Aus dem

CharitéCentrum 1 für Human- und Gesundheitswissenschaften

Institut für Sozialmedizin, Epidemiologie und Gesundheitsökonomie

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Habilitationsschrift

Zusammenhang zwischen einer veganen Ernährungsweise und inflammatorischen Biomarkern, Knochengesundheit sowie Per- und Polyfluoralkylsubstanzen

zur Erlangung der Lehrbefähigung

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1. Gutachter/in: ……

2. Gutachter/in: ……

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Abkürzungen

ALA	α-Linolensäure
95%-KI	95%-Konfidenzintervall
BUA	Breitband-Ultraschall-Abschwächung
COPLANT-Studie	Cohort on Plant-based Diets-Studie
CRP	C-reaktives Protein
DGE	Deutsche Gesellschaft für Ernährung
DHA	Docosahexaensäure
DEXA	Dual-Energy-Röntgen-Absorptiometrie
EPA	Eicosapentaensäure
EFSA	Europäische Behörde für Lebensmittelsicherheit
EPIC-Studie	European Prospective Investigation into Cancer and Nutrition-Studie
FGF23	Fibroblast growth factor 23
FFQ	Food-Frequency-Questionnaire
HWZ	Halbwertszeit
hsCRP	Hochsensitives C-reaktivem Protein
IL-1 RA	Interleukin-1 receptor antagonist
IL-18	Interleukin-18
IL-6	Interleukin-6
IQR	Interquartilsabstand
ICAM-1	Interzelluläres Adhäsionsmolekül-1
LDL-Cholesterin	Low-Density-Lipoprotein-Cholsterin
LA	Linolsäure
sICAM	Lösliches interzelluläres Adhäsionsmolekül-1
MW	Mittelwert
MCP-1	Monozytisches chemoattraktives Protein-1
PFAS	Per- und Polyfluoralkylsubstanzen
PFHxS	Perfluorhexansulfonsäure
PFNA	Perfluornonansäure
PFOA	Perfluoroctansäure
PFOS	Perfluoroctansulfonsäure
PTH	Parathormon
QUS	Quantitativer Ultraschall
RRR	Reduzierte Rang Regression
RBVD-Studie	Risks and Benefits of a Vegan Diet-Studie
SOS	Schallgeschwindigkeit
SD	Standardabweichung
SI	Steifigkeitsindex
TNF-α	Tumornekrosefaktor-α
T2D	Typ 2 Diabetes
uPRAL	Urinäre potenzielle renale Säurelast
WHO	Weltgesundheitsorganisation

1. Einleitung

1.1. Definitionen pflanzenbasierter Ernährungsformen

Grundsätzlich gibt es keine einheitliche Form einer pflanzenbasierten Ernährungsweise [1]. Unter Vegetarismus versteht man eine Vielzahl von Ernährungsweisen (Abbildung 1). Alle vegetarischen Ernährungsformen haben gemeinsam, dass sie Lebensmittel von getöteten Tieren ausschließen [2]. Dazu zählt neben Fleisch und Fisch, ebenso die daraus hergestellten Produkte, wie z.B. Wurstwaren oder Gelatine [2]. Eine Sonderform bilden die Pescetarier:innen, die Fisch aber kein Fleisch verzehren.

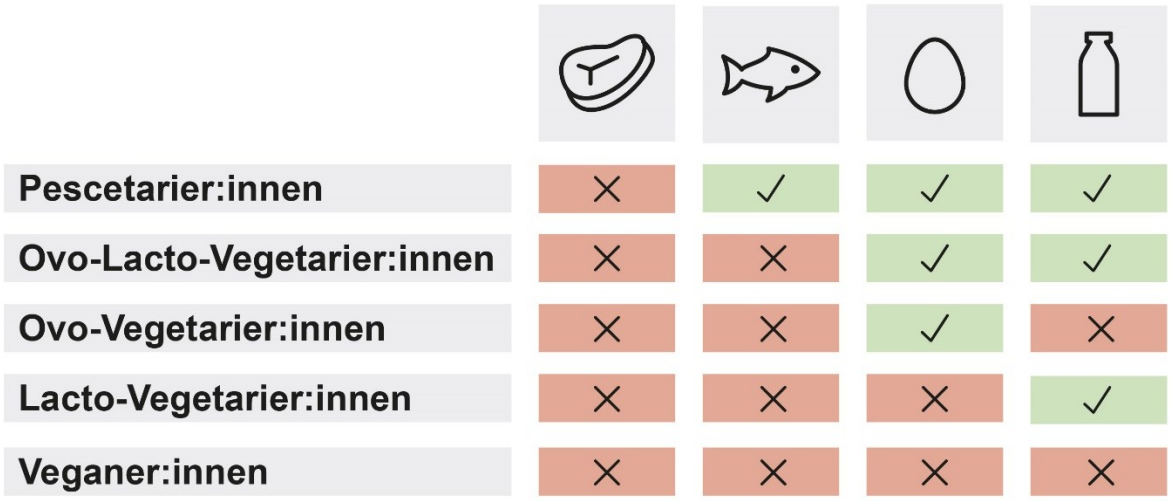

Abbildung 1: Formen der pflanzenbasierten Ernährung (eigene Abbildung)

Jedoch lassen sich nach Grad der Vermeidung tierischer Lebensmittel bzw. tierischer Produkte verschiedene pflanzenbasierte Ernährungsformen genauer definieren (Abbildung 1). So verzehren Ovo-Lakto-Vegetarier:innen neben pflanzlichen Lebensmitteln auch Milch und Eier, sowie daraus hergestellte Produkte [1]. Ovo-Vegetarier:innen essen neben pflanzlichen Nahrungsmitteln ebenfalls Eier, aber keine Milch oder Milchprodukte [1]. Der Veganismus ist die konsequenteste Ernährungsweise mit Ausschluss jeglicher tierischer Lebensmittel bzw. Produkte, es werden ausschließlich pflanzliche Lebensmittel verzehrt [1]. Zudem verwenden viele Veganer:innen auch keine Gebrauchsgegenstände oder Materialien, die von Tieren stammen, wie z.B. Wolle, Leder oder Seide [1].

1.2. Pflanzenbasierte Ernährungsformen in Deutschland

In Deutschland gibt es seit den 1990er Jahren einen Trend der vegetarischen und zunehmend veganen Ernährungsformen [1]. Aktuell liegt der geschätzte Anteil von Vegetarier:innen bei etwa 7-10% [2,3]. Laut dem Institut für Demoskopie Allensbach ernähren sich etwa 8 Millionen Menschen in Deutschland vegetarisch [4], eine halbe Million Menschen mehr als noch im Jahr

2021 [4]. Einer veganen Ernährungsweise folgten im Jahr 2022 1-2% (etwa 1.6 Millionen Personen) der deutschen Bevölkerung [3,5] mit einem Zuwachs von ca. 170.000 Personen als noch im Vorjahr [5]. Allerdings kann der Anteil vegan oder vegetarisch lebender Menschen in Deutschland nur geschätzt werden, die Angaben in wissenschaftlichen Studien sind dabei meist niedriger als die Angaben von Meinungsforschungsinstituten [1]. Dennoch lässt sich zusammenfassen, dass nach Schätzungen etwa 10 Millionen Menschen in Deutschland einer veganen oder vegetarischen Ernährungsweise folgen.

Zudem schränken immer mehr Menschen den Konsum von Fleisch in Deutschland ein. Laut dem Bundesministerium für Ernährung und Landwirtschaft bezeichneten sich 44% der deutschen Bevölkerung 2022 als flexitarisch [3]. So werden Menschen bezeichnet, die ganz bewusst den Konsum von Fleisch einschränken [3]. Eine einheitliche Definition wie hoch der maximale Verzehr sein darf, gibt es aber bisher nicht [1].

Die Einteilung nach der Lebensmittelauswahl erlaubt eine Klassifizierung der vegetarischen Ernährungsformen, doch die Gründe warum sich Menschen dazu entscheiden sind vielschichtig [2]. Die Entscheidung für eine pflanzenbasierte Ernährung ist oftmals Teil eines Lebensstilkonzeptes, dass über die Auswahl von Nahrungsmitteln hinausgeht [2]. Dabei werden auch Aspekte des Tierschutzes und Tierrecht, Umweltschutz, Klimawandel oder Perspektiven der Welternährung, sowie physische und mentale Gesundheit etc. einbezogen [2].

