprofil unterschiedliche Zusammenhänge mit Ernährungsfaktoren und MAFLD bestehen. Wenn dies so nachweisbar ist, sollten unterschiedliche Ernährungsempfehlungen je nach Stoffwechselbeeinträchtigung ausgesprochen werden.

Bisher besteht hauptsächlich für eine kalorienreduzierte Ernährung die beste Evidenz zur Vorbeugung der MAFLD [15]. Hierbei scheint insbesondere eine mediterrane Ernährungsweise mit einer Basis pflanzlicher Lebensmittelquellen vielversprechend, jedoch gibt es darüber hinaus hinsichtlich der genauen Makronährstoffverteilung oder

der genauen Lebensmittelmengen keine eindeutigen Empfehlungen [15]. Vielmehr kommt hier individuellen Faktoren eine besondere Rolle zu. Als mögliche Einflüsse auf individuell angepasste Ernährungsempfehlungen werden daher neben persönlichen Lebensmittelpräferenzen auch Effekte des zirkadianen Rhythmus, des Mikrobioms oder genetischen Faktoren diskutiert [15]. Eine weitere Möglichkeit stellt die Anpassung von Ernährungsempfehlungen an das individuelle Stoffwechselprofil, sog. Metabotypen dar [17]. Nachdem bereits im Jahr 2020 von Riedl et al. die unterschiedliche Auswirkung verschiedener Lebensmittelgruppen auf das Diabetesrisiko in Abhängigkeit vom Stoffwechselprofil beobachtet worden ist [97], konnten in der vorgelegten zweiten Publikation ebenfalls solche Interaktionseffekte in Bezug auf das Risiko für MAFLD gezeigt werden. Dies unterstützt die These, dass solche individuellen Faktoren bei Ernährungsempfehlungen hinsichtlich der Fettleber berücksichtigt werden sollten. Aufgrund des eingangs beschriebenen Zusammenhangs von Blutgerinnungsparametern mit der Fettleber, der in der Vergangenheit auch bereits mit Diabetes Typ 2 beschrieben wurde [98], liegt auch ein Einfluss des Metabotyps auf die beobachteten Effekte der Ernährung auf die Blutgerinnung nahe. Eine weitere Erforschung dieses Zusammenhangs könnte ebenfalls zur Entwicklung individuell angepasster Ernährungsempfehlungen hinsichtlich Gerinnungsstörungen und weiterer Herz-Kreislauf-Erkrankungen beitragen und zu akkurateren Empfehlungen in Bezug auf die Prävention und Behandlung solcher Krankheiten beitragen.

# 5. Zusammenfassung

## 5.1 Englisch

According to the WHO, non-communicable diseases (NCDs) are responsible for approximately 74% of deaths worldwide and became more prevalent at an alarming rate over the past decades. While the major role of nutrition in the primary prevention of such conditions has been established, not all aspects of nutrition on health and disease are completely understood yet. This cumulative dissertation focalizes on the effects of diet on parameters of blood coagulation and fatty liver disease which are both meaningful factors for NCDs. Both included publications are based on the KORA-Fit (S4) survey, a follow-up study of the population-based KORA cohort study in the region of Augsburg in Germany. The study subjects (n = 595 and n = 689) of both projects are born between 1945 and 1964 and living in the study region of Augsburg. Information about habitual dietary intakes was collected by combining repeated 24-h food lists (24HFLs) and a food frequency questionnaire (FFQ). Antithrombin III, D-dimers, factor VIII, fibrinogen, protein C, protein S, aPTT, Quick value and INR were determined in citrate plasma. Fatty liver was estimated by the calculation of the fatty liver index (FLI). Three metabotype clusters were determined by using a k-means clustering algorithm based on five parameters.

Regarding fatty liver, beneficial effects for the consumption of plant-based food items have been observed; on the other hand, the intakes of animal-based or processed products were associated with detrimental health outcomes. Especially the habitual intakes of butter and dairy were associated with a clinically relevant elevation of D-dimer values in the study participants while the consumption of meat, fish, eggs or soft drinks was accompanied with the presence of fatty liver. Conversely, nuts, whole grains and a Mediterranean dietary pattern which is characterized by a high consumption of plant-based food items, were associated with lower markers of fatty liver. Furthermore, interaction effects of the three metabotype clusters were observed which means that the observed effects are dependent on the metabolic profile of the study participants. These findings need more elaborate evaluation in further prospective studies to promote the development of guidelines and prevention strategies to lower the risk of cardio-metabolic diseases on a population-based level.

