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Der Zusammenhang zwischen Adipositas und Fettleber mit Parametern der Blutgerinnung in der erwachsenen Bevölkerung

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Augsburg, 10.09.2024

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Inhaltsverzeichnis

Abkürzungsverzeichnis

Abkürzung	Bedeutung
NAFLD	non-alcoholic fatty liver disease/ nichtalkoholische Fettleber-erkrankung
MAFLD	metabolic dysregulation-associated fatty liver disease
BMI	Body Mass Index
FLI	Fatty Liver Index
WHO	World Health Organisation
NAFL	nicht alkoholische Fettleber
NASH	nicht alkoholische Steatohepatitis
GGT	Gamma Glutamyl Transferase
WC	waist circumference
aPTT	activated partial thromboplastin time
INR	international normalized ratio
HIT	Heparin-induzierte Thrombozytopenie
KORA	Cooperative Health Research in the Region of Augsburg
MONICA	Monitoring Trends and Determinants in Cardiovascular Disease
PAI-1	plasminogen activator inhibitor
tPA	tissue plasminogen activator

Publikationsliste

1. Iglesias Morcillo M, Freuer D, Peters A, Heier M, Meisinger C, Linseisen J. Body Mass Index and Waist Circumference as Determinants of Hemostatic Factors in Participants of a Population-Based Study. Medicina (Kaunas). 2023 Jan 26;59(2):228. doi: 10.3390/medicina59020228.

2. Iglesias Morcillo M, Freuer D, Peters A, Heier M, Teupser D, Meisinger C, Linseisen J. Association between fatty liver index and blood coagulation markers: a population-based study. Lipids Health Dis. 2023 Jun 29;22(1):83. doi: 10.1186/s12944-023-01854-8.

1. Autorenbeitrag

Der Doktorand Maximilian Iglesias Morcillo führte alle folgenden Tätigkeiten selbstständig aus.

1.1 Beitrag zu Publikation I

Er übernahm die Literaturrecherche, entwickelte in Absprache mit seinem Doktorvater Prof. Dr. Jakob Linseisen ein Analysekonzept und führte die statistischen Analysen durch. Er interpretierte die Ergebnisse, erstellte die Tabellen und verfasste die Publikation. Beraten und unterstützt wurde er in jeder Phase von seinem Doktorvater Prof. Dr. Jakob Linseisen sowie von Dr. Dennis Freuer als statistischem Berater. Herr Prof. Dr. Jakob Linseisen fungierte während des Einreichungsprozesses als korrespondierender Autor und übernahm die Kommunikation mit den Gutachtern und dem Editor.

1.2 Beitrag zu Publikation II

Der Doktorand entwarf zusammen mit seinem Doktorvater Prof. Dr. Jakob Linseisen das Analysekonzept und führte die statistischen Analysen durch. Er interpretierte die Ergebnisse und erstellte die Tabellen. Er verfasste die Zusammenfassung, den Methodenteil und den Ergebnisteil. Er führte die Literaturrecherche durch und verfasste die Einleitung und Diskussion. Er redigierte und überarbeitete die Publikation. Beraten und unterstützt wurde er in jeder Phase von seinem Doktorvater, Herrn Prof. Dr. Jakob Linseisen, sowie von Herrn Dr. Dennis Freuer als statistischem Berater. Herr Prof. Dr. Jakob Linseisen fungierte während des Einreichungsprozesses als korrespondierender Autor und übernahm die Kommunikation mit den Gutachtern und dem Editor.

2 Einleitung

2.1 Adipositas und nicht-alkoholische Fettleber

Adipositas oder Fettleibigkeit ist eine chronische Erkrankung, von der in Deutschland mit einer Prävalenz von ca. 25% jeder Vierte betroffen ist [1]. Weltweit nimmt die Adipositas stetig zu und wird von der Weltgesundheitsorganisation seit einiger Zeit als Epidemie eingestuft [2]. Seit den 80er Jahren hat sich die Zahl der Adipösen verdoppelt, so dass heute weltweit ein Drittel aller Menschen als übergewichtig oder adipös eingestuft werden kann [3]. Neben der Belastung der Gesundheitssysteme spielen auch die volkswirtschaftlichen Folgen eine immer größere Rolle. Allein in Deutschland belaufen sich die Kosten für die medizinisch notwendige direkte Behandlung und Pflege nach einer Schätzung auf ca. 30 Milliarden Euro pro Jahr. Die indirekten Kosten durch Arbeitsunfähigkeit etc. belaufen sich auf weitere 30 Milliarden Euro [4].

Adipositas ist das Ergebnis des Zusammenspiels verschiedener Einflussfaktoren: ungünstige Ernährungsgewohnheiten, Bewegungsmangel, psychosoziale und sozioökonomische Faktoren, aber auch genetische, metabolische und endokrine Faktoren führen zu einer Zunahme der Körperfettmasse [5]. Letztlich entsteht Übergewicht als Folge einer nicht ausgeglichenen Energiebilanz. Einer zu hohen Kalorienaufnahme steht ein zu geringer Energieverbrauch gegenüber, was langfristig zur Einlagerung von Körperfett und damit zur Gewichtszunahme führt [6].

Adipositas ist mit schwerwiegenden Erkrankungen assoziiert und erhöht nicht nur das Risiko für Stoffwechselerkrankungen wie Diabetes, Fettstoffwechselstörungen oder Gicht, sondern auch für Herz-Kreislauf-Erkrankungen, bestimmte Tumorerkrankungen, Thrombosen, Arthritis und andere mehr [6].

Ein einfaches diagnostisches Maß zur Klassifizierung von Übergewicht ist der Body-Mass-Index, kurz BMI, der sich aus dem Körpergewicht geteilt durch die Körpergröße zum Quadrat errechnet. Nach der Klassifikation der WHO [7] gilt man ab einem BMI-Wert größer oder gleich 25 $\frac{kg}{m^2}$ als übergewichtig und von 30 $\frac{kg}{m^2}$ oder mehr als adipös (Tabelle 1).

Der BMI ist das am häufigsten verwendete Maß zur Definition der Adipositas. Mehrere Methoden stehen für eine genaue Messung des Körperfettanteils zur Verfügung; dazu gehören die Bioelektrische Impedanz-Analyse, die Unterwasserwiegung, oder die MRT-basierte Fettquantifizierung an verschiedenen Körperkompartimenten [8]–[10]. Metabolisch bedeutsam ist die Unterscheidung zwischen dem metabolisch aktiveren viszeralen Fettgewebe im Bauchraum und dem subkutanen Fettgewebe. Die Messung des Taillenumfangs mit einem flexiblen Maßband ist eine einfache Methode zur Bestimmung des viszeralen Fettgewebes. Auch hier gibt die WHO geschlechtsspezifische Angaben für einen Taillenumfang, der mit einem erhöhten Krankheitsrisiko verbunden ist [11].

Tabelle 1: Einteilung des Body Mass Index nach WHO-Klassifizierung für Erwachsene und des Taillenumfangs geschlechtsabhängig nach Krankheitsrisiko [7], [11]

Kategorie	**BMI [$\frac{kg}{m^2}$]**	
Untergewicht	BMI < 18,5	
Normalgewicht	18,5 ≤ BMI < 25	
Übergewicht	25 ≤ BMI < 30	
Adipositas (Grad I-III)	BMI ≥ 30	
Kategorie	**Taillenumfang [cm]**	
	Männer [cm]	**Frauen [cm]**
Leicht erhöht	≥ 94	≥ 80
Stark erhöht	≥ 102	≥ 88

Übergewicht und Adipositas erhöhen das Risiko an einer nicht-alkoholischen Fettleber zu erkranken. Die nichtalkoholische Fettlebererkrankung (NAFLD), mittlerweile auch MAFLD (metabolic dysregulation-associated fatty liver disease) genannt, entsteht durch ein multifaktorielles Zusammenwirken von Bewegungsmangel, Übergewicht und zahlreichen anderen Faktoren, wobei das Übergewicht den Hauptfaktor darstellt [12]. Als nicht-alkoholische Fettleber (NAFLD) wird ein Spektrum von Lebererkrankungen bezeichnet, das mit einer erhöhten Fettablagerung in der Leber einhergeht, das heißt mit einer Akkumulation von Lipiden in mehr als 5% der Hepatozyten [13]. Die isolierte Steatose, d.h. die nichtalkoholische Fettleber (NAFLD), wird von der nichtalkoholischen Steatohepatitis (NASH), den sekundären Steatosen und der Fettleberzirrhose unterschieden [13][14]. Ein einfaches Maß zur Ermittlung des Leberfettgehalts ist der Fettleberindex (fatty liver index, FLI). Dieser wurde von der Forschergruppe um Bedogni [15] entwickelt und nutzt Werte aus der Routinediagnostik, um den Index zu berechnen. Als Parameter werden der Body Mass Index, Nüchtern-Plasma-Triglyzeride, Gamma-Glutamyl-Transferase-Aktivität und Taillenumfang verwendet. Der FLI wird nach folgender Formel berechnet:

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

Die Klassifizierung der berechneten Werte erfolgt anhand von validierten Grenzwerten (Tabelle 2):

Tabelle 2: Einteilung des FLI nach Bedogni [15]

Kategorie	**FLI**
Keine Fettleber	<30
Grauzone	30-59
Fettleber	≥60

Neben dem FLI sind weitere Indizes zur Ermittlung des Leberfettgehaltes vorgeschlagen worden. Für die medizinische Diagnose werden exakte Messungen mittels bildgebender Verfahren oder anhand von Biopsien verwendet [16]–[18].

2.2 Blutgerinnung

Die Blutgerinnung oder Hämostase ist ein lebenswichtiger Prozess, der durch die Bildung eines Gerinnsels extravasale oder intravasale Blutungen stillt [19]. Man unterscheidet die primäre Hämostase von der sekundären Hämostase. Das Ergebnis ist die Bildung eines stabilen Thrombus aus Fibrin. Der Prozess der Blutgerinnung erfolgt nach einer Verletzung des Endothels, das ein Blutgefäß auskleidet, und umfasst zwei Phasen, die primäre und die sekundäre Hämostase. Die primäre Hämostase führt durch die Anhäufung von Blutplättchen und die Freisetzung von Gewebefaktoren zur Bildung von Fibrin an der unmittelbaren Verletzungsstelle. Die sekundäre Hämostase beginnt gleichzeitig mit der Aktivierung einer Kaskade von Gerinnungsfaktoren. Diese Kaskade wird in zwei Systeme unterteilt: den intrinsischen und den extrinsischen Weg. Bei beiden Wegen werden Plasmaproteine (meist Serinproteasen) durch proteolytische Spaltung aktiviert. Ein so aktivierter Gerinnungsfaktor aktiviert dann spezifisch einen weiteren Faktor. So entsteht ein sich selbst verstärkender Prozess. Bei der Blutstillung verlaufen beide Wege immer parallel und führen zur Aktivierung der Serinprotease Thrombin. Diese fördert schließlich die Umwandlung von Fibrinogen in Fibrin, und die Anhäufung von Fibrin und Thrombozyten bildet schließlich den stabilen Thrombus [20]. Die plasmatische Gerinnung wird aus Gründen der Didaktik immer noch in eine intrinsische und eine extrinsische Gerinnungskaskade unterteilt. Die Wechselwirkungen zwischen den beiden Systemen werden jedoch immer deutlicher. Um eine unerwünschte oder zu starke Gerinnung zu verhindern, verfügt der Körper auch über Mechanismen, um die Hämostase zu hemmen: Verschiedene Protease-Inhibitoren (u. a. Antithrombin, Protein C und Protein S) zirkulieren im Blut und hemmen die Bildung von Thrombin und anderen Gerinnungsfaktoren [21]–[23]. Der ordnungsgemäße Ablauf und die Zeit, die benötigt wird, um Fibrin zu bilden, können im Labor mit verschiedenen Tests gemessen werden, darunter die aktivierte partielle Thromboplastinzeit (aPTT). Diese Tests geben Aufschluss über die Ursache verschiedener Störungen im Rahmen der Blutstillung. Schließlich führen beide Wege zusammen zu einer gemeinsamen Endstecke: nach der der Aktivierung von Fibrinogen zu Fibrin, das die Thrombozyten aneinanderbindet und den Thrombozytenpfropf stabilisiert, kommt es zur Bildung eines vernetzten Fibringerinnsels. Die für die Indikation Thrombose üblicherweise verwendeten hämostatischen Faktoren sind hauptsächlich D-Dimere, Faktor VIII, Fibrinogen D, Protein C, Protein S und Antithrombin III, die entweder einen der beiden Wege fördern oder sie hemmen. Die Gerinnungstests, die zur Beschreibung des Versagens des intrinsischen oder extrinsischen Weges verwendet werden, sind aPTT, INR und Quick.