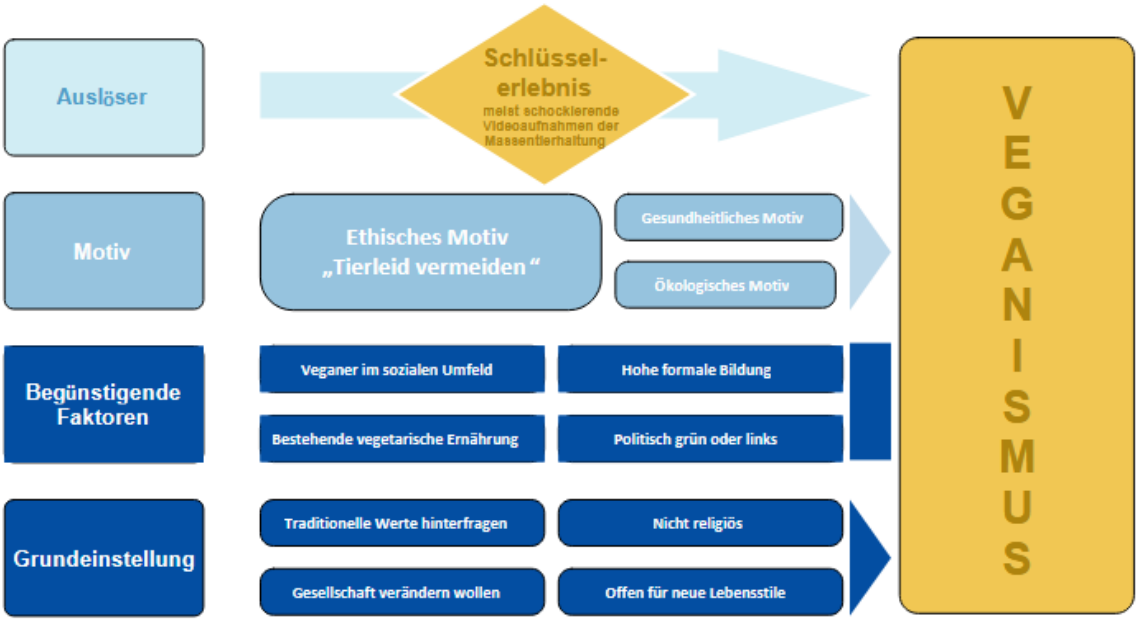

Abbildung 2: Individuelle Entstehung des Veganismus (übernommen aus Bundesinstitut für Riskobewertung [6])

Die Entscheidung für eine vegane Lebensweise ist meist durch das Zusammenwirken von Einstellungen, begünstigenden Faktoren und einem ethischen Motiv geprägt, zudem gibt es häufig ein Schlüsselereignis (Abbildung 2) [6]. Insgesamt sind vor allem ethische Motive eine der häufigsten Beweggründe [1,6], gefolgt von gesundheitlichen und ökologischen Motiven [1]. Dabei sind die persönlichen Motive nicht fixiert und können sich im Laufe der Zeit wandeln [1].

1.3. Gesundheitliche Aspekte einer veganen Ernährung

1.3.1. Nährstoffversorgung bei veganer Ernährungsweise

Grundsätzlich gibt es vielfältige Ausprägungen einer veganen Ernährungsweise [7], entsprechend abwechslungsreich bzw. auch eintönig kann die Lebensmittelauswahl gestaltet werden. Dennoch ist bei einigen Nährstoffen eine ausreichende Versorgung nicht oder nur schwer möglich [8]. Der kritischste Nährstoff ist hierbei Vitamin B12 [8,9], denn Cobalamine können von Pflanzen nicht gebildet werden [9]. Ein Vitamin B12 Mangel hat schwerwiegende Konsequenzen [9]. Dieser führt zu einer Störung der Methioninsynthese und folglich zu einem Mangel an reaktionsfähigem Folat [9]. In Folge kommt es zu einer unzureichenden DNA-Synthese mit Zellteilungsstörungen im gesamten Organismus [9]. Leitsymptom ist hierbei die makrozytäre hyperchrome Anämie [9]. Schwerwiegender wiegen neben den hämatologischen Symptomen die neurologischen Folgen der funikuläre Myelose [9]. Um potentielle irreversible neurologische Schäden zu vermeiden, wird empfohlen, dass Veganer:innen dauerhaft ein Vitamin-B12-Präparat einnehmen sollten [8,9]. Andere potenziell kritischen Nährstoffen sind laut der Deutschen Gesellschaft für Ernährung (DGE) bei einer veganen Ernährungsform Proteine bzw. unentbehrliche Aminosäuren und langkettige Omega-3-Fettsäuren wie Eicosapentaensäure (EPA) und Docosahexaensäure (DHA) [8]. Wichtige Vitamine wie Riboflavin (Vitamin B2) und Vitamin D, sowie einige Mineralstoffe und Spurenelemente (Calcium, Eisen, Jod, Zink, Selen) gelten ebenso als potentiell kritische Nährstoffe [8]. Dies bestätigt auch ein systematisches Review aus dem Jahr 2021 [10]. Das Review zeigte, dass Veganismus mit einer geringeren Aufnahme verschiedener Vitamine (B2, Niacin (B3), B12, D), sowie einiger Mineralstoffe und Spurenelemente (Calcium, Kalium, Jod, Zink, Selen) assoziiert ist [10].

Neben der Nährstoffzufuhr kann die Bewertung der Nährstoffversorgung auch über den Ernährungsstatus erfolgen [7]. Der Ernährungsstatus umschreibt den ernährungsbedingten körperlichen Zustand [7]. Dieser ergibt sich aus Bilanz von Bedarf und Verbrauch von Nährstoffen (und Nahrungsenergie) und kann anhand von Biomarkern gemessen werden [7]. Wenn diese Bilanz nicht ausgeglichen ist, kann der Ernährungsstatus auch Auswirkungen auf den Gesundheitsstatus haben, wobei Übergänge fließend sind und Wechselwirkungen bestehen [7]. Eine langfristige unzureichende Nährstoffzufuhr kann somit zu einem

unzureichenden Ernährungsstatus und infolgedessen zu einem schlechten Gesundheitsstatus führen, welcher sich durch latente oder manifeste Krankheitsbilder äußern kann [7].

Seit den 1990er Jahren fand ein Paradigmenwechsel statt, so wurde in der Vergangenheit in wissenschaftlichen Studien oft einseitig pflanzenbasierte Ernährungsformen hinsichtlich möglicher Nährstoffmängel untersucht [11]. Schließlich sind eher präventive und therapeutische Aspekte in den Mittelpunkt des wissenschaftlichen Interesses gerückt [11]. Dabei zeigte sich, dass pflanzenbasierte Kostformen (Abbildung 3) das Risiko für verschiedene Erkrankungen [12], wie Typ 2 Diabetes (T2D) [13], Herz-Kreislauferkrankungen [14] und für einige Krebserkrankungen [15] senken können. Hier muss jedoch betont werden, dass die vegane Ernährungsweise weniger umfangreich untersucht wurde, als im Vergleich zu vegetarischen Kostformen.