## 5.2 Deutsch

Nach Angaben der WHO sind nicht übertragbare Krankheiten (engl. non-communicable diseases, NCDs) für etwa 74 % der Todesfälle weltweit verantwortlich und haben sich in den letzten Jahrzehnten in alarmierendem Maße ausgebreitet. Wenngleich die bedeutsame Rolle der Ernährung bei der Primärprävention solcher Krankheiten inzwischen oft gut belegt ist, sind bestehende Forschungslücken weiter zu schließen. In der vorliegenden Arbeit wurden die Zusammenhänge zwischen Ernährungsfaktoren mit Parametern der Blutgerinnung und der nicht-alkoholischen Fettlebererkrankung, welches beide bedeutende Faktoren bei der Entstehung von NCDs sind, untersucht. Beide Arbeiten basieren auf Daten aus der KORA-Fit (S4)-Studie, einer Follow-Up-Studie der bevölkerungsbezogenen KORA-Kohortenstudie. Die 595 bzw. 689 Studienteilnehmenden in den beiden Projekten sind zwischen 1945 und 1964 geboren und leben in der Studienregion Augsburg. Daten zum Lebensmittelverzehr stammen aus wiederholten 24-Stunden-Erinnerungsprotokollen (24HFLs) und einem Verzehrshäufigkeitsfragebogen (FFQ). Antithrombin III, D-Dimere, Faktor VIII, Fibrinogen, Protein C, Protein S, aPTT, Quick-Wert und INR wurden im Citratplasma bestimmt. Die Ausprägung der Fettleber wurde durch die Berechnung des Fettleber-Index (FLI) erfasst. Drei Metabotyp-Cluster wurden mit Hilfe des K means-Verfahrens auf der Basis von fünf Parametern bestimmt.

Die Ergebnisse zum Einfluss auf die Entwicklung einer Fettleber zeigen vorteilhafte gesundheitliche Zusammenhänge für den Verzehr pflanzlicher Lebensmitteln; dagegen zeigen ein vermehrter Verzehr von Lebensmitteln tierischen Ursprungs oder verarbeiteten Produkten eher ungünstige Folgen. Insbesondere der Verzehr von Butter und Milchprodukten war bei den Studienteilnehmenden mit einer klinisch relevanten Erhöhung der D-Dimer-Werte verbunden. Ein erhöhter Verzehr von Fleisch, Fisch, Eiern oder Softdrinks ging mit dem Auftreten einer Fettleber einher. Nüsse, Vollkornprodukte und eine mediterrane Ernährungsweise, die durch einen hohen Verzehr von pflanzlichen Lebensmitteln gekennzeichnet ist, zeigten eine signifikant inverse Assoziation mit dem Fettleber-Index. Zusätzlich wurden Interaktionseffekte mit den drei Metabotyp-Clustern beobachtet; dies bedeutet, dass die beobachteten Zusammenhänge von der Stoffwechselsituation der Studienteilnehmenden abhängen. Diese Ergebnisse sollten in weiteren prospektiven Studien genauer untersucht werden, um die Weiterentwicklung von Präventionsstrategien zur Verringerung des Risikos von kardio-metabolischen Krankheiten auf Bevölkerungsebene zu befördern.

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# Appendix

## I. Abkürzungsverzeichnis

| | |
|---|---|
| BMI | Body-Mass-Index |
| CT | Computertomographie |
| CVD | Krankheiten des Herz-Kreislauf-Systems (engl. cardiovascular diseases) |
| DALYs | für Krankheit adjustierte Lebensjahre (engl. disability-adjusted life-years) |
| DGE | Deutsche Gesellschaft für Ernährung |
| FLI | Fettleberindex (engl. fatty liver index) |
| GBD | Global Burden of Disease study |
| KORA | Kooperative Gesundheitsforschung in der Region Augsburg |
| MAFLD | Stoffwechsel-bedingte Fettlebererkrankung (engl. metabolic-dysfunction associated fatty liver disease) |
| MRT | Magnet-Resonanz-Tomographie |
| NCDs | nicht-übertragbare Erkrankungen (engl. non-communicable diseases) |

## II. Abbildungsverzeichnis



## III. Tabellenverzeichnis

## IV. Danksagung

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