Tabelle 3: Übersicht der wichtigsten Faktoren des intrinsischen und extrinsischen Systems und ihrer gemeinsamen Endstrecke

Intrinsisches System			**Extrinsisches System**		
Faktor	**Synonym**	**Aufgabe/ Einflussfaktoren**	**Faktor**	**Synonym**	**Aufgabe/ Einflussfaktoren**
XII	Hageman-Faktor	löst in seiner aktivierten Form das intrinsische System aus	VII	Prokonvertin	Bindet an Gewebefaktor, aktiviert Faktor X, IX
XI	Plasma-Thromboplastin-Vorläufer (PTA)	aktiviert Faktor IX	III	Gewebsthromboplastin	löst nach Schädigung des Endothels das extrinsische System aus
IX	Christmas-Faktor	Aktivierung zu Faktor IXa	X	Stuart-Prower-Faktor	Aktivierung zu Faktor Xa
Protein C		Hemmt aktivierte Faktoren V und VIII, löst Fibrinolyse aus	Antithrombin		Inaktivierung von Thrombin und Faktor Xa
Protein S		Cofaktor des aktivierten Protein C, hemmt Blutgerinnung			
VIII	Antihämophiles Globulin	Aktivierung zu Faktor VIIIa			
X	Stuart-Prower-Faktor	Aktivierung zu Faktor Xa			

Gemeinsame Endstrecke		
Faktor	**Synonym**	**Aufgabe**
II	Prothrombin	Vorläufer von Thrombin, wandelt Fibrinogen zu Fibrin; aktiviert Faktoren V, VIII, XI und XIII; an Thrombomodulin gebundenes Thrombin aktiviert Protein C; Vitamin-K-abhängig
I	Fibrinogen	Vorläufer von Fibrinmonomeren
XIII	Fibrinstabilisierender Faktor	durch Thrombin aktiviert und katalysiert Bildung von Peptidbindungen zwischen benachbarten Fibrinmonomeren
V	Proaccelerin	Cofaktor von Faktor X, beschleunigt Umwandlung von Prothrombin

Die Fibrinolyse stellt den gegenläufigen Prozess dar. Das Fibrinolysesystem hat die Aufgabe, überschüssige Fibrinbildung zu verhindern und damit Gefäße, Drüsen oder andere Röhrensysteme im Körper vor Fibrinablagerungen zu schützen [24]. Die Fibrinolyse wird direkt durch körpereigene Aktivatoren wie die Protease Plasminogen aktiviert, die in ihrer aktiven Form Plasmin der Gerinnung entgegenwirkt. Weitere körpereigene Aktivatoren sind die Streptokinase und die Urokinase. Blutgerinnung und Fibrinolyse laufen im Gefäßsystem immer parallel ab, um unnötige Fibrinbildung zu vermeiden und alte Thromben abzubauen [25]. Die klinisch verwendeten Parameter zur Beurteilung der Fibrinolyse sind die D-Dimere [26], deren Abbauprodukte bei der Auflösung eines Blutgerinnsels vermehrt im Plasma zirkulieren.

2.3 Blutgerinnungsstörungen

Störungen der Blutgerinnung können entweder zu einer übermäßigen, hämorrhagischen Blutung oder zu einer verstärkten Blutgerinnung oder Thrombophilie führen [27].

Hämorrhagische Diathesen sind dabei meist durch eine zu lange oder zu starke Blutung gekennzeichnet. Viele Ursachen für eine erhöhte Blutungsneigung sind genetisch bedingt, also angeboren, können aber auch medikamentös induziert sein. Grundsätzlich können Blutungen aufgrund von Thrombozytopathien, Thrombozytopenie, Koagulopathien oder Diathesen auftreten [28].

Thrombozytopathien sind meist angeboren, wie z. B. das Bernard-Soulier-Syndrom, können aber auch medikamentös erworben sein, z. B. durch die Gabe von Acetylsäure oder Clopidogrel. Sie sind durch eine Störung der Thrombozytenaktivierung gekennzeichnet [29].

Der Mangel an Thrombozyten im Blut wird als Thrombozytopenie (<150000/µl) bezeichnet. Ätiologisch kommen viele Ursachen für diesen Mangel in Frage: Bildungsstörungen, Anämien, Substrat- oder Faktorenmangel, exzessive Gerinnungsaktivierung oder auch Verteilungsstörungen, z.B. in der Schwangerschaft [30].

Koagulopathien stören die plasmatische Gerinnung oder die Fibrinolyse. Hypokoagulopathien führen zu Blutungen, Hyperkoagulopathien zu Thrombophilie. Die Störungen können angeboren oder erworben sein. Angeborene Koagulopathien machen den größten Teil aus. Zu den bekanntesten Syndromen gehören das von Willebrand-Jürgens-Syndrom oder die Hämophilie A oder B, bei denen die Bildung oder das Vorhandensein bestimmter Faktoren krankhaft verändert ist. Erworbene Störungen können z. B. durch Vitamin-K-Mangel, durch Medikamente oder auch durch Leberschäden hervorgerufen werden. Koagulopathien sind häufig durch ein vermehrtes Auftreten von Blutungen gekennzeichnet [31].

Vaskuläre Diathesen zeichnen sich durch eine erhöhte Blutungsneigung vor allem kleiner Blutgefäße aus, wobei vor allem punktförmige, petechiale Blutungen auf der Haut oder Schleimhaut sichtbar werden. Die Ursachen sind überwiegend erblicher oder erworbener Natur.

Unter Thrombophilie versteht man eine erhöhte Neigung zur Bildung von Thrombosen. Die Ursachen können vererbt oder erworben sein, wobei bestimmte Faktoren und vor allem Lebensgewohnheiten das Thromboserisiko erhöhen. So erhöhen Risikofaktoren wie Rauchen, Übergewicht, Bewegungsmangel, aber auch das Altern das Thromboserisiko. Zu den erblichen Ursachen zählen ein Mangel an den Faktoren Antithrombin III, Protein C und S, zu den erworbenen Störungen unter anderem die Heparin-induzierte Thrombozytopenie (HIT), Herzinsuffizienz, maligne Tumore, aber auch Schwangerschaft [27], [28].

Zur Früherkennung möglicher krankhafter Veränderungen oder Störungen des Blutgerinnungssystems haben neben spezifischen Gerinnungstests auch die Bestimmung einzelner Faktoren Einzug in den klinischen Alltag gehalten. In der Routinediagnostik sind Bestimmungsmethoden wie die partielle Thromboplastinzeit (aPTT), die international normalisierte Ratio (INR) und der Quick-Wert seit langem etabliert und dienen vor allem der Darstellung von Veränderungen im intrinsischen oder extrinsischen System (Tabelle 3). Um bestimmte Störungen innerhalb der Hämostase festzustellen, werden auch einzelne Faktoren, die an der Blutgerinnung beteiligt sind, bestimmt. So kann der Gehalt an Antikoagulantien im Blut wie Antithrombin III, Protein C und Protein S [21]–[23] bestimmt werden, um mögliche Thromboserisiken zu erkennen und zu behandeln. Ein weiterer Faktor zum Ausschluss einer Thrombose, aber auch einer Lungenembolie, ist die Konzentration von D-Dimeren. Diese entstehen als Spaltprodukt im Rahmen der Fibrinolyse und treten daher bei einer über das natürliche Maß hinausgehenden Auflösung von Fibrin als Spaltprodukt vermehrt im Plasma auf [32]. Ebenso wird ein Überschuss an Faktor VIII im Plasma als Hinweis auf eine mögliche Thrombose herangezogen [33]. Ein Mangel des Faktors wird vor allem bei der Hämophilie A nachgewiesen. Ein weiterer wichtiger Faktor ist Fibrinogen, das durch Thrombin enzymatisch in Fibrin umgewandelt wird. Eine niedrige Fibrinogen Konzentration lässt Rückschlüsse auf eine erhöhte Blutungsneigung zu [34].

Tabelle 4: Übersicht über diagnostische Blutgerinnungstests sowie Beispiele für Ursachen und Folgen bei deren Veränderungen

	Einheit	**Referenzbereich**[1]	**Verlängert bei**	**Verkürzt**	**Abschnitt der Blutgerinnung**
aPTT	[s]	26-36	Hämophilie, Antikoagulation, Mangel an Gerinnungsfaktoren, Leberinsuffizienz, Vitamin K-Mangel	Hyperkoagulabilität	Intrinsisch
INR	keine	0,90-1,15	Blutungsneigung Therapeutische Werte z.B. für das Vorliegen einer Lungenembolie, Vorhofflimmern oder tiefer Beinvenenthrombosen liegen bei 2,0-3,5, s. aPTT		Extrinsisch
Quick	[%]	82-125	Medikamentengabe z.B. Phenprocoumon, Warfarin	Antikoagulation, Vitamin K-Mangel, Leberschädigung	Extrinsisch

[1]Referenzwerte des Klinikums Augsburg

2.4 KORA Studie

Die Kooperative Gesundheitsforschung in der Region Augsburg (KORA) ist eine Plattform für bevölkerungsbasierte Gesundheitssurveys. KORA wurde 1996 als Fortsetzung des MONICA-Projekts (Monitoring trends and determinants in cardiovascular disease) ins Leben gerufen, das von 1984 bis 1995 von der WHO in Augsburg durchgeführt wurde [35]. Seit 1984 wurden im Abstand von fünf Jahren vier Basiserhebungen durchgeführt (S1-S4). In der Folgezeit wurden alle Teilnehmerinnen und Teilnehmer in regelmäßigen Abständen schriftlich befragt und zum Teil erneut im Studienzentrum untersucht. Auf diese Weise wurde der Gesundheitsverlauf über 30 Jahre hinweg erfasst, mit dem Ziel, Daten und Stichproben für zukünftige Forschungsprojekte zu gewinnen und damit Risikofaktoren zu identifizieren und Präventionsmaßnahmen auf dieser Basis zu entwickeln; Schwerpunkte waren und sind Herz-Kreislauf-Erkrankungen, Diabetes und Umweltfaktoren [36]. Insgesamt wurden nahezu 18.000 Teilnehmer nach dem Zufallsprinzip aus den Einwohnermelderegistern der Studienregion ausgewählt. Einschlusskriterien für die Teilnahme an der Studie waren der Hauptwohnsitz im Untersuchungsgebiet, die deutsche Staatsangehörigkeit und ein Alter zwischen 25 und 74 Jahren. Das Studiengebiet umfasst die Stadt Augsburg und die beiden angrenzenden Landkreise, Landkreise Augsburg und Aichach-Friedberg. Die Datenerhebung erfolgt in Kooperation mit dem Universitätsklinikum Augsburg. Die Sammlung von Bioproben umfasst hauptsächlich Blut, Serum, DNA, Urin und Stuhl. Seit mehr als 38 Jahren werden in regelmäßigen Abständen Folgestudien durchgeführt [35], [36]. Die KORA Fit-Studie wurde in den Jahren 2018 und 2019 durchgeführt; im S4-Anteil von KORA Fit wurden Citrat-Plasmaproben gesammelt, in denen Parameter der Blutgerinnung gemessen wurden.

2.5 Fragestellung und Ziele der Arbeit

2.5.1 Blutgerinnung und BMI

Der Zusammenhang zwischen Adipositas bzw. zunehmendem Übergewicht und Veränderungen des Blutgerinnungssystems ist seit langem bekannt [37]. Zahlreiche Studien belegen, dass eine Gewichtszunahme einen prokoagulatorischen Zustand begünstigt und das Risiko für Erkrankungen, wie etwa Thrombosen oder Herz-Kreislauf-Erkrankungen, entsprechend ansteigt [38].

Im Jahr 2004 wurde versucht, anhand der Daten von Teilnehmern der Framingham Offspring Study, einer prospektiven Langzeitstudie zur Untersuchung von Risikofaktoren für Herz-Kreislauf-Erkrankungen, festzustellen, ob ein Zusammenhang zwischen Übergewicht und bestimmten hämostatischen Faktoren besteht [39]. Übergewicht wurde anhand des Body Mass Index, des Taillenumfangs und des Verhältnisses von Taillen- zu Hüftumfang dargestellt, die Gerinnungsfaktoren Fibrinogen, Plasminogenaktivatorinhibitor (PAI-I), Tissue Plasminogen Activator (tPA), Faktor VII und der von-Willebrand-Faktor (VWF) wurden bestimmt. Es konnten signifikante Zusammenhänge zwischen steigendem BMI und dem Verhältnis von Taillen- zu Hüftumfang und den genannten Faktoren für Männer und Frauen gefunden werden, wobei nur der Taillenumfang geschlechtsspezifische Unterschiede aufwies und nicht für alle Faktoren signifikant war. Es konnte auch gezeigt werden, dass der BMI einen prothrombotischen Zustand begünstigt.

Eine patientenbezogene Studie aus dem Jahr 2015, die 377 Traumapatienten untersuchte, konnte zeigen, dass übergewichtige Traumapatienten im Vergleich zu Normalgewichtigen einen

Zustand der Hyperkoagulation aufweisen. Darüber hinaus konnte gezeigt werden, dass ein erhöhter BMI die Wahrscheinlichkeit, eine thromboembolische Komplikation zu erleiden, um 85 % erhöht [40].

Da der Body-Mass-Index standardmäßig zur Diagnose von Übergewicht herangezogen wird, liegt der Rückschluss auf die Blutgerinnung nahe. Bei genauerer Betrachtung wird jedoch auch deutlich, dass Studien, die einzelne Gerinnungsfaktoren untersuchen und bevölkerungsbezogen sind, noch fehlen. Als einfaches Maß zur Bestimmung des viszeralen Fettgewebes wird der Taillenumfang gemessen. Sind die Assoziationen für BMI und Taillenumfang mit den Parametern der Blutgerinnung einheitlich, ist die Gesamtmenge an Körperfett ausschlaggebend. Im Rahmen der vorliegenden Arbeit wurde daher der BMI und Taillenumfang auf einen Zusammenhang mit einer Reihe von Gerinnungsmarkern in der erwachsenen Bevölkerung untersucht. Die folgenden Gerinnungsmarker und Gerinnungstests wurden in der Arbeit untersucht: Antithrombin III, D-Dimer, Faktor VIII, Fibrinogen D, Protein S, Quick-Wert, aPTT und INR.