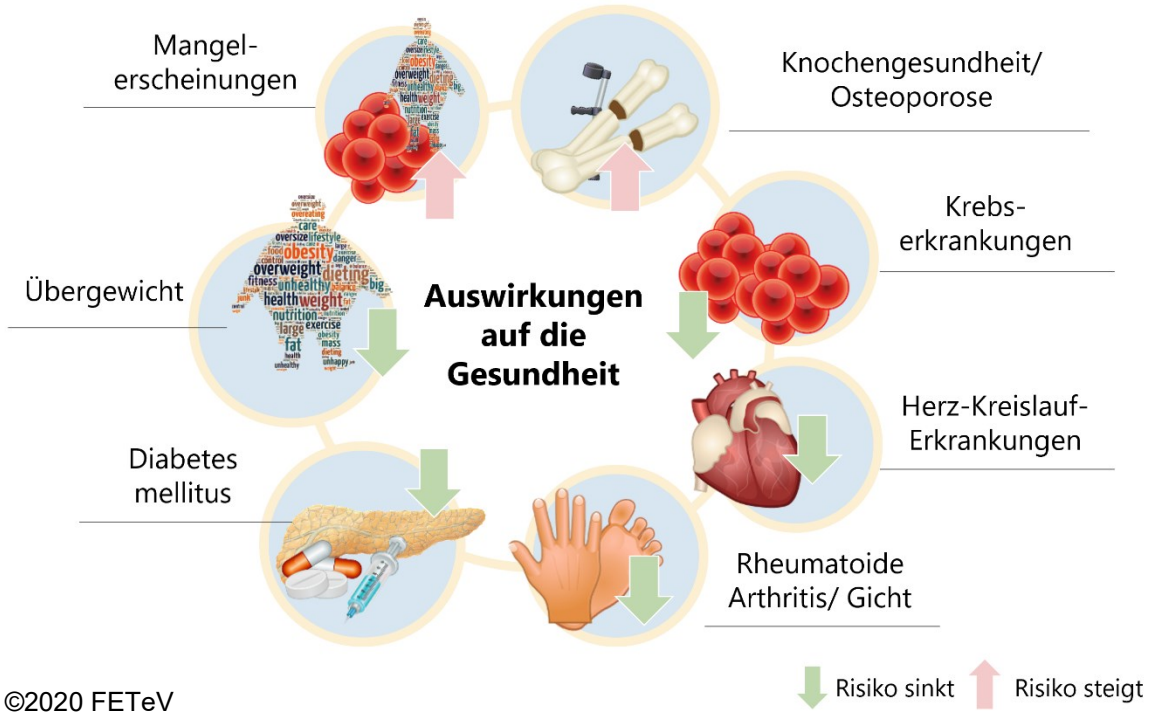

Abbildung 3: Mögliche Auswirkungen auf die Gesundheit bei veganer Ernährungsform (übernommen aus Fachgesellschaft für Ernährungstherapie und Prävention (FET) e.V. [16], mit freundlicher Genehmigung)

1.3.2. Vegane Ernährung und inflammatorische Biomarker

Anders als bei akuten Entzündungsreaktionen, welche durch eine begrenzte Hochregulierung der Entzündungsaktivität gekennzeichnet sind und einen wichtigen Schutzmechanismus des Körpers darstellen [17], sind chronisch-persistierende subklinische Entzündungsreaktionen (sogenannte low-grade Inflammation (niedrigschwellig) oder silent (stille) Inflammation) [18] geprägt durch anhaltende Entzündungsaktivitäten [19]. Damit ist unter anderem die anhaltende

Produktion von proinflammatorischen Zytokinen, aber auch proinflammatorischen Lipiden wie Eicosanoiden gemeint [19].

Es gilt als bekannt, dass es einen Zusammenhang zwischen low-grade Inflammationen und einem erhöhten Risiko für verschiedene chronische Krankheiten gibt [17,19,20]. Somit können inflammatorische Biomarker auch als intermediäre Risikofaktoren für die Entwicklung chronischer Krankheiten dienen. Tatsächlich wurden Assoziationen zwischen erhöhten Konzentrationen von inflammatorischen Biomarkern wie hochsensitivem C-reaktivem Protein (hsCRP) oder Interleukin-18 (IL-18) mit den pathogenetischen Mechanismen chronischer Erkrankung wie T2D [21] oder Herz-Kreislauf-Erkrankungen aufgezeigt [22,23]. Im Gegensatz dazu wurde berichtet, dass die Konzentration des antiinflammatorischen Hormons Adiponektin in inversem Zusammenhang mit diesen Krankheiten in Verbindung steht [24,25].

Verschiedene modifizierbare Einflussfaktoren gelten als Risikofaktor für low-grade Inflammation [17,20]. Neben der körperlichen Inaktivität, (viszeraler) Adipositas oder psychischem Stress, gilt auch die Ernährung als ein möglicher Einflussfaktor [17,20]. Zwei systematische Übersichtsarbeiten mit Metaanalysen zeigten, dass pflanzenbetonte Ernährungsformen, im Vergleich zu nicht pflanzenbetonten Ernährungsformen, mit niedrigeren CRP-Konzentrationen assoziiert sind [26,27]. Diese Übersichtsarbeiten schlossen dabei nicht nur Studien mit den Ernährungsformen des Vegetarismus ein, sondern auch Ernährungsformen der mediterranen Ernährung, Nordic Diet und Dietary Approaches to Stop Hypertension (DASH) [26,27]. In gewissem Maße konnte Eichelmann et al. auch für Interleukin-6 (IL-6) (-0.25 ng/l, 95%-KI: -0.56 bis 0.06), sowie lösliches interzelluläres Adhäsionsmolekül-1 (sICAM) (-25.1 ng/ml, 95%-KI: -52.3 bis 2.17) eine Verringerung der durchschnittlichen Konzentrationen bei einer pflanzenbetonten Ernährungsform aufzeigen [26]. Für Tumornekrosefaktor-α (TNF-α), Resistin, Adiponectin und Leptin wurden keine wesentlichen Veränderungen festgestellt [26,27]. Hierbei muss jedoch betont werden, dass CRP wissenschaftlich am umfassendsten im Vergleich zu anderen inflammatorischen Biomarkern untersucht wurde.

Weitere Metaanalysen [28,29] fokussierten sich explizit auf den Vegetarismus und berichteten ebenfalls, dass eine vegetarische Lebensweise mit niedrigeren CRP-Konzentrationen assoziiert ist [28,29]. Auch hier ist die Studienlage in Hinblick auf andere inflammatorischen Biomarker sehr limitiert [28,29].

Es lässt sich vermuten, dass ein vorteilhaftes inflammatorisches Biomarkerprofil von Personen mit pflanzenbasierten Ernährungsformen einen protektiven Einfluss auf das Erkrankungsrisiko von T2D, Herz-Kreislauferkrankungen oder für bestimmte Krebserkrankungen haben könnte. Denn wie oben beschrieben, gilt es als bekannt, dass Menschen die einer pflanzenbasierten Kostform folgen, ein verringertes Risiko dieser chronischen Krankheiten haben [12].

Es lässt sich zusammenfassen, dass bisher einige Studien die Zusammenhänge zwischen einer pflanzenbasierten Ernährungsweise und inflammatorischen Biomarkern untersucht haben. Dabei fokussieren sich diese Studien jedoch fast ausschließlich auf CRP sowie auf vegetarische Ernährungsformen. Wissenschaftliche Studien, die neben CRP noch andere inflammatorische Biomarker oder vegane Ernährungsweisen untersuchen, sind bisher sehr limitiert [30,31].

1.3.3. Vegane Ernährung und Knochengesundheit

Im Gegensatz zu den beschriebenen vorteilhaften Auswirkungen einer pflanzenbasierten Ernährung auf die Gesundheit, wird berichtet, dass vegane Ernährungsformen in Hinblick auf die Knochengesundheit negative Auswirkungen haben können (Abbildung 3). So wurde eine geringere Knochengesundheit (niedrigere Knochendichte und höhere Frakturraten) bei Veganer:innen im Vergleich zu Mischköstler:innen in aktuellen Übersichtsarbeiten [32,33] gezeigt, ebenso in drei Metaanalysen mit Beobachtungsstudien [34-36] und in einer aktuellen großen prospektiven Studie (n=54.898) [37].

An der Knochengesundheit sind eine Vielzahl von Nährstoffen beteiligt (Abbildung 4) [33]. Das Skelett ist kein starres, sondern ein sehr dynamisches und stoffwechselaktives Gewebe [38] und reagiert äußerst empfindlich auf seine Mikroumgebung [39]. So kann ein anhaltendendes Ungleichgewicht zu einem beträchtlichen Knochenverlust führen [39]. In diesem Zusammenhang werden Ernährungsgewohnheiten und die damit verbundene Aufnahme wichtiger Nährstoffe als ein wichtiger modifizierbarer Einflussfaktor für die Knochengesundheit angesehen [39,40].