2.5.2 Blutgerinnung und FLI

Der Syntheseort der meisten Gerinnungsfaktoren ist die Leber [41]. Umso wichtiger erscheint es, den Zusammenhang zwischen bestimmten Gerinnungsfaktoren und krankhaften Veränderungen der Leber zu beleuchten. Die als Wohlstandskrankheit bezeichnete nicht-alkoholische Fettleber macht einen erheblichen Anteil der Leberveränderungen aus. Schätzungen gehen davon aus, dass etwa ein Viertel der Weltbevölkerung darunter leidet [42]. Ein diagnostisches Instrument zur Beurteilung krankhafter Leberveränderungen infolge Fetteinlagerung ist der Fatty Liver Index.

In einer Vergleichsstudie [43] zum Einfluss der nicht-alkoholischen Fettlebererkrankung auf die Blutgerinnung konnte gezeigt werden, dass zahlreiche Gerinnungsfaktoren, eine endotheliale Gefäßdysfunktion, Thrombozytenanomalien und Veränderungen von Faktoren, die an der Gerinnungskaskade und der Fibrinolyse beteiligt sind, zu einem prothrombotischen Zustand bei Patienten mit nichtalkoholischer Fettlebererkrankung beitragen können.

Eine koreanische Studie [44] zeigte ebenfalls, dass ein höherer FLI-Wert das Risiko für Gesamtmortalität, Herzinfarkt und Schlaganfall erhöht. Die Forscher stellten fest, dass eine einfache (wiederholte) Bewertung des NAFLD-Status auf der Grundlage des FLI den Ärzten helfen könnte, Gruppen mit einem höheren Risiko für Gesamtmortalität, Herzinfarkt und Schlaganfall zu identifizieren. An der Studie nahmen rund 3 Millionen Menschen teil, die zwischen 2009 und 2013 viermal an der Gesundheitsuntersuchung teilgenommen hatten. NAFLD wurde definiert als FLI ≥60.

Ein Vergleich mit bevölkerungsbasierten Studien, die den Zusammenhang zwischen Blutgerinnungsmarkern und dem Fettleber-Index aufzeigen, ist schwierig, da bisher kaum entsprechende Arbeiten durchgeführt wurden. Diese Lücke soll mit der vorliegenden Arbeit anhand von Daten der erwachsenen Bevölkerung geschlossen werden, wobei die folgenden Gerinnungsmarker und Gerinnungstests untersucht wurden: Antithrombin III, D-Dimer, Faktor VIII, Fibrinogen D, Protein C, Protein S, Quick-Wert, aPTT und INR.

2.6 Spezifizierung und Inhalt der Arbeit

Diese Dissertation besteht aus zwei Erstautor-Publikationen, die im Rahmen der kumulativen Promotion in peer-reviewed Fachzeitschriften veröffentlicht wurden.

2.6.1 Publikation I

In der ersten Publikation wurde der Zusammenhang zwischen Body Mass Index und Taillenumfang mit den Plasmakonzentrationen von Antithrombin III, D-Dimeren, Fibrinogen D, Protein S, Faktor VIII, aPTT, Quick-Wert und INR in der Allgemeinbevölkerung untersucht. Multivariable lineare Regressionsmodelle wurden zur Darstellung der Zusammenhänge zwischen BMI und Taillenumfang mit den hämostatischen Markern verwendet. Diese wurden in zwei unterschiedlich adjustierten Modellen dargestellt. Es wurden Plasmaproben aus der bevölkerungsbasierten KORA Fit S4-Studie verwendet, wobei alle Teilnehmerinnen und Teilnehmer im Alter von 54 bis 74 Jahren mit Vorliegen einer Citrat-Plasmaprobe in die Analysen eingeschlossen wurden, die zudem keine Antikoagulanzien einnahmen.

2.6.2 Publikation II

Ziel der zweiten Publikation ist die Untersuchung des Zusammenhangs zwischen dem Fettleber-Index (FLI) als Maß für eine Fettleber und den Plasmakonzentrationen von Antithrombin III, D-Dimer, Fibrinogen D, Protein C, Protein S, Faktor VIII, aktivierter partieller Thromboplastinzeit (aPTT), Quick-Wert und internationaler Thromboplastinzeit (INR) in einer süddeutschen Allgemeinbevölkerung. Für die linearen Regressionen wurden zwei unterschiedlich adjustierte Modelle eingesetzt. Zusätzlich wurden die Analysen getrennt nach Glukosetoleranzstatus (Normal, Prädiabetes, Typ 2-Diabetes) untersucht. Für die statistische Analyse wurden die Ergebnisse der Plasmaproben von 776 Teilnehmerinnen und Teilnehmern der KORA Fit S4-Studie verwendet. Die Personen waren zwischen 54 und 74 Jahre alt und Personen, die Antikoagulantien einnahmen, wurden ausgeschlossen.

3 Zusammenfassung:

Die Prävalenz der Adipositas mit ihren zahlreichen Begleit- und Folgeerkrankungen hat in den letzten Jahrzehnten rasant zugenommen und stellt eine Belastung für das Gesundheitssystem dar; eine genaue Analyse der gesundheitlichen Folgen und deren Bewertung ist damit wichtiger denn je. In bevölkerungsbasierten Studien ist der Zusammenhang zwischen einer Reihe von ausgewählten Gerinnungsparametern und der Körperfettansammlung sowie der Leberfetteinlagerung bisher unzureichend untersucht worden.

Die vorliegende Arbeit analysierte den Zusammenhang ausgewählter Gerinnungsparameter mit dem BMI und Taillenumfang einerseits und dem Leberfettgehalt, bestimmt über den Fatty Liver Index (FLI) andererseits auf der Basis von Daten der KORA-Fit-Studie und stellte die Ergebnisse im Rahmen von zwei Publikationen vor.

In der ersten Publikation wurden die Zusammenhänge zwischen den Blutgerinnungsparametern Antithrombin III, D-Dimere, Faktor VIII, Fibrinogen D, aPTT, Protein S, Quick und INR einerseits und dem Body Mass Index sowie dem Taillenumfang als Parameter für die Bauchfettverteilung andererseits mittels multivariabler linearer Regressionsmodelle untersucht. Beide Modelle waren für Geschlecht, Alter, Alkoholkonsum, Ausbildungsdauer, Rauchen und sportliche Aktivität adjustiert, das zweite Modell adjustierte zusätzlich für Schlaganfall, Hypertonie, Myokardinfarkt, Non-HDL-Cholesterin und Plasma-Triglyceride. Die Parameter D-Dimere, Faktor VIII, Fibrinogen D, Protein S und Quick-Wert waren signifikant positiv und INR und Antithrombin III signifikant negativ mit dem BMI und dem Taillenumfang assoziiert. Nur für aPTT-Wert bestand keine signifikante Assoziation. Damit konnte der Einfluss von steigendem BMI und Taillenumfang auf die Blutgerinnung deutlich gemacht werden. Die nachgewiesene prothrombotische Veränderung des Gerinnungsprofils durch Adipositas, analysiert als Body-Mass-Index und Taillenumfang, stellt somit ein potentielles Ziel für die Primärprävention bei adipösen Personen dar.

In der zweiten Arbeit wurden wiederum zwei unterschiedlich adjustierte lineare Regressionsmodelle verwendet, um die Zusammenhänge zwischen den Blutgerinnungsmarkern und dem FLI darzustellen. Im zweiten Modell wurde zusätzlich zu den oben beschriebenen Adjustierungsfaktoren für den Diabetesstatus adjustiert. Mit Ausnahme der aPTT waren in beiden Modellen signifikante Assoziationen des Fatty Liver Index mit den untersuchten Gerinnungsparametern zu beobachten. Antithrombin III und INR waren signifikant invers mit dem FLI assoziiert. Nach dem Vorliegen von signifikanten Interaktionsthermen mit dem Diabetes-Status (kein Diabetes, Prädiabetes, bekannter Typ 2- Diabetes und neu diagnostizierter Typ 2-Diabetes) erfolgte zusätzlich eine stratifizierte Auswertung. Für die Teilnehmer mit normalem Blutzucker ergaben sich vergleichbare Werte wie in der Gesamtanalyse, die Ergebnisse bei Prädiabetikern und insbesondere bei Diabetikern wichen jedoch deutlich davon ab. Die Bestimmung des Fettleber-Index als zusätzliches Instrument in der Routinediagnostik zur Prävention der nachweislich durch eine Fettleber induzierten Gesundheitsrisiken wird durch die vorliegenden Ergebnisse zum Zusammenhang mit einem prothrombotischen Gerinnungsprofil unterstrichen.

Die Dissertation erweitert den Kenntnisstand zur Assoziation von einfach zu bestimmenden Indizes wie dem Body Mass Index und dem Fatty Liver Index, mit einem veränderten Profil der Blutgerinnungsfaktoren in der erwachsenen Bevölkerung. Diese Ergebnisse liefern auch neue Ansatzpunkte für die Prävention der Folgen von Adipositas und Fettleber.

4 Abstract (English):

In recent decades, the prevalence of obesity, with its many comorbidities and secondary diseases, rapidly increased and represents a burden for the health care system, making a detailed analysis of the health consequences and their assessment more important than ever. Population-based studies have insufficiently investigated the association of a number of selected coagulation parameters with body fat accumulation and hepatic steatosis.

Thus, the present study analysed the association of selected coagulation parameters with BMI and waist circumference on the one hand and liver fat content, determined by the Fatty Liver Index (FLI) on the other hand, based on data from the KORA-Fit study and presented the results in two publications.

In the first publication, the associations between the coagulation parameters antithrombin III, D-dimer, factor VIII, fibrinogen D, aPTT, protein S, Quick and INR on the one hand and body mass index and waist circumference as parameters of abdominal fat distribution on the other hand were analysed using multivariable linear regression models. Both models were adjusted for sex, age, alcohol consumption, years of education, smoking and physical activity; the second model was additionally adjusted for stroke, hypertension, myocardial infarction, non-HDL cholesterol and plasma triglycerides. D-dimer, factor VIII, fibrinogen D, protein S and quick values were significantly positively, and INR and antithrombin III significantly inversely associated with BMI and waist circumference. Only aPTT showed no significant association. This clearly demonstrated the influence of increasing BMI and waist circumference on blood coagulation. The demonstrated alterations towards a prothrombotic coagulation profile in obesity, analysed as body mass index and waist circumference, thus represent a potential target for primary prevention in obese individuals.

In the second paper, two differently adjusted linear regression models were used to analyse the relationships between blood coagulation markers and the FLI. In the second model, adjustments were made for diabetes status in addition to the adjustment factors described above. With the exception of aPTT, significant associations of the Fatty Liver Index with the investigated coagulation parameters were observed in both models. Antithrombin III and INR were significantly negatively associated with the FLI. After the presence of significant interaction terms with diabetes status (no diabetes, prediabetes, known type 2 diabetes and newly diagnosed type 2 diabetes), an additional stratified evaluation was performed. The results for participants with normal blood glucose were comparable to those in the overall analysis, but the results for prediabetics and especially diabetics were significantly different. The observed results on the association with a prothrombotic coagulation profile support the use of the Fatty Liver Index as an additional tool in routine diagnostics for the prevention of health risks associated with fatty liver.

The thesis extends the knowledge on the association of easily measured indices such as Body Mass Index and Fatty Liver Index with an altered profile of blood coagulation factors in the adult population. These results also provide new avenues for the prevention of the consequences of obesity and fatty liver.

5 Publikaton I: Body Mass Index and waist circumference as determinants of hemostatic factors in participants of a population-based study

Iglesias Morcillo M, Freuer D, Peters A, Heier M, Meisinger C, Linseisen J. Body Mass Index and Waist Circumference as Determinants of Hemostatic Factors in Participants of a Population-Based Study. Medicina (Kaunas). 2023 Jan 26;59(2):228. doi: 10.3390/medicina59020228.

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Body Mass Index and Waist Circumference as Determinants of Hemostatic Factors in Participants of a Population-Based Study

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Abstract: *Background*: In contrast to studies in patients, an association between obesity and blood coagulation factors has not been established in the population. If confirmed it could become a target for primary prevention. *Objective*: To investigate the relationship between Body Mass Index (BMI) and waist circumference (WC) with plasma concentrations of antithrombin III, D-dimers, fibrinogen D, protein S, factor VIII, activated partial thromboplastin time (aPTT), quick value, and international normalized ratio (INR) in the general population. Materials and *Methods*: Participants of the Cooperative Health Research in the Region of Augsburg (KORA) S4 study who took part in the KORA Fit follow-up (2018–2019, aged 54–74 years) examination were eligible. Citrate plasma samples were collected in fasted participants. After the exclusion of participants with anticoagulative treatment, 776 participants (420 women and 356 men) with analytic data on hemostatic factors were included in the present analysis. Linear regression models were used to explore the association between BMI or WC with hemostatic markers, adjusted for sex, age, alcohol consumption, education, smoking status, and physical activity. In a second model, additional adjustments were made for the prevalence of stroke, hypertension, myocardial infarction, serum non-HDL cholesterol, and serum triglycerides. *Results*: In the multivariable models (with or without health conditions), significant positive associations with BMI were obtained for plasma concentrations of D-dimers, factor VIII, fibrinogen D, protein S, and quick value, while INR and antithrombin III were inversely associated. Similar to BMI, WC was significantly associated with all hemostatic factors, except for aPTT. *Conclusion*: In this population-based study, both increasing BMI and WC affect the blood coagulation system. Thus, modification of a prothrombotic coagulation profile emerged as a potential target for primary prevention in obese subjects.