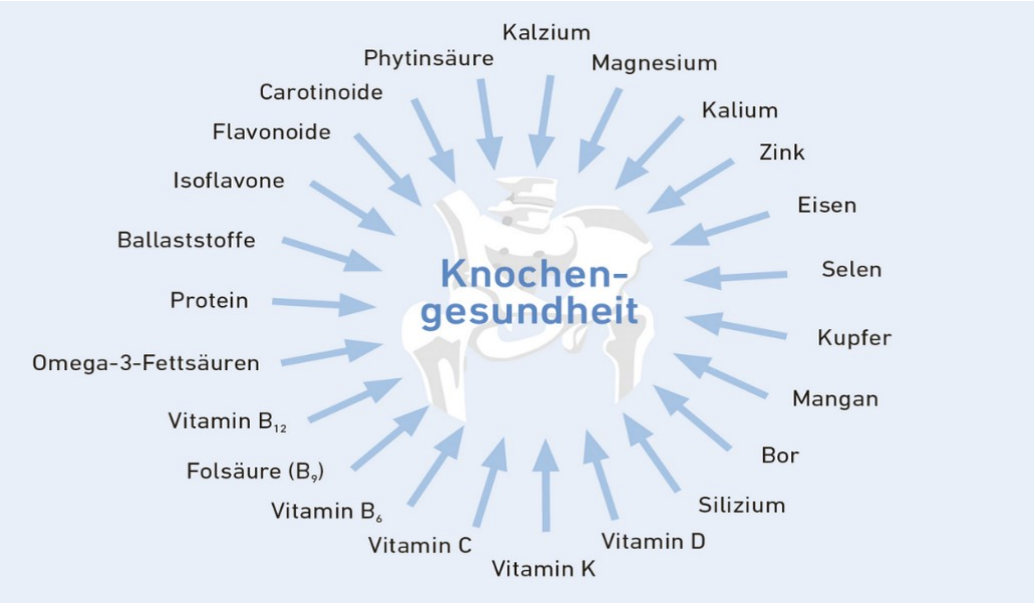

Abbildung 4: Nährstoffe für gesunde Knochen (übernommen aus Rittenau [41], mit freundlicher Genehmigung)

Wie bereits dargestellt, kann es bei einer veganen Ernährungsform zu einer unzureichenden Versorgung einiger Nährstoffe kommen [8,9]. Dies kann zu einer verminderten Knochengesundheit bei Veganer:innen beitragen. So sind beispielsweise Calcium und Vitamin D als wichtige Determinanten der Knochengesundheit bekannt [40], doch gelten gleichzeitig als potenziell kritische Nährstoffe bei Veganer:innen [8,9]. Auch andere potentiell kritische Nährstoffe einer veganen Ernährung, wie z.B. langkettige Omega-3 Fettsäuren, Vitamine B12, Vitamin A oder Spurenelemente (Zink, Selen, Jod) [8] sind mit der Knochengesundheit assoziiert (Abbildung 4) [39]. Andererseits kann eine vegetarische und vegane Ernährungsweise auch wichtige Nährstoffe liefern, die einen protektiven Einfluss auf den Knochen haben, z. B. Vitamin K [38,39,42] und Folat [38,43-45].

Demzufolge erfordern mehrere Nährstoffe besondere Aufmerksamkeit für die Knochengesundheit bei Veganer:innen. Eine geringere Knochengesundheit einem einzigen ernährungsbedingten Biomarker zuzuschreiben, wäre jedoch angesichts der Komplexität der homöostatischen Regulationsmechanismen des Knochens zu vereinfacht [46]. Bisher fehlen jedoch Studien, die den Einfluss verschiedener Nährstoffe und anderer knochenrelevanter Biomarker auf die Knochengesundheit in Kombination untersucht haben.

1.3.3.1. Einfluss der Säurelast auf den Knochen

Der physiologische pH-Wert im Blut liegt im Bereich von 7.35 bis 7.45 und ist von höchster Bedeutung für die Funktion der Stoffwechselprozesse im menschlichen Organismus [47]. Daher wird der pH-Wert des Blutes durch umfangreiche Puffersysteme nahezu konstant gehalten [47]. Abweichungen vom Normbereich können den Ablauf physiologischer Prozesse im Organismus erheblich beeinträchtigen, dabei führen schon geringe Abweichungen zu massiven Störungen im Stoffwechsel [47]. Die Regulation des pH-Wertes erfolgt neben den Puffereigenschaften des Blutes, sowie der extra- und intrazellularen Kompartimente, auch durch die Lunge, Niere und Leber, die für die Metabolisierung und Ausscheidung von Säuren verantwortlich sind [47].

In den letzten Jahrzehnten zeigte sich, dass eine westliche Ernährung eine Ursache für eine latente Azidose sein kann [48]. So entsteht bei einer westlichen Ernährung oft ein Überschuss an Säuren durch potente Säurebildner in unseren Lebensmitteln, z.B. Fleisch und Fleischwaren, Fisch, Käse, Eier und bestimmte Getreide wie Hafer und verarbeitete Weizenprodukte [49]. Viele Obst- und Gemüsesorten gelten dagegen als Basenbildner [49]. Zum Ausgleich einer ernährungsbedingten latenten Azidose sind vor allem die Adaptationsmechanismen der Niere wichtig, da die Pufferkapazitäten im Bindegewebe, Blut und Körperzellen vermindert bzw. übermäßig beansprucht sind [50]. Ein typisches Anzeichen der latenten Azidose ist daher eine erhöhte Nettosäureausscheidung über die Nieren und eine

Abnahme des Harn-pH-Wertes [50]. Eine tendenziell azidotische Stoffwechsellage über einen längeren Zeitraum kann Ursache für vielseitige endokrin-metabolische Veränderungen mit langfristigen gesundheitlichen Folgen sein, wie z.B. negative Folgen für die Knochengesundheit [51]. Denn eine leichte Azidose kann zu einer gesteigerten Aktivität der Osteoklasten und damit zu erhöhten die Resorption des Knochens führen [51]. Dennoch sind die Ergebnisse wissenschaftlicher Studien uneindeutig. Tatsächlich zeigen einige Studien, dass eine hohe Säurelast durch die Nahrung mit einer schlechteren Knochengesundheit (ungünstige Knochenparameter, niedrigere Knochendichte oder Frakturen) assoziiert ist [52-55], eine Metaanalyse konnte jedoch nur schwache oder keine Assoziationen aufzeigen [56].

Durch pflanzenbasierte Ernährungsformen, insbesondere eine vegane Ernährung ist ein Überschuss von Basenbildnern durch Obst und Gemüse im Vergleich zu potenten Säurebildnern zu erwarten. Tatsächlich zeigten wissenschaftliche Publikationen, dass vegane Ernährungsformen mit einer niedrigen Säurelast der Nahrung assoziiert sind [49,57-60]**.** In Hinblick auf die Knochengesundheit lässt sich vermuten, dass die geringe Säurelast für Veganer:innen möglicherweise einen wichtigen Einflussfaktor für die Knochengesundheit darstellen könnte. Tatsächlich kommt ein narratives Review zum Schluss, dass die geringe Säurelast einer veganen Ernährung die Knochen von Veganer:innen schützt [61], zumindest bei einer ausreichenden Nährstoffzufuhr [33]. In der wissenschaftlichen Debatte wird aber auch diskutiert, dass die Vorteile einer Alkalisierung der Ernährung für die Knochengesundheit nur auf Menschen beschränkt sein könnten, die gewöhnlich eine Ernährung mit einem hohen Gehalt an potenzieller renaler Säurelast (PRAL) zu sich nehmen, eine verminderte Säureausscheidungsfähigkeit der Nieren haben und/oder Menschen mit Prädispositionen z.B. (altersbedingte) eigeschränkte Nierenfunktion oder ein metabolisches Syndrom [62,63]. Das würde bedeuten, dass Veganer:innen mit ihrer vermuteten geringen Säurelast nicht zusätzlich von einer weiteren Alkalisierung der Ernährung in Hinblick auf die Knochengesundheit profitieren. Daher muss der komplexe Zusammenhang einer veganen Ernährung, Säure- oder Basenlast durch die Nahrung und der Knochengesundheit weiter untersucht werden.

1.4. PFAS in der veganen Ernährung

Per- und Polyfluoralkylsubstanzen (PFAS) sind eine komplexe Gruppe von künstlich hergestellten Industriechemikalien, die nicht natürlich vorkommen [64]. Es handelt sich um organischen Verbindungen, bei denen die am Kohlenstoff gebundenen Wasserstoffatome vollständig (perfluoriert) oder teilweise (polyfluoriert) durch Fluoratome ersetzt wurden [64]. Die Gruppe der PFAS unterscheiden sich hinsichtlich der Kohlenstoffkettenlängen und den im Molekül vorhandenen funktionellen Gruppen, z.B. einer Carboxygruppe oder Sulfonatgruppe [64]. Aufgrund ihrer wasser-, fett- und schmutzabweisenden Eigenschaften kommen sie seit Jahrzehnten für die Herstellung zahlreicher Verbraucherprodukte zum Einsatz, z.B.

antihaftbeschichtetes Kochgeschirr, Sport- und Outdoorbekleidung, sowie Schutzbeschichtungen für Papier, Lebensmittelverpackungsmaterialien oder Kosmetika [64].

Aus diesen Alltagsgegenständen werden PFAS in die Umwelt freigesetzt und gelangen so in die Nahrungskette [65]. Neben der externen Exposition durch Luft und Staub ist der Verzehr von Lebensmitteln und Trinkwasser für den Menschen ein Hauptweg der Exposition (Abbildung 5) [65,66].

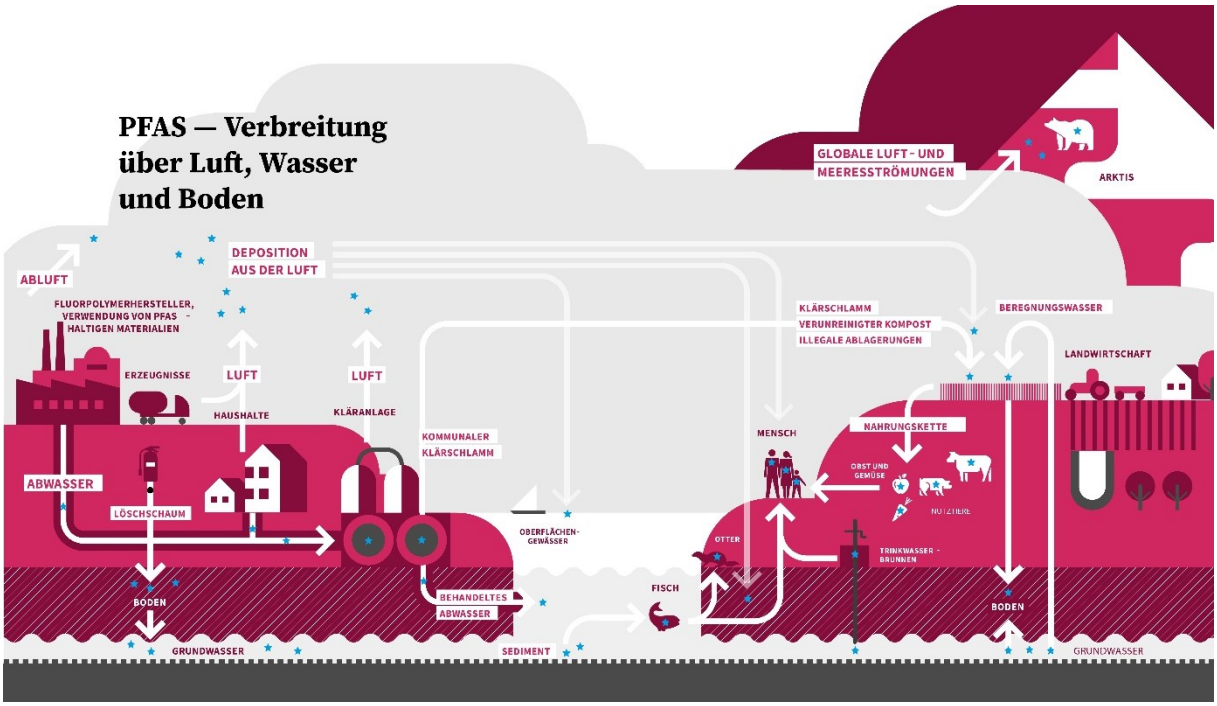

Abbildung 5: Verbreitung von PFAS über Luft, Wasser und Boden (verändert nach Umweltbundesamt [66], mit freundlicher Genehmigung)

Langkettige PFAS haben eine lange physiologische Halbwertszeit (HWZ) und akkumulieren nach der Aufnahme über Trinkwasser, Lebensmittel oder über andere Quellen im Körper [64]. Dabei sind vier Verbindungen mit ihren langen HWZ besonders relevant: Perfluoroctansäure (PFOA, HWZ: 2.7-8.5 Jahre), Perfluoroctansulfonsäure (PFOS, HWZ: 3.1-5.4 Jahre), Perfluornonansäure (PFNA, HWZ: 1.7-3.2 Jahre) und Perfluorhexansulfonsäure (PFHxS, HWZ: 4.7-8.5 Jahre) [64]. In Summe repräsentieren diese vier PFAS mehr als 90 % der nachweisbaren PFAS im Serum/Plasma von Erwachsenen in Industrieländern [64].

Jüngste Bewertungen der Europäischen Behörde für Lebensmittelsicherheit (EFSA) zeigten, dass die Lebensmittelhauptgruppen "Fisch und andere Meeresfrüchte", "Eier und Eiprodukte", "Fleisch und Fleischerzeugnisse" und "Obst und Fruchterzeugnisse" maßgeblich zur Exposition gegenüber PFAS (insbesondere für PFOS und PFOA) beitragen [64,67]. Daher kann vermutet werden, dass die interne Exposition durch die Ernährungsweisen beeinflusst werden könnte. Durch den vollständigen Verzicht auf tierische Produkte im Rahmen einer

veganen Ernährungsweise, könnten Veganer:innen niedrigere PFAS-Konzentrationen im Blut, im Vergleich zu Mischköstler:innen, haben. Studien zu diesem Thema fehlen jedoch noch.

Das öffentliche Bewusstsein für mögliche gesundheitliche Risiken von PFAS ist noch relativ neu [68]. Die EFSA sieht die überzeugendste Evidenz für die folgenden Gesundheitseffekte: Anstieg des Leberenzyms Alanintransaminase, reduziertes Geburtsgewicht von Säuglingen, verringerter Antikörperbildung nach Impfung bei Säuglingen und einen Anstieg des Gesamt- und LDL-Cholesterins (Low-Density-Lipoprotein) [67]. Tatsächlich zeigte ein aktuelles systematisches Review, dass die PFAS Exposition mit höheren Konzentrationen von Gesamt- und LDL-Cholesterins assoziiert waren, insbesondere für PFOA (Gesamt- und LDL-Cholesterins), PFOS (Gesamtcholesterin) und PFNA (LDL-Cholesterin) [69]. Durch die fehlende Aufnahme von tierischen Fetten ist bekannt, dass Veganer:innen niedrigere Konzentrationen von Gesamt- und LDL-Cholesterin, im Vergleich zu Mischköstler:innen, haben [70]. Zudem könnten Veganer:innen, wie oben beschrieben, auch eine geringere externe und interne Exposition von PFAS aufweisen. Hinsichtlich der möglichen Assoziationen von PFAS auf Konzentrationen von Gesamt- und LDL-Cholesterins, kann eine vegane Ernährungsweise einen wichtigen Confounder darstellen, der bisher nur wenig oder gar nicht in epidemiologische Studien berücksichtigt bzw. untersucht wurde.