Keywords: Body Mass Index; waist circumference; coagulation; hemostatic factors; obesity; KORA

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1. Introduction

The WHO declared obesity an epidemic as it became a worldwide health threat affecting people of all ages, sex, and various socioeconomic status [1]. According to the WHO, between 1975 and 2016 the number of obese individuals nearly tripled [2]. Due to obesity being associated with many diseases such as type-2 diabetes, cardiovascular diseases, or cancers of different sites, the consequences of this development are important for health and the economy [3]. The factors contributing to obesity are manifold. Eventually, a positive energy balance results in the storage of fat in adipocytes and other cells, such as hepatocytes. Physical activity and dietary behaviour, as well as genetics and physiology, have a strong impact on excess body fat accumulation [4]. It is well known that the effect

of obesity on atherosclerosis is mediated by chronic inflammation, hyperlipidemia, and endothelial dysfunction with the formation of foam cells and atherosclerotic plaques [5,6]. Also, the effects of obesity on the risk of thrombosis due to an impaired hemostatic balance leading to procoagulant and hypofibrinolytic states is well known [7]. So far, the impact of obesity on coagulation factors has been mainly investigated in patient groups [8]. It is, however, unclear whether such associations also exist at the population level which is the level for primary prevention.

Thus, the present study investigated whether Body Mass Index (BMI), a measure of relative body weight also used to classify overweight and obesity, is related to parameters of the blood coagulation system. In addition, waist circumference (WC) as a measure of visceral body fat accumulation was examined. For the analyses, data from the population-based KORA (Cooperative Health Research in the Region of Augsburg) Fit study were utilized.

2. Materials and Methods

2.1. Study Sample

KORA is a research platform established in 1996 for population-based surveys. It is the continuation and development of the MONICA (monitoring trends and determinants in cardiovascular disease) studies, which were conducted in the study region of Augsburg from 1984 to 1995 [9]. The MONICA/KORA project comprises four cross-sectional surveys S1 to S4 (S1 1984/85, S2 1989/90, S3 1994/95, and S4 1999/2001) and assessed the health status of the population in the city of Augsburg and the surrounding districts with an emphasis on cardiovascular diseases, lung diseases and diabetes, as well as environmental exposure factors [9].

The KORA S1–S4 studies consist of around 18,000 participants and include a random sample of the inhabitants of the study region between the ages of 25 and 74 years. Since recruitment, all participants are being followed up by questionnaires to assess incident diseases and changes in exposure factors. In the KORA Fit follow-up study (2018–2019), MONICA/KORA study participants born between 1945 to 1964 were re-invited to the study centre for interviews and physical examination. For the present analysis, all participants of KORA S4 who took part in KORA Fit and for which citrate plasma could be collected were considered, leaving 805 study participants. After the exclusion of those with anticoagulative treatment, 776 participants (420 women and 356 men) with analytic data on hemostatic parameters measured in citrate plasma were included in the present analysis. All study participants gave written informed consent. The study protocol was approved by the Ethics Committee of the Bavarian Chamber of Physicians and conducted according to data protection requirements. The investigations were conducted in accordance with the Declaration of Helsinki.

2.2. Data Collection

The KORA Fit study consists of an interview and self-administered questionnaires, on, for example, health conditions, and physical measurements.

Anthropometric measurements were performed with the subjects in light clothing and without shoes, according to the World Health Organization MONICA protocol [10]. Body Mass Index was calculated as weight in kg (measured to the nearest 0.1 kg) divided by height in square meters (measured to the nearest 0.5 cm). BMI was categorized according to WHO guidelines as underweight (BMI < 18.5 kg/m^2), normal weight (BMI $\geq$ 18.5–<25 kg/m^2), overweight (BMI $\geq$ 25–<30 kg/m^2), and obese (BMI $\geq$ 30 kg/m^2) [2,11]. Waist circumference was measured to the nearest 0.1 cm at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest using stretch-resistant tapes [12]. Habitual consumption of alcoholic beverages was assessed for the previous workday and the previous weekend. Alcohol consumption was calculated in grams per day. Self-reported information on prevalent type-2 diabetes, myocardial infarction, and stroke was verified against medical records. Arterial hypertension was defined as systolic blood pressure

of ≥140 mm HG, diastolic blood pressure of ≥90 mm HG or known hypertension with the use of antihypertensive drugs. Education years were divided into two groups: less or equal to 12 years and more than 12 years of education. In addition, the smoking habits of participants were classified as current smokers (including irregular smokers), former smokers, and never smokers. Physical activity was categorized into four groups: participation in physical activity for more than two hours per week, at least one hour per week, less than one hour per week, and (almost) no physical activity. Non-HDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol.

Detailed information on the data collection procedures and examinations in the KORA studies has been described elsewhere [13].

2.3. Laboratory Measurements

Hemostatic factors were analyzed in citrate plasma samples collected after an overnight fast. Plasma samples were centrifuged (10 min. at 15°) immediately after collection and then aliquoted and stored at −80 °C. The separation of plasma from the other blood components was completed after 30 min. at the latest. The following hemostatic factors were analyzed: antithrombin III, D-dimers, factor VIII, fibrinogen, protein S, partial thromboplastin time (aPTT), quick value, and international normalized ratio (INR).

Antithrombin III (reference value: 83–118%) was determined by chromogenic activity assay (Innovance Antithrombin, SCS cleaner, Siemens Healthcare Diagnostics, Eschborn, Germany). D-dimers (reference value: <500 µg/dL) were measured by means of a particle-enhanced immunoturbidimetric assay (Innovance D-Dimer Kit, Siemens Healthcare Diagnostics). Factor VIII activity (reference value: 70–150%) was measured photometrically (coagulation factor VIII deficient plasma, Pathromtin SL, CaCl2, Siemens Healthcare Diagnostics), as well as quick value (reference value: 82–125%; Thromborel S, Siemens Healthcare Diagnostics), aPTT (reference value: 26–36 s; Pathromtin SL, CaCl Lösung, Actin FS, Siemens Healthcare Diagnostics), and protein S (reference value: men: 73–130%, women: 52–126%; Hemoclot protein S, OVB-Puffer, $CaCl_2$, SCS Cleaner). Fibrinogen (reference value: 210–400 mg/dL) was measured photometrically and turbidimetrically (Multifibren U, Siemens Healthcare Diagnostics). INR (reference value: 0.9–1.15) was calculated from the prothrombin ratio (Thromborel S, Siemens Healthcare Diagnostics) by dividing the thromboplastin time of the subject by that of normal plasma squared with International Sensitivity Index (ISI) as defined by the World Health Organization [14]. Reference values of all hemostatic factors were taken from University Clinic Augsburg, while all other serum parameters were measured at the clinical laboratory of the University Hospital (Klinikum Großhadern) of the Ludwig-Maximilians University in Munich. Measurement procedures were performed and controlled by trained laboratory personnel according to standardized protocols.

2.4. Statistical Analysis

For data analysis, the statistical software SPSS, version 26 (IBM Inc., Armonk, New York, USA) was used. The Kolmogorov–Smirnov and Shapiro-Wilk tests were used to test for the normal distribution of data. Normally distributed variables were presented as arithmetic mean and standard deviation. None of the blood coagulation markers were normally distributed and thus described by the median and interquartile range (25–75% percentile). Non-parametric tests were used to test for differences between two (Mann–Whitney U) or more (Kruskal–Wallis) independent samples. A p-value of <0.05 was regarded as statistically significant.

Linear regression models with BMI or WC as independent variables were created. The models included the blood coagulation markers described above as dependent variables. The models were first adjusted for age (continuous), alcohol consumption (continuous), sex (male; female), education (<12 years; ≥12 years), smoking status (never; former; current), physical activity (physical activity for more than two hours per week, at least one hour per week, less than one hour per week, and (almost) no physical activity), and in a second

model further adjusted for prevalent stroke (yes/no), hypertension (yes/no), myocardial infarction (yes/no), serum non-HDL cholesterol, and triglycerides (continuous).

3. Results

The baseline characteristics of the study population are given in Table 1. The mean BMI was higher in men with 28.40 ± 4.23 kg/m^2 as compared to women with 27.39 ± 5.18 kg/m^2, leaving more men than women in the overweight and obese categories.

Table 1. Characteristics of the study participants [a]; overall and by sex.

		Total (n = 776)		**Males (n = 356)**		**Females (n = 420)**	
				Mean	SD		
Age [years]		63	6	63	6	63	6
BMI [kg/m^2]		27.86	4.79	28.40	4.23	27.39	5.18
Waist circumference [cm]		94.0	13.8	100.5	12.2	88.4	12.6
				Median (25th–75th percentile)			
Alcohol consumption [b] [g/d]		5.71 (0.00; 22.86)		5.71 (0.00; 21.50)		6.70 (0.00; 22.86)	
				N	%		
BMI [kg/m^2]	Underweight	5	0.6%	0	0%	5	1.0%
	Normal weight	229	29.5%	80	22.5%	149	35.5%
	Overweight	307	39.6%	156	43.8%	151	36.0%
	Obese	235	30.3%	120	33.7%	115	27.4%
Education [years]	≤12 years	471	60.7%	198	56.0%	273	63.0%
	>12 years	305	39.3%	158	44.0%	147	37.0%
Physical activity	≥2 h/week	284	36.6%	133	37.5%	151	36.0%
	1 h/week	264	34.0%	115	32.5%	149	35.5%
	<1 h/week	94	12.1%	50	14.0%	44	10.5%
	(almost) no activity	134	17.3%	58	16.0%	76	18.0%
Smoking	Current smoker	106	13.6%	53	15.0%	53	13.0%
	Former smoker	335	43.2%	176	49.5%	159	38.0%
	Never smoker	335	43.2%	127	35.5%	208	49.0%
Hypertension [c]	Yes	361	46.5%	196	55.0%	165	39.0%
	No	414	53.5%	159	45.0%	255	61.0%
Myocardial infarction	Yes	22	2.8%	18	5.0%	4	1.0%
	No	754	97.2%	338	95.0%	416	99.0%
Stroke	Yes	18	2.3%	12	3.4%	6	1.5%
	No	758	97.7%	344	96.6%	414	98.5%
Type-2 diabetes [c]	Yes	64	8.3%	32	9.0%	32	7.6%
	No	710	91.7%	324	91.0%	386	92.4%

[a] After exclusion of individuals with anticoagulation therapy (n = 29); [b] n = 775; [c] n = 775.

The median concentrations of hemostatic parameters differed between men and women, except for D-dimers, factor VIII, and fibrinogen (Table 2). Median concentrations of all markers were in a normal range, except for protein S.

Table 3 describes the median plasma concentrations of coagulation markers by BMI categories (Table 3). Obese subjects showed the highest concentrations of D-dimers, factor VIII, fibrinogen, and protein S, while antithrombin III concentrations were the lowest. Whereas all other coagulation factors showed no deviation from the normal range, median protein S concentration in obese subjects was outside the normal range (median: 131.70; interquartile range: 113.20; 148.80).

Table 2. Plasma concentrations of blood coagulation factors in all participants and by sex [a,d].

	Total	Males	Females	*p*-Value [d]
	Median (25th–75th percentile)			
Antithrombin III [mg/dL]	102.30 (95.50; 108.90)	98.80 (93.30; 105.55)	104.70 (98.30; 110.60)	0.001
D-dimers [µg/L]	407 (309; 556)	408 (316; 563)	406 (306; 554)	0.642
Faktor VIII [%]	121.70 (97.40; 143.00)	119.80 (94.90; 141.80)	123.75 (100.90; 143.55)	0.181
Fibrinogen D [mg/dL]	294.55 (260.40; 334.20)	288.00 (257.50; 326.70)	299.55 (263.25; 339.75)	0.041
aPTT [s] [b]	30.70 (28.70; 32.90)	31.15 (29.30; 33.50)	30.25 (28.70; 32.90)	0.001
Protein S [%]	125.70 (105.45; 146.10)	131.70 (111.00; 153.90)	120.00 (101.30; 114.50)	0.001
Quick value [%]	108.80 (102.20; 114.50)	106.70 (100.20; 112.50)	110.35 (104.50; 115.70)	0.001
INR [c]	0.95 (0.92; 0.99)	0.97 (0.93; 1.01)	0.95 (0.91; 0.98)	0.001

[a] After exclusion of individuals with anticoagulation therapy (n = 29); [b] activated partial thromboplastin time; [c] international normalized ratio; [d] Mann–Whitney U Test $p < 0.05$.

Table 3. Plasma concentrations of hemostatic factors [a] (median, IQR) according to Body Mass Index (BMI) categories.