1.5. „Risks and Benefits of a Vegan Diet" Studie

Bis auf die Ausnahme des systematischen Reviews mit Metaanalyse (Originalarbeit 2) basieren alle Originalarbeiten dieser Habilitationsschrift auf der „Risks and Benefits of a Vegan Diet" (RBVD)-Studie. Die Studienteilnehmer:innen wurden per Aushang in (veganen) Supermärkten auf die Studie aufmerksam gemacht [71]. Teilnehmer:innen für die RBVD-Studie waren Personen, die auf diese Aushänge reagierten und das Studienzentrum am Bundesinstitut für Risikobewertung (BfR) per Telefon oder Mail kontaktierten (n=161) [71]. In einem telefonischen Screening wurde die Studie erläutert und die Einschlusskriterien (Alter 30-60 Jahre, Ernährungsform seit mindestens 1 Jahr) und Ausschlusskriterien (BMI ≥ 30 kg/m^2, Herz-Kreislauf-Erkrankungen, T2D, Krebs, Schwangerschaft, Stillen, aktuelle Infektionen) überprüft (Abbildung 6) [71]. Eine Mischkost wurde in der RBVD-Studie als ein Verzehr von mindestens drei Portionen Fleisch oder zwei Portionen Fleisch und zwei Portionen Wurst pro Woche definiert [71]. Veganer:innen verzichteten definitionsgemäß auf den Konsum aller tierischen Lebensmittel [71]. Die Studie wurde in Übereinstimmung mit der Deklaration von Helsinki durchgeführt und wurde von der Ethikkommission der Charité - Universitätsmedizin Berlin genehmigt (Nr. EA4/121/16) [71].

Die Teilnehmerzahl basiert auf einer Powerkalkulation zur primären Fragestellung (Knochengesundheit) der RBVD-Studie (G-Power, t-Test für unabhängige Stichproben) [71].

Basierend auf Unterschieden in den Mittelwerten der Breitband-Ultraschall-Abschwächung (BUA) wurde die Teilnehmerzahl unter der Annahme eines klinisch relevanten Unterschieds von mindestens 5% zwischen Veganer:innen und Mischköstler:innen (mit Signifikanzniveau von 5%, Power von 80%) berechnet [71]. Somit wurden insgesamt 72 Teilnehmer:innen (n=36 Veganer:innen und n=36 Mischköstler:innen) im Alter zwischen 30 und 57 Jahren in die RBVD-Studie eingeschlossen und von Januar bis Juli 2017 in Berlin am BfR untersucht (Abbildung 6) [71]. Die Gruppe der Veganer:innen und der Mischköstler:innen wurden dabei nach Alter und Geschlecht gematcht [71]. Wie erwartet, wurden durch das Matching keine Unterschiede in Hinblick auf das Geschlecht und Alter zwischen Veganer:innen und Mischkostgruppe festgestellt (Veganer:innen: 50% männlich, medianes Alter: 37.5 Jahre; Mischköstler:innen: 50% männlich, medianes Alter: 38.5 Jahre) [71]. Die Veganer:innen folgten ihrer Ernährungsweise im Median 4.8 Jahre (Interquartilsabstand (IQR): 3.1-8.7) [71].

Im Rahmen der Studie wurden umfangreiche Daten der Teilnehmer:innen erhoben (Abbildung 6), dazu zählen Fragebögen zum Lebensstil, verschiedene Instrumente der Ernährungserhebung (Food-Frequency-Questionnaire (FFQ), 3-Tage-Wiegeprotokoll), aber auch klinische Parameter, wie anthropometrische Maße, Blutdruck oder Knochengesundheit. Die Knochengesundheit wurde mit einem quantitativen Ultraschall (QUS) am Fersenbein gemessen. Dabei wird die frequenzabhängige BUA und die Schallgeschwindigkeit (SOS) einer Schallwelle gemessen, während sie den Fersenknochen durchdringt. Aus beiden Werten wird unter Berücksichtigung des Alters der Steifigkeitsindex (SI) berechnet. Auch Bioproben (Blut, Urin und Stuhl) wurden gesammelt, auf deren Basis verschiedene Biomarker gemessen wurden.

Weikert et al. [71] zeigte in der Basispublikation der RBVD-Studie Unterschiede in der Aufnahme verschiedener Makronutrienten als auch der Mikronutrienten zwischen Veganer:innen und Mischköstler:innen bei vergleichbarer Energieaufnahme [71]. Bei Veganer:innen sind dabei die höheren Aufnahmemengen von Ballaststoffen, aber auch verschiedener Vitamine (Vitamin E, K, Folat), sowie Eisen, hervorzuheben [71]. Die Aufnahme von Vitamin B12, D und Jod war dagegen geringer im Vergleich zu der Mischkostgruppe [71].

In Hinblick auf die Konzentrationen im Blut hatten Veganer:innen, im Vergleich zu den Mischköstler:innen, geringere Konzentrationen der Vitaminen A, B2, B3 und E, sowie Selenprotein P und Zink. [71]. Im 24-Stunden-Sammelurin hatten Veganer:innen, im Vergleich zur Mischkostgruppe, eine geringere Calciumausscheidung sowie eine deutlich geringere Jodausscheidung [71]. Die Jodausscheidung lag bei 31% der Veganer:innen der RBVD-Studie unterhalb von 20 µg/l, dem Grenzwert der Weltgesundheitsorganisation (WHO) für eine schwere Unterversorgung [71]. Zudem zeigte sich, dass in der RBVD-Studie 97.2% aller Veganer:innen innerhalb der letzten vier Wochen Supplemente eingenommen hatten, mit der

häufigsten Supplementierung von Vitamin B12 (92%) [71]. Bei den Mischköstler:innen supplementierten insgesamt nur ein Drittel (33.3%) verschiedene Nährstoffe [71].

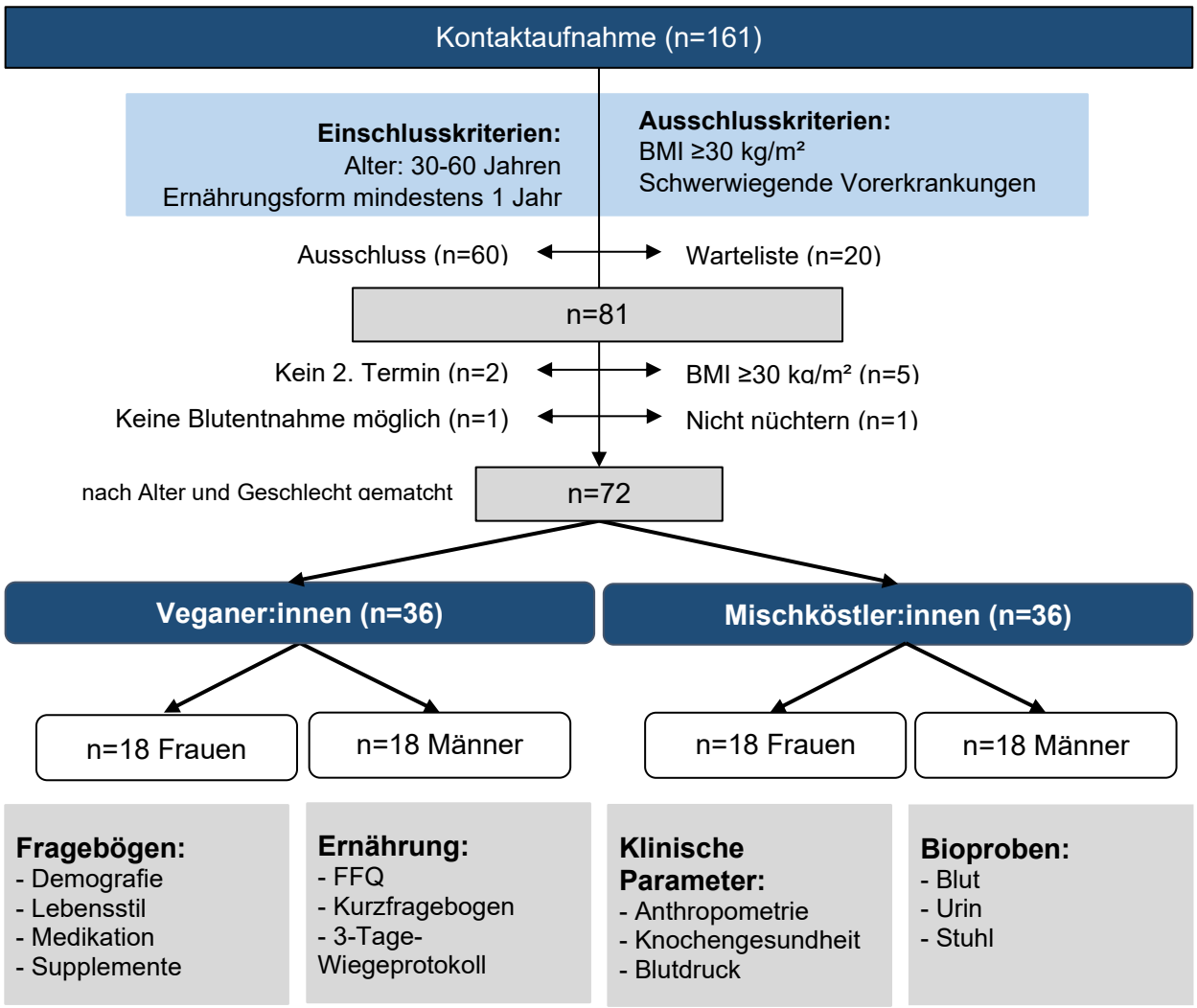

Abbildung 6: Überblick Studiendesign der RBVD-Studie (verändert nach Weikert et al. [71])

1.6. Fragestellungen der vorliegenden Originalarbeiten

Ein Ziel der Originalarbeiten dieser Habilitationsschrift war es, inflammatorische Biomarker umfassend bei Veganer:innen im Vergleich zu Mischköstler:innen zu untersuchen (Originalarbeit 1). Ziel dabei war es nicht nur hsCRP in der RBVD-Studie zu untersuchen, sondern auch viele andere wichtige inflammatorische Biomarker: IL-18, Interleukin-1 receptor antagonist (IL-1 RA), Intercellular adhesion molecule-1 (ICAM-1), Adiponectin, Omentin-1 oder Resistin. Zudem wurde eine umfassende systematische Übersichtsarbeit (Originalarbeit 2) mit Metaanalyse angefertigt, die den Zusammenhang dieser inflammatorischen Biomarker zwischen Vegetarismus und Veganismus im Vergleich zur Mischkost getrennt untersuchte.

Weitere Originalarbeiten hatten zum Ziel, die Knochengesundheit, welche mit quantitativem Ultraschall gemessen wurde, zwischen Veganer:innen im Vergleich der Mischköstler:innen in der RBVD-Studie zu untersuchen (Originalarbeit 3 und 4). Darüberhinausgehend wurden ernährungsbedingte Biomarker in Blut oder Urin untersucht, die mit der Knochengesundheit zusammenhängen. Dazu zählen verschiedene Vitamine, Mineralstoffe, Fettsäuren und Aminosäuren. Es wurden aber auch andere knochenrelevante Biomarker untersucht, wie Biomarker des Knochenumsatzes, Calciumhomöostase, Inflammation und der Fibroblast Growth Faktor 23 (FGF23) /α-Klotho-Signalachse. Durch die Anwendung der reduzierten Rang Regression (RRR) wurde das Ziel verfolgt, ein exploratives Biomarkermuster zu erkennen, dass möglicherweise eine Kombination von Biomarkern aufdeckt, die zur Knochengesundheit beitragen (Originalarbeit 3). Eine weitere Originalarbeit (Originalarbeit 4) untersuchte weiterführend die Säurelast als einen möglichen Einflussfaktor auf die Knochengesundheit bei Veganer:innen und Mischköstler:innen. Dabei hatte die Studie das Ziel die urinäre potenzielle renale Säurelast (uPRAL) zu charakterisieren, sowie die 24-Stunden-Harnausscheidungsprofile von säure- und basenrelevanten Ionen und Urin-pH von Veganer:innen im Vergleich zu Mischköstler:innen zu untersuchen. Zudem hatte die Studie das Ziel zu untersuchen, ob die erwartete niedrige Säurelast bei einer veganen Ernährung einen Einfluss auf die Knochengesundheit hat.

Eine weitere Originalarbeit (Originalarbeit 5) hatte zum Ziel, die Unterschiede der internen Exposition (Konzentration im Blut) verschiedener einzelner PFAS und ihrer Summe zwischen Veganer:innen und Mischköstler:innen zu untersuchen. Auch Korrelationen von relevanten Lebensmittelgruppen mit PFAS-Konzentrationen sollten untersucht werden. Zudem untersuchte diese Arbeit auch den Einfluss einer veganen Ernährung und der internen PFAS-Exposition in Hinblick auf Cholesterinwerte.

2. Eigene Arbeiten

2.1. Vegane Ernährung und inflammatorische Biomarker

2.1.1. Unterschiede in den Konzentrationen von sieben inflammatorischen Biomarkern zwischen Veganer:innen und Mischköstler:innen

***Menzel J**, Biemann R, Longree A, Isermann B, Mai K, Schulze MB, Abraham K, Weikert C, Associations of a vegan diet with inflammatory biomarkers, Scientific Reports, 2020 Feb 6;10(1):1933. doi: 10.1038/s41598-020-58875-x.*

Die Ergebnisse der Originalpublikation werden im Folgenden zusammengefasst und können dem Abstract der Publikation ähneln. Die deutsche Übersetzung erfolgte durch die Autorin.

Da sich die aktuellen wissenschaftlichen Studien fast ausschließlich auf CRP, sowie auf vegetarischen Ernährungsformen fokussieren, wurde in dieser Orginalarbeit nicht nur hsCRP untersucht, sondern auch andere wichtige inflammatorische Biomarker: Adiponektin, ICAM-1, IL-18, IL-1 RA, Omentin-1 und Resistin [72]. Dabei wurden Unterschiede dieser inflammatorische Marker zwischen Veganer:innen (n=36) im Vergleich zu Mischköstler:innen (n=36) in der RBVD-Studie untersucht [72].

Bezüglich hsCRP wurden keine Unterschiede in den Konzentrationen zwischen Veganer:innen und Mischköstler:innen im adjustierten Modell gefunden (Adjustierung wichtiger Lebensstilfaktoren) [72]. Dennoch zeigte sich, dass Mischköstler:innen im unadjustierten Modell tendenziell höhere hsCRP Werte (geometrischer Mittelwert: 0.94 mg/l (95%-Konfidenzintervall (95%-KI): 0.65-1.28) im Vergleich zu Veganer:innen (geometrischer Mittelwert: 0.60 mg/l, 95%-KI: 0.36-0.87, p=0.09) hatten [72]. Weiterführend zeigte diese Arbeit keine signifikanten Unterschiede zwischen Veganer:innen und Mischköstler:innen bei anderen untersuchten inflammatorischen Biomarkern Adiponektin, ICAM-1, IL-18, IL-1 RA, Omentin-1 oder Resistin (alle $p>0.05$) [72].

In weiteren Analysen zeigte diese Arbeit auch, dass die Dauer der veganen Ernährung positiv mit IL-18 (Spearman $r=0.44$, $p=0.02$) korreliert [72]. Außerdem hatten Veganer:innen, die bereits mehr als 4.8 Jahre vegan lebten, tendenziell eher niedrigere hsCRP-Werte (geometrischer Mittelwert: 0.50 mg/l, 95%-KI: 0.21-0.85) im Vergleich zu Veganer:innen, die sich ≤ 4.8 Jahre vegan ernährten (geometrischer Mittelwert: 0.85 mg/l, 95%-KI:0.46-1.33, $p=0.09$) [72]. Darüber hinausgehend waren auch Resistin-Konzentrationen stark mit der Dauer einer veganen Ernährung korreliert (Spearman $r=0.59$, $p=0.0008$) [72]. Der Taillenumfang und BMI waren hingegen positiv mit hsCRP, ICAM-1, IL-1 RA und invers mit Adiponektin und Omentin-1 korreliert [72].