BMI [kg/m^2]	Underweight [<18.5]	Normal Weight [≥18.5–<25]	Overweight [≥25–<30]	Obesity [≥30]	*p*-Value [d]
	(n = 5)	(n = 229)	(n = 307)	(n = 235)	
	Median (25th–75th percentile)				
Antithrombin III [mg/dL]	104.7 (94.0; 112.0)	106.5 (99.5; 113.10)	100.60 (94.70; 107.30)	99.90 (93.40; 106.10)	0.001
D-dimers [µg/L]	335 (289; 476)	368 (276; 503)	392 (308; 552)	449 (348; 691)	0.001
Faktor VIII [%]	112.40 (100.30; 125.30)	119.80 (9740; 140.35)	116.00 (93.70; 137.90)	132.50 (107.30; 154.30)	0.001
Fibrinogen D [mg/dL]	306.20 (296.10; 336.10)	278.60 (247.10; 316.20)	288.30 (261.90; 325.30)	317.70 (279.10; 357.30)	0.001
aPTT [s] [b]	28.80 (28.10; 30.30)	30.60 (28.60; 32.90)	31.10 (29.1; 33.30)	30.40 (28.40; 32.70)	0.070
Protein S [%]	128.50 (98.90; 154.50)	115.50 (98.80; 133.20)	129.25 (105.70; 148.80)	131.70 (113.20; 148.80)	0.001
Quick value [%]	111.40 (107.80; 121.00)	108.05 (100.45; 113.70)	108.00 (101.7; 113.90)	110.20 (104.25; 115.90)	0.004
INR [c]	0.94 (0.89; 0.96)	0.96 (0.93; 1.00)	0.96 (0.92; 1.00)	0.95 (0.91; 0.98)	0.004

[a] After exclusion of individuals with anticoagulation therapy (n = 29); [b] activated partial thromboplastin time; [c] international normalized ratio; [d] Kruskal–Wallis test $p < 0.05$.

The results of the linear regression models that explored the association between BMI and each of the blood coagulation factors are shown in Table 4. All analyses were adjusted for sex, age, alcohol consumption, education, smoking status, and physical activity (Model 1). In a second model, additional adjustments were made for the prevalence of stroke, hypertension, and myocardial infarction, as well as for plasma non-HDL cholesterol and plasma triglycerides (Model 2). In Model 1, statistically significant positive associations were obtained for plasma concentrations of D-dimers, factor VIII, fibrinogen D, protein S, and quick value, while INR and antithrombin III were inversely associated. The results were slightly attenuated after additional adjustments for health conditions (Model 2). Only aPTT

was not significantly related to BMI. Similar associations were seen in linear regression models with WC as the independent variable (Table 5).

Table 4. Association between Body Mass Index and blood coagulation parameters [a].

	Model 1 [b]			Model 2 [c]		
	β Estimate	95% Confidence Interval	*p*-Value	β Estimate	95% Confidence Interval	*p*-Value
Antithrombin III [mg/dL]	−0.435	−0.592; −0.279	<0.001	−0.468	−0.633; −0.302	<0.001
Ln D-dimers	0.020	0.013; 0.028	<0.001	0.020	0.012; 0.028	<0.001
Faktor VIII [%]	1.273	0.740; 1.806	<0.001	1.145	0.581; 1.710	<0.001
Fibrinogen D [mg/dL]	2.987	2.045; 3.929	<0.001	2.923	1.921; 3.926	<0.001
aPTT [s] [d]	−0.036	−0.087; 0.014	0.160	−0.043	−0.097; 0.010	0.114
Protein S [%]	0.943	0.425; 1.460	<0.001	0.609	0.070; 1.147	0.027
Quick value [%]	0.214	0.070; 0.358	0.004	0.177	0.026; 0.328	0.021
INR [e]	−0.001	−0.002; 0.000	0.005	−0.001	−0.002; 0.000	0.022

[a] After exclusion of individuals with anticoagulation therapy (n = 29); [b] dependent variables: blood coagulation markers, independent variable: Body Mass Index, adjusted for age, alcohol consumption, sex, education, smoking, and physical activity; [c] dependent variables: blood coagulation markers, independent variable: Body Mass Index, additionally adjusted for stroke, hypertension, myocardial infarction, non-HDL cholesterol, triglycerides; [d] activated partial thromboplastin time; [e] international normalized ratio.

Table 5. Association between waist circumference and blood coagulation parameters [a].

	Model 1 [b]			Model 2 [c]		
	β Estimate	95% Confidence Interval	*p*-Value	β Estimate	95% Confidence Interval	*p*-Value
Antithrombin III [mg/dL]	−0.180	−0.241; −0.118	<0.001	−0.191	−0.256; −0.126	<0.001
Ln D-dimers	0.008	0.005; 0.011	<0.001	0.008	0.005; 0.011	<0.001
Faktor VIII [%]	0.441	0.231; 0.651	<0.001	0.385	0.162; 0.608	0.001
Fibrinogen D [mg/dL]	1.035	0.664; 1.406	<0.001	1.021	0.623; 1.418	<0.001
aPTT [s] [d]	−0.016	−0.036; 0.004	0.114	−0.020	−0.041; 0.001	0.064
Protein S [%]	0.395	0.193; 0.597	<0.001	0.265	0.054; 0.477	0.014
Quick value [%]	0.098	0.042; 0.154	0.001	0.086	0.026; 0.146	0.005
INR [e]	−0.001	−0.001; 0.000	0.001	−0.001	−0.001; 0.000	0.005

[a] After exclusion of individuals with anticoagulation therapy (n = 29); [b] dependent variables: Blood coagulation markers, independent variable: Body Mass Index, adjusted for age, alcohol consumption, sex, education, smoking, and physical activity; [c] dependent variables: blood coagulation markers, independent variable: Body Mass Index, additionally adjusted for stroke, hypertension, myocardial infarction, non-HDL cholesterol, triglycerides; [d] activated partial thromboplastin time; [e] international normalized ratio.

4. Discussion

This population-based study identified significant associations between nearly all analyzed blood coagulation markers and BMI as well as WC. Increased BMI or WC were related to higher concentrations of D-dimers, factor VIII, fibrinogen, protein S, and quick value and decreased values of antithrombin III and INR.

Many studies confirmed a higher risk of early mortality for people with a high body fat mass (often expressed as BMI) [15] but data on hemostatic factors and mortality is rare. Ageno and co-workers [16] searched databases from 1996 to 2006 and found 15 studies of sufficient quality to summarize the evidence for the link between obesity and venous thromboembolism. Eight case-control studies and one cohort study were combined in a meta-analysis; the pooled relative risk of venous thromboembolism was 2.33 (95% CI: 1.68–2.34) in obese subjects versus subjects with normal body weight.

The basis for the correct interpretation of our findings is the physiologic role each hemostatic factor has in the complex blood coagulation system. D-dimers reflect both activations of fibrinolysis and thrombin production [17]. It is clinically used to indicate hypercoagulability in various disorders like venous thromboembolism, ischemic heart

disease, trauma, and infection [18,19]. Coagulation factor VIII is a glycoprotein synthesized mainly in hepatocytes, but also in kidneys, endothelial cells, and lymphatic tissue [20]. Higher plasma concentrations of both factors indicate thrombotic events, and lower levels eliminate the risk of thrombolytic events. Another glycoprotein produced in the liver is fibrinogen. Fibrinogen gets converted enzymatically by thrombin to fibrin and contributes to the formation of blood clots, in reaction to vascular or tissue injury [21]. Protein S, a co-factor of protein C, is created in the liver and influences blood coagulation by increasing fibrinolysis; it is dependent on vitamin K. Low levels of protein S lead to a higher risk of thrombophilia, which itself is associated with a higher occurrence of thrombosis. Differences in protein S levels can be due to low vitamin K levels, pregnancy, or several chronic diseases [22]. Antithrombin III is a glycoprotein belonging to the group of serine protease inhibitors. By inhibiting several factors of the coagulation system, especially thrombin, and factors VIIa, IXa, Xa, Xia, and XIIa, antithrombin III holds a central role in regulating the coagulation cascade [23]. Low levels of antithrombin III lead to a very high risk of thrombosis [24] and can also suggest reduced synthesis and underlying liver disease [25]. Antithrombin III is a physiologic anticoagulant and its physiologic effects are increased by a factor of 1000 in the presence of heparin.

A few other investigations have also confirmed positive associations of coagulation markers with rising BMI as observed in our study. Hörber et al. [26] investigated the relationship between obesity, in particular, body fat distribution, and hemostatic parameters. They used data from 150 subjects with impaired glucose tolerance and/or impaired fasting glucose, which were randomly selected from the "Prediabetes Lifestyle Intervention Study" (PLIS). The participants were exclusively recruited at the University Hospital in Tübingen. Regression models were not fully adjusted for possible confounders except age and sex. They reported positive associations between BMI and factor VIII, protein S, and D-dimers, while antithrombin III was inversely associated with BMI. These findings are in line with our results in a middle-aged population. However, the literature also reports positive associations of antithrombin III levels with rising BMI [27]. Christensen et al. [28] described the influence of BMI on the rise of factor VIII through adipose tissue influencing hepatic metabolism and the production of hemostatic factors in the liver.

Clotting tests used in clinical practice to describe failures in the intrinsic or extrinsic pathway are aPTT, INR, and quick value. They mostly depend on partial thromboplastin time. Quick and INR are inversely proportional to each other, meaning that a rise in one causes a decrease in the other. This was also confirmed in the present study. While Quick and INR detect influences on extrinsic blood coagulation, aPTT detects those in the intrinsic system. To the best of our knowledge, the influence of BMI or WC on the results of clotting tests has not been reported so far.

Since, in our study, BMI- and WC-based analyses indicated similar associations with markers of coagulation, it appears to be the extent of body fat in general—and not limited to visceral fat—that affects the coagulation system. In a review article, De Pergola et al. [29] described coagulation and fibrinolysis abnormalities in obese patients who showed higher plasma concentrations of all prothrombotic factors, especially fibrinogen, compared to non-obese, and with a positive association with central fatty tissue accumulation. Their review also confirms the great influence of plasminogen activator inhibitor (PAI-1) on the risk of coronary events and atherothrombosis. With PAI-1 being the primary physiological inhibitor of plasminogen activation, it has a significant effect on fibrinolysis [30]. Several more studies cited in this review showed that plasma levels of PAI-1 in obese people directly correlate with visceral fat independent of metabolic and non-metabolic variables. In a sub-study of the Prediabetes Lifestyle Intervention Study, Hörber et al. [31] examined the effect of lifestyle intervention on hemostatic factors. They showed that lifestyle intervention can improve prothrombotic state and decrease several coagulation factors, among others, protein S, factor VIII, and PAI-1. They used data from 100 individuals with impaired glucose tolerance or impaired fasting blood glucose, who participated in a one-year lifestyle intervention, including precise metabolic phenotyping, MR-based determination of liver

fat content, and analysis of coagulation parameters before and after this intervention. The results show a potential way to improve prothrombotic status through lifestyle intervention and provide insight into possible future treatment targets.

The strengths of our study are a large sample size, the population-based design, the availability of standardized data on cardiovascular risk factors, and standardized anthropometric and laboratory measurements. However, several limitations of the study also need to be considered. The lack of measurement of other important coagulation factors like thrombin, von Willebrand factor, and plasminogen activator inhibitor limits the significance of the study. Furthermore, follow-up studies need to consider the distribution of subcutaneous fat and visceral fat by bioelectrical impedance analysis (BIA) dual-energy X-ray absorptiometry (DEXA), or magnetic resonance imaging (MRI) measurements. Finally, the results may not be transferable to persons of other ethnicity or other ages, since only participants in the Augsburg region ranging in age from 53 to 74 years were considered.

5. Conclusions

Overall, our results in this population-based study indicated that an increase in BMI and WC impacts the blood coagulation system in a sense that the balance between coagulation and fibrinolysis is shifted towards a more prothrombotic constellation, which is confirmed by the increase in pro-coagulative factors and the result of clotting tests in our study. As an additional mechanism to explain the effect of obesity on the development of selected chronic diseases, the coagulation system could be considered a target for the primary prevention of thrombotic events.

Author Contributions: M.I.M. conducted the statistical analysis, interpreted the data, and drafted and revised the manuscript; J.L. designed the study, financed the citrate plasma collection and laboratory analysis, 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; A.P. and M.H. were responsible for the design and conduct of the KORA FIT study. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the Bavarian Chamber of Physicians (KORA-Fit 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 25 January 2023).

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

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6 Publikation II: Association between fatty liver index and blood coagulation markers: a population-based study

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Lipids in Health and Disease

RESEARCH Open Access

Association between fatty liver index and blood coagulation markers: a population-based study

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Abstract

Background Population-based studies investigating the association between blood coagulation markers and non-alcoholic fatty liver disease (NAFLD) are rare. Thus, we aimed to investigate the relationship between the Fatty Liver Index (FLI) as a measure of hepatic steatosis and plasma concentrations of antithrombin III, D-dimer, fibrinogen D, protein C, protein S, factor VIII, activated partial thromboplastin time (aPTT), quick value and international thromboplastin time (INR) in the general population.

Methods After the exclusion of participants with anticoagulative treatment, 776 participants (420 women and 356 men, aged 54–74 years) of the population-based KORA Fit study with analytic data on hemostatic factors were included in the present analysis. Linear regression models were used to explore the associations between FLI and hemostatic markers, adjusted for sex, age, alcohol consumption, education, smoking status, and physical activity. In a second model, additional adjustments were made for the history of stroke, hypertension, myocardial infarction, serum non-HDL cholesterol levels, and diabetes status. In addition, analyses were stratified by diabetes status.

Results In the multivariable models (with or without health conditions), significantly positive associations with FLI were obtained for plasma concentrations of D-dimers, factor VIII, fibrinogen D, protein C, protein S, and quick value, while INR and antithrombin III were inversely associated. These associations were weaker in pre-diabetic subjects and largely disappeared in diabetic patients.

Conclusion In this population-based study, an increased FLI is clearly related to changes in the blood coagulation system, possibly increasing the risk of thrombotic events. Due to a generally more pro-coagulative profile of hemostatic factors, such an association is not visible in diabetic subjects.

Keywords Fatty liver index, Coagulation, Hemostatic factors, Hepatic steatosis, KORA

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Background

Non-alcoholic fatty liver disease (NAFLD) is highly prevalent and includes a spectrum of abnormalities ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) to cirrhosis. With a prevalence of around 25% worldwide, it has become the most frequent chronic liver disease in recent decades [1]. Fatty liver, also called steatotic hepatitis, is a common disease in industrialized nations [1, 2]. NAFLD often remains undiagnosed for a long time, and there is still no specific medication available [2]It is closely related to type 2 diabetes mellitus (T2DM) or impaired fasting glucose, dyslipidemia, obesity, and hypertension. Excessive dietary energy intake and progressive obesity lead to the accumulation of body fat stores, including lipid deposits in the liver, resulting in alterations of glucose and lipid metabolism, and inflammation. Thus, environmental factors, including an energy-dense diet and low physical activity, in combination with genetic factors, promote the development of metabolic derangements and eventually NAFLD. Without medical intervention and a comprehensive change in lifestyle and body fat mass, fatty liver disease may develop into fatty liver inflammation (steatohepatitis), liver fibrosis, cirrhosis, and eventually liver cancer [2–5]. However, this is not a strict sequence as, e.g., fibrosis may already occur at the early stages of NAFLD [6]In addition, in subjects with NAFLD the risk of cardiovascular diseases, including heart attack and type 2 diabetes, considerably increases [7].

Bedogni and coworkers were the first to describe a simple formula for calculating the so-called Fatty Liver Index (FLI). By including data on waist circumference, body mass index, serum triglycerides and the liver enzyme gamma-glutamyl transferase, they were able to develop and validate a score [8]. This simple tool can be used to determine the likelihood of fatty liver disease quickly and easily. However, it lacks the precision of histologic analysis of liver biopsies. Liver biopsy analysis remains the diagnostic gold standard, but its use is limited by high costs and procedural risk [9]. The high incidence of NAFLD makes routine biopsies both impractical and impossible in all patients [10] Imaging techniques, including ultrasonography of the liver, are also used to analyze fat accumulation in the liver, and the FLI was validated against the results of liver ultrasonography. Also, the application of imaging techniques requires specific devices and trained personnel [10] Among many other functions of the liver, this organ also produces most of the coagulation markers and thus plays a key role in blood coagulation [11]; abnormalities of the liver, including a high liver cell fat content, may result in modifications of liver-derived hemostatic factors and impact the blood coagulation system [12]. Blood clotting markers that initiate or drive blood clotting or lead to the formation of a clot, such as fibrinogen D or factor VIII, are mainly formed in the liver. In addition, proteins and enzymes such as antithrombin III, protein C and protein S are also produced in the liver and play a major role in inhibiting blood clotting, thus acting as natural anticoagulants [11]. Simplified speaking, blood clotting is triggered by the ladder-like activation of individual enzymes, which, through their activation, trigger a cascade that results in the formation of a thrombus [13].

The prevalence of insulin resistance is high in NAFLD and even higher in patients with NASH [14]. As there is an indication that treatment of prediabetic patients also leads to the normalization of coagulation markers [15], the relationship between glucose tolerance status and FLI needs exploration. Thus, the purpose of this study was to investigate the relationship between FLI and a large set of clinically relevant blood coagulation markers in the general population as FLI could be used as a screening tool to identify NAFLD (followed by clinical characterization, and eventually intervention). Additionally, the situation in diabetic and prediabetic subjects will be explored. If an influence of the FLI on hemostatic factors - and thus the coagulation cascade - can be confirmed at the population level, this would provide further support for preventive measures to decrease the liver fat content and thus avoid the development of liver fibrosis and metabolic (and cardio-vascular) complications.

Materials and methods

Study sample

The KORA (Cooperative Health Research in the region of Augsburg) project continued the MONICA (Monitoring trends and determinants in cardiovascular disease) project in 1996. Together, they had the objective of investigating the health status of the adult inhabitants of the Augsburg region, with a special focus on cardiovascular diseases, lung diseases, diabetes, and environmental exposure factors [16]. Four cross-sectional surveys, KORA survey 1 (S1) to KORA survey 4 (S4), were carried out in a period from 1984 to 2001, and 18,000 participants aged 25–74 years were recruited and their health status was assessed; several follow-up studies were conducted [17]. In the 2018–2019 KORA Fit Follow-up Study, all living participants of the KORA cohort born between 1945 and 1964 were enrolled to get information on the health status of all middle-agers in the full KORA cohort. All living KORA S4 participants who had participated in KORA Fit and for whom citrated plasma samples were available were eligible for the present analyses. A total of 776 participants, 420 women and 356 men, were considered after the exclusion of participants undergoing anticoagulative treatment (n=29). All study participants gave written informed consent. The study protocol was approved by the Ethics Committee

of the Bavarian Chamber of Physicians (KORA Fit EC No. 17040) and conducted according to the data protection requirements. The investigations were carried out in accordance with the Declaration of Helsinki.

Data collection

Participants of the KORA Fit study were interviewed by certified and specially trained personnel in a standardized interview about lifestyle factors, such as smoking behavior, physical activity, alcohol consumption, pre-existing diseases, medication intake and socio-demographic variables. Alcohol consumption was calculated in grams per day assessed for the previous workday and the previous weekend. Education was recorded in completed school years (≤12 years and >12 years). Physical activity was estimated using two separate four-item interview questions asking about the time per week spent on leisure-time sports (including cycling) in summer and winter. The winter and summer responses were combined to create one variable of leisure-time physical activity. First, participants were classified into one of four categories for frequency of leisure time activity: 'No activity' was defined as no sport in either summer or winter; 'Low activity' was irregular participation in sport for approximately 1 hour per week in at least one season; 'Moderate activity' was regular participation in sport for approximately 1 hour per week in at least one season; 'High activity' was regular participation in sport in both summer and winter for more than 2 h per week in both seasons. The previous existence of myocardial infarction and stroke (yes/no) was recorded but not verified against medical records. Smoking behavior was recorded as current, former and never smoker. Persons aware of having hypertension, taking antihypertensive medication, and/or having blood pressure values of 140/90 mmHg or higher at baseline were defined as actual hypertensives (yes/no). Diabetes status was assessed by HbA1c (%) and serum fasting glucose (mg/dl) and categorized into five subgroups: normal status, prediabetes (fasting plasma glucose levels: ≥100- <126 and/or HbA1c: ≥5.7- <6.5), newly diagnosed diabetes (fasting plasma glucose levels: ≥126 or HbA1c: ≥6.5), known diagnosis of diabetes 2, and other type or vague status. Body mass index was calculated as weight in kilograms divided by height in square meters [18]. Waist circumference was measured to the nearest 0.1 cm at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest using stretch-resistant tape [19]. The interview process and all measurements were done according to the standard operating procedures; the devices used were regularly calibrated according to the manufacturer's advice. Study nurses were trained according to SOPs, certified and regularly re-certified for each examination module. Quality assurance measures were applied throughout the field phase, and for the final data control, all laid down in the quality assurance report. Detailed information on the data collection, examination procedures and variable definitions in the KORA studies is described in detail elsewhere [20].

Laboratory measurements

All hemostatic measurements were performed in citrated plasma collected after an overnight fasting state. Samples were processed immediately (centrifugation 10 min at 15 °C), aliquoted and stored at -80 °C.

Antithrombin III activity (reference value: 83–118%) was measured by chromogenic activity assay (Innovance Antithrombin, SCS cleaner, Siemens Eschborn). D-Dimers were determined by means of a particle-enhanced immunoturbidimetric assay (ref. value: <500 µg/dL; Innovance D-Dimer Kit, Siemens Eschborn). Faktor VIII activity (ref. value: 70–150%) was analyzed using photometry (coagulation factor VIII deficient plasma, Pathromtin SL, CaCl2, Siemens Eschborn). Fibrinogen (ref. value: 210–400 mg/dL) was quantified photometrically and turbidimetrically (Multifibern U, Siemens Eschborn). The parameters of aPTT (ref. value: 26–36 s; Pathromtin SL, CaCl Lösung, Actin FS, Siemens Eschborn), protein C and S activity (ref. value for protein C: 70–140%; ref. value for protein S: 73–130% for men, 52–126% for women; Berichrom Protein C, Siemens Healthcare; Hemoclot Protein S, OVB-Puffer, CaCl2, SCS-Cleaner) and quick value (ref. value: 82–125%; Thromborel S Siemens Eschborn) were determined photometrically. INR (ref. value: 0.9–1.15) was calculated from the prothrombin ratio (Thromborel S, Siemens Eschborn) by dividing the thromboplastin time of the subject by the thromboplastin time of normal plasma squared with the International Sensitivity Index (ISI) according to the WHO [21]. Serum triglycerides (ref. value: 0-200 mg/dl) were determined by an enzymatic color test (Hoffman-La Roche AG Basel/Switzerland) on a Cobas 8000 c702 (Hoffman-La Roche AG Basel/Switzerland). Serum total cholesterol levels (ref. value: 0-200 mg/dL) and HDL cholesterol (ref. value: >45 mg/dL) were analyzed enzymatically (Cobas 8000 c702 Roche chemistry analyzer; Hoffman-La Roche AG, Basel/Switzerland). Non-HDL cholesterol was built by subtracting HDL cholesterol from total cholesterol. Gamma-glutamyl transferase (GGT) was analyzed according to the IFCC method (Hoffmann-La Roche AG Basel/Switzerland), and serum glucose was quantified using the hexokinase method (Hoffmann-La Roche AG Basel/Switzerland). For the determination of glycosylated haemoglobin (HbA1c), an HPLC method with UV detection (Variant II Turbo Hemoglobin Test System; Biorad Hercules, California/USA) was used.

Reference values of all blood coagulation markers were taken from Hospital Augsburg, and all other parameters were measured at the clinical laboratory of the University Hospital of the Ludwig-Maximilians University in Munich (Klinikum Großhadern). All measurement procedures were performed and controlled according to standardized protocols by trained laboratory personnel.

The Fatty Liver Index was calculated according to the formula of Bedogni et al. [8]:

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

FLI was categorized into three groups. Values between 0 and 29 rule out fatty liver disease and thus hepatic steatosis, values between 30 and 59 are defined as unclear, and a score of 60 and higher indicates the presence of fatty liver disease (hepatic steatosis).

Statistical analyses

Continuous variables were presented by means and standard deviation, and nonnormally distributed variables were described by median and interquartile range. The Shapiro–Wilk test was applied to test for normal distribution. The Kruskal–Wallis test was used to test for differences between the medians of three or more independent groups.

Linear regression models were applied to investigate associations between FLI (continuous) as an exposure variable and blood coagulation markers as dependent variables. The models were adjusted for age (continuous), alcohol consumption (continuous), sex (male; female), education (<12 years; ≥12 years), smoking status (never; former; current) and physical activity (≥2 h/week; 1 h/week; <1 h/week; (almost) no activity). In a second model, additional adjustments were made for stroke (yes/no), hypertension (yes/no), myocardial infarction (yes/no), serum non-HDL cholesterol (continuous) and diabetes status (normal; prediabetes; newly diagnosed; known type of diabetes; other type or vague). We tested for interaction effects with diabetes status by including a multiplicative term in the fully adjusted models. Stratified analysis by diabetes status (normal; prediabetes; newly diagnosed and known diabetes) was conducted to describe differential associations as indicated by significant interaction results. All model assumptions were ensured, including homoscedasticity. Multicollinearity and autocorrelation were assessed using the variance inflation factor (VIF) and the Durbin-Watson statistic, respectively. A value of $P<0.05$ was considered statistically significant. All analyses were performed with IBM SPSS 29 (IBM Inc., Armonk, New York; USA).

Results

Table 1 shows the baseline characteristics of the 776 participants included in the analyses, stratified by sex. The mean age was the same for both males and females (mean total: 62.6; SD: 5.6). Men (mean: 60.0; SD: 27.6) showed a higher average FLI than women (mean: 39.1; SD: 30.1). Thus, 201 (56.5%) men were regarded as having fatty liver disease. Among women, only 125 (29.7%) were classified as subjects with fatty liver disease. The proportion of females with normal glucose tolerance (233; 55.5%) was higher than that of males (144; 40.5%). Accordingly, the prevalence of prediabetes or diabetes was higher in men than in women.

Median plasma concentrations of coagulation markers are described in Table 2, stratified by FLI categories and glucose tolerance status. Overall, the mean concentrations of blood coagulation markers were in a normal range, except for protein S. Protein S concentrations were elevated for the total sample in the intermediate FLI category (median: 131.3; IQR: 107.8;150.3) and in subjects with hepatic steatosis (median: 132.1; IQR: 112.9; 153.0). When the models were stratified according to glucose tolerance status, the same results were found in all categories, namely, an increased protein S level in the intermediate status and in hepatic steatosis. In the case of newly diagnosed and known diabetes, an increased level of D-dimer was also found in the intermediate status group of FLI (median: 511.5; IQR: 429.0;874.0).

Table 3 shows the results of the linear regression models exploring the association between FLI and blood coagulation markers in the total sample. In the models adjusted for age, alcohol consumption, sex, education, smoking status, and physical activity, significant positive associations were detected for D-dimers, factor VIII, fibrinogen D, protein C, protein S and quick value; inverse associations were obtained for antithrombin III and INR, and aPTT was not significantly associated with FLI (model 1). Similar results were obtained with the second adjustment model, with all hemostatic factors showing significant relationships, but aPTT was also significantly inversely associated with FLI (model 2).

Testing for interaction between FLI and diabetes status revealed several significant results. When stratifying the analysis by diabetes status, the significant associations between FLI and coagulation parameters observed in non-diabetic subjects were not observed in diabetic subjects (except for antithrombin III in model 1). Subjects with normal glucose tolerance (Table 4) showed almost the same results as reported for the whole sample (Table 3). In prediabetic subjects, significant positive associations were obtained for factor VIII, fibrinogen

Table 1 Characteristics of the study participants[a]

		Total (n = 776)		Males (n = 356)		Females (n = 420)	
				Mean	SD		
Age [years]		62.6	5.6	62.6	5.8	62.6	5.5
FLI		48.7	30.8	60.0	27.6	39.1	30.1
BMI [kg/m^2]		27.9	4.8	28.4	4.2	27.4	5.2
Waist circumference [cm]		94.0	13.8	100.5	12.2	88.4	12.6
Gamma glutamyl transferase [U/l]		35.5	40.2	42.7	34.8	29.4	43.4
Glutamate oxalate transaminase [U/l]		26.1	12.9	27.6	10.2	24.9	14.6
Glutamate pyruvate transaminase [U/l]		28.2	16.0	32.3	14.7	24.7	16.4
Total cholesterol [mg/dL]		212.1	40.2	201.4	39.8	221.1	38.3
HDL cholesterol [mg/dL]		64.3	18.9	55.3	15.2	71.9	18.4
Non-HDL cholesterol [mg/dL]		147.8	39.1	146.1	39.6	149.3	38.6
		Median (25th – 75th percentile)					
Alcohol consumption[b] [g/d]		5.7 (0.0;22.9)		5.7(0.0;21.5)		6.7 (0.0;22.9)	
Triglycerides [mg/dL]		106.0 (77.0;148.0)		114.0 (80.5;164.0)		100.0 (72.8;136.0)	
				N	%		
FLI	<30, no hepatic steatosis	287	37.0%	73	20.5%	214	51.0%
	30–59, intermediate status	163	21.0%	82	23.0%	81	19.3%
	≥ 60, hepatic steatosis	326	42.0%	201	56.5%	125	29.7%
Education [years]	≤ 12 years	471	61.0%	198	56.0%	273	63.0%
	> 12 years	305	39.0%	158	44.0%	147	37.0%
Physical activity	high activity (≥ 2 h/week)	284	37.0%	133	37.5%	151	36.0%
	moderate activity (1 h/week)	264	34.0%	115	32.5%	149	35.5%
	low activity (< 1 h/week)	94	12.0%	50	14.0%	44	10.5%
	(almost) no activity	134	17.0%	58	16.0%	76	18.0%
Smoking	current smoker	106	14.0%	53	15.0%	53	13.0%
	former smoker	335	43.0%	176	49.5%	159	38.0%
	never smoker	335	43.0%	127	35.5%	208	49.0%
Hypertension[b]	yes	361	46.5%	196	55.0%	165	39.0%
	no	414	53.5%	159	45.0%	255	61.0%
Myocardial infarction	yes	22	2.8%	18	5.0%	4	1.0%
	no	754	97.2%	338	95.0%	416	99.0%
Stroke	yes	18	2.3%	12	3.4%	6	1.5%
	no	758	97.7%	344	96.6%	414	98.5%
Glucose tolerance status	normal	377	48.6%	144	40.5	233	55.5%
	prediabetes	303	39.0%	159	44.7%	144	34.3%
	newly diagnosed diabetes	16	2.0%	12	3.4%	4	0.9%
	known type 2 diabetes	68	8.8%	37	10.4%	31	7.4%
	other type of diabetes or vague	12	1.6%	4	1.0%	8	1.9%

[a] After exclusion of individuals with anticoagulation therapy (n = 29); [b] n = 775, 1 = missing

D, protein C and protein S. Antithrombin III and aPTT were significantly inversely associated (model 1). In the second adjusted model (model 2), antithrombin III, factor VIII and aPTT were the only significant coagulation markers, with aPTT being inversely associated.

Discussion

The findings of the present study clearly show statistically significant associations between the FLI and a range of blood coagulation markers. All hemostatic factors and clotting tests (except for aPTT) were affected by increasing liver fat content.

In their study, Hörber et al. [22] followed a similar approach, and the coagulation markers tested in relation to fatty liver showed the same trends as those found in our analyses. They demonstrated that fatty liver is significantly positively associated with protein C, protein S, fibrinogen, and factor VIII and negatively associated with antithrombin III and aPTT. According to their interpretation, a fatty liver leads to an increased synthesis of proteins C and S in the liver, with protein S being a

Table 2 Blood coagulation markers (median; IQR) according to Fatty Liver Index (FLI) categories in all participants[a] and stratified by glucose tolerance status

Total sample (n = 776)	**FLI[b]**			
	no hepatic steatosis [FLI < 30]	**intermediate status [FLI 30–59]**	**hepatic steatosis [FLI ≥ 60]**	***P* value[e]**
	n = 287	**n = 163**	**n = 326**	
Antithrombin III [mg/dL]	105.5 (99.0;112.5)	102.1 (96.0;107.9)	98.9 (92.5;106.0)	< 0.001
D-dimers[µg[l/	367.0 (276.0;506.0)	408.0 (330.0;557.0)	438.5 (334.0;624.0)	< 0.001
Faktor VIII [%]	116.8 (93.8;139.3)	117.3 (94.1;137.9)	125.8 (103.4;151.4)	< 0.001
Fibrinogen D [mg/dL]	280.6 (249.1;316.0)	298.4 (265.4;336.1)	307.4 (268.5;345.4)	< 0.001
aPTT [s][c]	30.7 (28.6;33.1)	30.8 (29.1;32.8)	30.6 (28.6;32.8)	0.671
Protein C [%]	122.5 (111.7;136.8)	124.9 (112.0;140.9)	124.2 (111.8;140.3)	0.207
Protein S [%]	115.9 (98.8;132.9)	131.3 (107.8;150.3)	132.1 (112.9;153.0)	< 0.001
Quick value [%]	107.3 (100.2;112.6)	109.2 (103.1;115.5)	109.4 (103.7;115.7)	0.001
INR[d]	1.0 (0.9;1.0)	1.0 (0.9;1.0)	1.0 (0.9;1.0)	0.001
Normal glucose tolerance (n = 377)	n = 194	n = 78	n = 105	
Antithrombin III [mg/dL]	105.5 (99.1;112.5)	102.4 (96.2;108.7)	100.7 (95.2;107.5)	< 0.001
D-dimers[µg[l/	348.0 (270.0;470.0)	379.0 (296.0;607.0)	429.0 (332.0;602.0)	< 0.001
Faktor VIII [%]	116.1 (91.7;139.0)	113.0 (95.6;134.8)	123.5 (100.3;143.5)	0.134
Fibrinogen D [mg/dL]	277.3 (248.8;305.6)	297.5 (260.2;334.6)	302.3 (262.3;342.8)	< 0.001
aPTT [s][c]	30.7 (28.7;33.1)	30.08 (29.1;33.3)	31.3 (29.0;33.3)	0.644
Protein C [%]	122.4 (109.6;136.5)	127.8 (114.8;140.9)	127.5 (113.7;139.2)	0.055
Protein S [%]	111.5 (96.7;129.6)	132.2 (106.6;152.5)	132.1 (110.1;162.3)	< 0.001
Quick value [%]	106.4 (99.7;111.6)	109.5 (103.2;115.8)	108.9 (103.0;117.7)	0.006
INR[d]	1.0 (0.9;1.0)	1.0 (0.9;1.0)	1.0 (0.9;1.0)	0.005
Prediabetes (n = 303)	n = 84	n = 72	n = 147	
Antithrombin III [mg/dL]	105.6 (97.0;110.4)	100.9 (95.4;106.2)	98.5 (91.2;105.3)	< 0.001
D-dimers[µg[l/	414.5 (333.0;562.0)	415.5 (349.0;516.0)	430.0 (326.0;606.0)	0.871
Faktor VIII [%]	116.8 (95.9;139.4)	119.0 (93.4;142.1)	125.5 (101.7;150.9)	0.056
Fibrinogen D [mg/dL]	296.3 (250.3;347.5)	296.8 (269.7;340.0)	310.3 (271.0;341.6)	0.468
aPTT [s][c]	31.1 (28.6;33.6)	30.8 (28.8;32.8)	30.1 (28.2;32.6)	0.201
Protein C [%]	123.5 (112.4;137.5)	122.3 (111.4;133.4)	123.5 (111.6;140.1)	0.959
Protein S [%]	123.8 (105.1;140.7)	130.2 (109.5;148.9)	134.2 (113.9;150.2)	0.050
Quick value [%]	108.8 (102.8;115.0)	108.8 (101.9;114.8)	110.5 (104.6;115.5)	0.381
INR[d]	1.0 (0.9;1.0)	1.0 (0.9;1.0)	0.9 (0.9;1.0)	0.396
Diabetes newly diagnosed and known (n = 84)	n = 6	n = 10	n = 68	
Antithrombin III [mg/dL]	109.8 (98.3;121.0)	104.4 (97.8;109.7)	96.3 (91.4;104.0)	0.038
D-dimers[µg[l/	419.0 (289.0;552.0)	511.5 (429.0;874.0)	499.5 (374.5;716.0)	0.486
Faktor VIII [%]	137.1 (97.4;177.7)	122.3 (115.8;139.8)	136.6 (114.1;162.5)	0.355
Fibrinogen D [mg/dL]	299.9 (274.8;356.5)	304.0 (284.0;318.3)	313.2 (277.1;349.9)	0.786
aPTT [s][c]	28.4 (25.5;30.4)	31.1 (29.6;32.9)	30.6 (28.8;32.7)	0.108
Protein C [%]	124.3 (119.7;133.1)	124.6 (114.3;141.1)	120.9 (110.6;142.0)	0.919
Protein S [%]	104.9 (94.4;137.3)	134.5 (122.3;156.7)	130.9 (112.8;146.4)	0.242
Quick value [%]	113.4 (108.4;121.0)	111.1 (103.6;116.1)	108.7 (101.6;115.7)	0.297
INR[d]	0.9 (0.9;1.0)	0.9 (0.9;1.0)	1.0 (0.9;1.0)	0.286

[a] After exclusion of individuals with anticoagulation therapy (n = 29); [b] Fatty Liver Index; [c] activated partial thromboplastin time [d] international normalized ratio [e] Kruskal–Wallis test

coproduct of protein C. As natural anticoagulants, they inhibit the coagulation cascade, which would counteract a prothrombotic state promoted by fatty liver [23, 24]. This hypothesis is supported by our data since both proteins were positively associated with an increasing FLI. As in our analyses, Hörber et al. also reported increased fibrinogen and factor VIII levels with increasing liver steatosis. Fibrinogen is a glycoprotein that is produced in the liver and contributes to the formation of a thrombus at the end of blood clotting [25]. Factor VIII is produced not only in the hepatocytes of the liver but also in the kidneys, lymphatic tissue, and endothelial cells [26]. Hörber et al. [22] also reported a negative association between antithrombin III, aPTT, and fatty liver, which was confirmed by our study results. Since antithrombin III is a natural anticoagulant produced in the liver and

Table 3 Association between the Fatty Liver Index (FLI) and blood coagulation parameters in all study participants[a]

	Model 1[b]			Model 2[c]		
	β estimate	95% confidence interval	*P* value	β estimate	95% confidence interval	*P* value
Antithrombin III [mg/dl]	-0.069	-0.095; -0.042	< 0.001	-0.080	-0.109; -0.051	< 0.001
Ln D-dimers	0.003	0.002; 0.004	< 0.001	0.002	0.001; 0.004	0.002
Faktor VIII [%]	0.232	0.144; 0.321	< 0.001	0.215	0.115; 0.314	< 0.001
Fibrinogen D [mg/dL]	0.418	0.260; 0.576	< 0.001	0.373	0.196; 0.551	< 0.001
aPTT [s][d]	-0.008	-0.017; 0.000	0.055	-0.010	-0.020; -0.001	0.032
Protein C [%]	0.108	0.064;0.153	< 0.001	0.083	0.035; 0.132	< 0.001
Protein S [%]	0.240	0.155; 0.325	< 0.001	0.184	0.089; 0.279	< 0.001
Quick value [%]	0.054	0.030; 0.078	< 0.001	0.048	0.021; 0.074	< 0.001
INR[e]	-0.032	-0.048; −0.017	< 0.001	-0.031	-0.048; -0.014	< 0.001

[a] After exclusion of individuals with anticoagulation therapy (n = 29); [b] dependent variables: blood coagulation markers, exposure variable: Fatty Liver Index, adjusted for age, alcohol consumption, sex, education, smoking and physical activity; [c] dependent variables: blood coagulation markers, exposure variable: Fatty Liver Index, additionally adjusted for stroke, hypertension, myocardial infarction, non HDL cholesterol, diabetes; [d] activated partial thromboplastin time; [e] international normalized ratio, INR times 100

inhibits numerous clotting factors, reduced production with increasing FLI leads to increased interference with the coagulation cascade. This may eventually increase the risk of thrombosis and subsequently of other diseases [27]. aPTT is a coagulation test based on partial thromboplastin time and is used clinically to indicate abnormalities within the coagulation system [11]. An elevated aPTT value indicates an increased bleeding tendency, and a low value indicates a decreased bleeding tendency [28]. aPTT reflects the clotting time of the intrinsic pathway, and a shortened clotting time and thus faster thrombus formation makes sense from a physiological point of view [29]. This effect is evident based on the present results.

Since only a few studies have investigated the relationship between FLI and blood coagulation markers, our results are compared to studies using NAFLD as an endpoint. It is therefore important to understand and explain the term "NAFLD". According to the European Association for the Study of the Liver (EASL) [4], NAFLD is characterized by excessive hepatic fat accumulation (associated with insulin resistance) and defined by the presence of steatosis in more than 5% of hepatocytes in association with metabolic risk factors, particularly obesity and type 2 diabetes, and in the absence of excessive alcohol consumption or other chronic liver diseases. There are two types of NAFLD: non-alcoholic fatty liver (NAFL) and the progressive form non-alcoholic steatohepatitis (NASH), which is characterized by chronic inflammation and death of liver cells that can progress to fibrosis and even the most severe form of metabolic cirrhosis. People usually develop one or the other form of NAFLD, although the transition is fluid. Kotronen et al. [30] measured various coagulation factors in a retrospective study comparing subjects with and without previously diagnosed non-alcoholic fatty liver disease (NAFLD). Liver fat was visualized in their work by H-magnetic resonance spectroscopy (H-MRS). An increase in the coagulation factors examined was found for subjects with NAFLD, but D-dimer values remained almost unchanged. However, since D-dimers are formed physiologically as cleavage products during the dissolution of fibrin [31], an increased blood concentration, as seen in our study, can be expected (in parallel with increasing fibrinogen). D-dimer concentrations are clinically relevant when excessive blood clotting and/or thrombus formation is suspected and provide important information as to whether clots were actually formed (and dissolved) in the body [32, 33]. The assumption that an elevated FLI may cause a procoagulant or prothrombotic state is also supported by the findings of Orgestra et al. [34]. In their review article, they compared published reports of changes in coagulation parameters associated with NAFLD. They confirmed that endothelial vascular dysfunction, platelet abnormalities and alterations in factors involved in the coagulation cascade and fibrinolysis may contribute to a prothrombotic state in patients with NAFLD. At the same time, they noted that currently available data on the role of platelets and changes in the coagulation system are sparse and often contradictory, highlighting the growing need for larger, prospective studies with well-defined patient groups and comprehensive tests to assess the hemostatic profile. In their paper, Virovic et al. [35] confirm the above statement that, despite research efforts, the link between NAFLD and coagulation disorders has not yet been established, in part because of the inadequacy of the tests used to assess these disorders. They argue that the close association of NAFLD and metabolic syndrome makes it difficult to distinguish the extent to which the two entities contribute independently to a procoagulant state. However, they concluded that there is growing evidence that NAFLD

Table 4 Association between the Fatty Liver Index (FLI) and blood coagulation parameters in study participants[a] stratified by glucose tolerance status

	Model 1[b]			Model 2[c]		
	β estimate	95% confidence interval	*P* value	β estimate	95% confidence interval	*P* value
Normal glucose tolerance						
Antithrombin III [mg/dL]	-0.042	-0.080; -0.004	0.032	-0.061	-0.103; -0.019	0.004
Ln D-dimers	0.003	0.001; 0.005	0.003	0.004	0.002; 0.006	< 0.001
Faktor VIII [%]	0.206	0.068; 0.343	0.003	0.239	0.087; 0.392	0.002
Fibrinogen D [mg/dL]	0.544	0.303; 0.785	< 0.001	0.502	0.235; 0.769	< 0.001
aPTT [s][d]	0.001	-0.013; 0.012	0.940	-0.009	-0.023; 0.005	0.203
Protein C [%]	0.163	0.098; 0.229	< 0.001	0.116	0.045; 0.187	0.002
Protein S [%]	0.305	0.177; 0.433	< 0.001	0.215	0.074; 0.355	0.003
Quick value [%]	0.082	0.047; 0.117	< 0.001	0.076	0.037; 0.115	< 0.001
INR[e]	-0.049	-0.070; − 0.028	< 0.001	-0.045	-0.069; -0.022	< 0.001
Prediabetes						
Antithrombin III [mg/dL]	-0.072	-0.113; -0.031	< 0.001	-0.097	-0.139; -0.054	< 0.001
Ln D-dimers	0.001	-0.001; 0.003	0.251	0.001	-0.001; 0.003	0.528
Faktor VIII [%]	0.279	0.132; 0.427	< 0.001	0.251	0.097; 0.405	0.002
Fibrinogen D [mg/dL]	0.210	-0.055; 0.474	< 0.001	0.169	-0.112; 0.450	0.237
aPTT [s][d]	-0.017	-0.031; -0.003	0.021	-0.018	-0.033; -0.002	0.023
Protein C [%]	0.078	0.007; 0.149	0.032	0.055	-0.018; 0.129	0.141
Protein S [%]	0.149	0.011; 0.287	0.035	0.127	-0.017; 0.272	0.083
Quick value [%]	0.030	-0.005; 0.065	0.096	0.032	-0.005; 0.070	0.088
INR[e]	-0.018	-0.039; 0.003	0.089	-0.020	-0.042; 0.002	0.077
Diabetes, newly diagnosed and known						
Antithrombin III [mg/dL]	-0.175	-0.318; -0.032	0.017	-0.120	-0.275; 0.034	0.125
Ln D-dimers	0.003	-0.003; 0.008	0.306	0.003	-0.003; 0.009	0.319
Faktor VIII [%]	0.152	-0.179; 0.483	0.364	0.114	-0.250; 0.479	0.533
Fibrinogen D [mg/dL]	0.160	-0.437; 0.758	0.594	0.320	-0.330; 0.971	0.329
aPTT [s][d]	0.002	-0.035; 0.040	0.903	-0,002	-0.043; -0.039	0.919
Protein C [%]	-0.019	-0.241;0.203	0.865	0.007	-0.228; 0.242	0.952
Protein S [%]	0.119	-0.267; 0.506	0.540	0.035	-0.385; 0.454	0.869
Quick value [%]	-0.047	-0.186; 0.092	0.499	-0.023	-0.170; 0.125	0.758
INR[e]	0.010	-0.100; − 0.121	0.851	-0.016	-0.134; 0.101	0.786

[a] After exclusion of individuals with anticoagulation therapy (n = 29); [b] dependent variables: blood coagulation markers, exposure variable: Fatty Liver Index, adjusted for age, alcohol consumption, sex, education, smoking and physical activity; [c] dependent variables: blood coagulation markers, exposure variable: Fatty Liver Index, additionally adjusted for stroke, hypertension, myocardial infarction, non HDL cholesterol [d] activated partial thromboplastin time; [e] international normalized ratio, INR times 100

and NASH in particular may contribute significantly and independently to a procoagulant and prothrombotic state, independent of the presence of other factors. Finally, they also argue for the urgent need for larger population-based studies with careful selection of the methods and factors studied to clarify the complex changes in the coagulation process that occur in vivo in NAFLD, their interactions and clinical outcomes. In our study, we used FLI for screening NAFDL, an approach that is easily applicable for screening a large number of subjects. Thus, the relationship between this index and markers of the coagulation system is important information for its future application.

Hörber et al. [15] showed in another study that early prevention through lifestyle intervention had a massive influence on changes in the blood coagulation system. They studied 100 individuals with impaired glucose tolerance or impaired fasting plasma glucose (i.e., prediabetic subjects) who participated in a one-year lifestyle intervention. In addition to precise metabolic phenotyping and MRS-based determination of liver fat content, they also conducted a comprehensive analysis of blood coagulation parameters before and after the intervention. They were able to establish a causal link between a decrease in liver fat content and an improvement in hemostasis and thus indicate a future preventive and therapeutic target. Improvement of the prothrombotic status by lifestyle modifications that lead to decreased FLI seems promising and should be regarded as a disease-preventive measure. Since the information for the calculation of the FLI

can be collected easily and quickly by taking simple measurements available in everyday clinical practice and the correlation of the FLI with the development of various disease patterns could already be demonstrated, the FLI represents a simple tool for diagnostics. In a population-based cohort study using the UK Biobank database, Zou et al. [36] found that the FLI was not the best measure for detecting liver steatosis; however, the FLI was significantly associated with prevalent and incident cardiovascular diseases. Additionally, Chang-Hoon Lee et al. [37] reported that a higher FLI score increases the risk of all-cause mortality and the risk of myocardial infarction and stroke. They observed that a simple (repeated) assessment of NAFLD status based on FLI could help clinicians identify groups at higher risk for all-cause mortality, myocardial infarction and stroke. The association between improving (i.e., decreasing) FLI and the decrease in cardiovascular disease risk also supports the clinical benefits of NAFLD prevention and treatment. Thus, the reported associations between FLI and coagulation markers in the present study may indicate a mechanistic pathway that could partly mediate such effects.

The observed lack of association between FLI and coagulation factors in patients with diabetes confirms that the specific metabolic situation dominates over the enrichment of fat in the liver [35, 38]. Patients with a diagnosis of diabetes show an increased thrombotic risk mainly mediated by the presence of insulin resistance, dysglycemia, and an increased inflammatory state, among other alterations [39]. This prothrombotic state in diabetic subjects is related to an overall increased cardiovascular disease risk [40]. According to our results, the relationships between FLI and hemostatic factors are weakened in prediabetic subjects (versus normoglycemic subjects); however, as discussed above, lifestyle intervention may restore metabolic and blood clotting derangements [41].

It is well described that a prothrombotic state could be the consequence of malignancies when tumor cells activate the coagulation system [42]. Besides, NAFLD is closely associated with hepatic and recently with extrahepatic cancers, such as bladder cancer [43]. Our study participants did not suffer from acute malignancies at the time of blood collection; however, as for other serious diseases, former cancer cases were not excluded from this cross-sectional analysis.

Study strengths and limitations

The strengths of our study are a large sample size, the population-based design, the availability of standardized data on cardiovascular risk factors, and standardized anthropometric and laboratory measurements. Possible confounders were available and could be considered as adjustment variables in the regression models. However, some limitations must be considered. As the analysis is based on the follow-up of a population-based study, it is possible that the participants are not representative of the original sample. It cannot be completely excluded that selection bias may have influenced the present results. Furthermore, future studies should also identify other existing liver diseases such as hepatitis to avoid false positive FLI results. The results cannot be related to people of other ethnic origins or age groups, as only study participants born in the period from 1945 to 1964 were included. Furthermore, an exact measurement of liver fat content (e.g., by MR imaging) is more precise but much more cost-intensive than the calculated FLI.

Conclusion

Our results demonstrate a relationship between the FLI and blood clotting markers, with a high FLI being associated with a pro-coagulative state. Patients at high risk of developing conditions such as thrombosis, CVD or stroke may benefit from this simple and fast diagnostic measure. However, further population-based studies are needed to clarify the complex changes in the coagulation process associated with the fatty liver index and their interactions with other metabolic derangements and subsequent clinical outcomes. The FLI may serve as a screening test to identify subjects that should undergo precise characterization of the liver and suitable intervention.

Abbreviations

aPTT	Activated thromboplastin time
EALS	European Association for the Study of the Liver
FLI	Fatty Liver Index
H-MRS	H-magnetic resonance spectroscopy
INR	International thromboplastin time
KORA	Cooperative Health Research in the region of Augsburg
MONICA	Monitoring trends and determinants in cardiovascular disease
MRS	Magnetic resonance spectroscopy
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis

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Authors' contributions

MIM conducted the statistical analysis, interpreted the data, and drafted and revised the manuscript; JL designed the study, financed the citrate plasma collection and laboratory analysis, and supervised the work; CM contributed to the design of the study, the data interpretation and the revision of the draft manuscript; DF supervised the statistical analysis; AP and MH were responsible for the design and conduct of the KORA FIT study; DT was in charge of the laboratory analyses; all authors read and approved the final version of the manuscript.

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Data Availability
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://epi.helmholtz-muenchen.de/).

Declarations

Ethics approval and consent to participate
The study protocol was approved by the Ethics Committee of the Bavarian Chamber of Physicians and conducted according to the data protection requirements. The investigations were carried out in accordance with the Declaration of Helsinki. Study participants gave written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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