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Associations of a vegan diet with inflammatory biomarkers

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Vegetarian or vegan nutrition might influence inflammatory processes, thereby reducing the risk of chronic diseases. As the vegan diet becomes more importance in modern societies, data from the "Risks and Benefits of a Vegan Diet"-study has been used to investigate the associations of veganism with a comprehensive spectrum of inflammatory biomarkers, compared to omnivores. This cross-sectional study comprises 36 vegans and 36 omnivores (18 men and 18 women each) aged 30–60 years. No significant differences in any of the investigated inflammatory biomarkers (high-sensitivity C-reactive protein (hsCRP), interleukin-18 (IL-18), interleukin-1 receptor antagonist (IL-1 RA), intercellular adhesion molecule-1 (ICAM-1), adiponectin, omentin-1 and resistin) were observed between vegans and omnivores. However, the duration of a vegan diet was positively correlated with resistin (Spearman r = 0.59), IL-18 concentrations (Spearman r = 0.44) and IL-1 RA (Spearman r = 0.34). Moreover, the present study supports BMI and waist circumference as important factors influencing the inflammatory state. Further research is needed to evaluate associations between a vegan diet and inflammatory biomarkers to provide more evidence about the inflammatory state as underlying mechanisms of a vegan diet to influence the risk of numerous chronic diseases.

There is a growing trend for vegetarian and vegan diets in Germany and many Western countries[1]. These diets are typically higher in fruits, vegetables, whole-grains, legumes, nuts, and various soy products[2], corresponding to larger amounts of antioxidant micronutrients such as vitamins C and E, phytochemicals and dietary fibre[2,3]. Especially, a growing trend toward veganism has arisen in the recent years, due to increasing awareness of the compassion for animals and environmental problems associated with livestock farming. Furthermore, more and more people are turning to a vegan diet also for the potential health benefits. Indeed, scientific evidence suggests that a vegan or vegetarian diet may be protective against many chronic diseases like type 2 diabetes[4], cardiovascular diseases[5], or cancer[6]. Recent research hypothesized links between low-grade inflammation and increased risk of various diseases, which are known to be triggered by underlying inflammation. Thus, inflammatory biomarkers may also act as intermediate risk factors in the development of chronic diseases. Actually, elevated levels of inflammatory markers like high-sensitivity C-reactive protein (hsCRP), or interleukin 18 (IL-18) are found to be associated with pathogenetic mechanisms of chronic diseases in type 2 diabetes[7] or cardiovascular disease[8,9]. In contrast, concentrations of the anti-inflammatory hormone adiponectin were found to be inversely associated with these diseases[10,11].

Recent evidence proposed that inflammatory biomarker profiles can be modulated by plant-based diets, showing an attenuation of inflammation markers as for instance CRP[12,13] or soluble intercellular adhesion molecule 1 (sICAM-1)[12]. Similarly, meta-analyses noticed that vegetarian patterns were associated with lowered CRP concentrations[3,14]. Indeed, while the associations between vegetarian patterns on inflammatory biomarkers have been investigated in some studies, comprehensive scientific research on the impact of an exclusive vegan diet on inflammatory biomarkers is still missing. As the vegan diet becomes more importance in modern societies, the present "Risks and Benefits of a Vegan Diet" study (RBVD-Study) aimed to investigate the association between a vegan diet and inflammation by analyzing data of vegans and omnivores on a comprehensive spectrum of

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	Vegans (n = 36)	Omnivores (n = 36)	*p-value*
Duration vegan diet [years]	4.8 (3.1–8.7)		
Men [%]	50% (18)	50% (18)	
Age [years]	37.5 (32.5–44.0)	38.5 (32.0–46.0)	*0.75*
Waist circumference [cm]			
Women	73.1 ± 6.9	77.2 ± 6.2	*0.07*
Men	84.5 ± 8.9	86.0 ± 6.1	*0.56*
Physical Activity [h/week]	2.8 (0.9–3.8)	2.3 (1.2–4.1)	*0.69*
Walking [h/week]	7.0 (5.0–12.0)	5.5 (3.5–11.8)	*0.15*
Smoking status [%]			*0.30*
Non-smoker	66.7% (24)	58.3% (21)	
Ex-Smoker	22.2% (8)	16.7% (6)	
Smoker	11.1% (4)	25.0% (9)	
Education [%]			*0.60*
Low	0.0% (0)	2.8% (1)	
Intermediate	30.6% (11)	30.6% (11)	
High	69.4% (25)	66.7% (24)	
Diet			
Alcohol consumption [g/d]			
Women	0.10 (0.00–4.69)	0.21 (0.02–4.88)	*0.22*
Men	0.04 (0.00–2.00)	3.85 (0.36–16.2)	*0.09*
Consumption fruits and vegetables [g/d]			
Fruits	185.5 (94.7–344.8)	152.3 (62.2–215.2)	*0.14*
Vegetables	459.5 (228.8–635.8)	226.8 (114.0–302.2)	*0.0001*
Total consumption	683.0 (463.0–887.8)	378.3 (216.0–523.0)	*<0.0001*
Plasma phospholipid fatty acids proportions [%]			
Total saturated fatty acids	46.0 (44.6–46.6)	47.3 (46.8–47.6)	*<0.0001*
Total mono-unsaturated fatty acids	12.8 (12.0–14.3)	12.3 (11.6–13.8)	*0.22*
Total poly-unsaturated fatty acids	41.0 (39.9–42.4)	39.9 (38.9–41.0)	*0.008*

Table 1. Characteristics of the study population according to a vegan or omnivorous diet (n = 72). Variables expressed as percentage (n) or mean ± SD or median (IQR).

inflammatory biomarkers, i.e. high-sensitivity C-reactive protein (hsCRP), interleukin-18 (IL-18), interleukin-1 receptor antagonist (IL-1 RA), intercellular adhesion molecule-1 (ICAM-1), adiponectin, omentin-1 and resistin.

Results

The distribution of general characteristics of the 72 sex- and age matched participants is shown in Table 1, according to vegan (n = 36) or omnivorous diet (n = 36). The median duration of veganism was 4.8 years (IQR: 3.1–8.7). As expected, we observed no differences in sex and age between vegans and omnivores (vegans: 50% male, median age: 37.5 years (min-max: 30.0–57.0); omnivores: 50% male, median age: 38.5 years (min-max: 30.0–57.0)). Moreover, we observed no differences in waist circumference, physical activity, smoking status, education status or alcohol consumption (all $p > 0.05$). However, vegans had a higher intake of vegetables, whereas no difference in fruit intake has been observed. Further, compared to omnivores, the plasma levels of saturated fatty acids (SFA) were lower in vegans, while levels of poly-unsaturated fatty acids (PUFA) were higher.

As depicted in Table 2, we observed no significant differences of the inflammatory biomarkers i.e. adiponectin, ICAM-1, IL-18, IL-1 RA, omentin-1 or resistin between vegans in comparison to omnivores (all > 0.05). Regarding hsCRP in the unadjusted model, omnivores were with a tendency more likely to have higher hsCRP levels (0.94 mg/l (95%-CI 0.65–1.28)) compared to vegans (0.60 mg/l (95%-CI 0.36–0.87), $p = 0.09$), nevertheless, with additionally adjustment of lifestyle factors this difference was further diminished ($p = 0.35$, Table 2). Interestingly, the duration of a vegan diet was positively correlated with IL-18 (Spearman $r = 0.44$, $p = 0.02$, Table 3). Further, long-time vegans (>4.8 years) were with a tendency more likely to have lower hsCRP level (0.50 mg/l (95%-CI 0.21-0.85) compared to vegans adhering to a vegan diet less than 4.8 years (0.85 mg/l (95%-CI 0.46-1.33), $p = 0.09$). IL-1 RA was positively correlated with the duration of a vegan diet in model 1 (Spearman $r = 0.35$, $p = 0.03$), however, this association was diminished with additionally adjustment (Table 3). Moreover, increased resistin concentrations were highly correlated with the duration of a vegan diet (Spearman $r = 0.59$, $p = 0.0008$, model 2). Moreover, waist circumference and BMI were positively correlated with hsCRP, ICAM-1, IL-1 RA and inversely correlated with adiponectin and omentin-1, although the correlations were less pronounced for BMI (Table 3). As shown in Table 3, plasma concentrations of SFA were positively correlated to resistin, and inversely correlated to the anti-inflammatory biomarker adiponectin and omentin-1. Higher levels of PUFA were correlated with lower levels of hsCRP (Table 3). For consumption of fruits and vegetables we noticed no association to any of the investigated inflammatory biomarkers (Table 3). In sensitivity analyses, the observed associations were not substantially altered neither by the exclusion of participants taking anti-rheumatic

	Vegans (n = 36)	Omnivores (n = 36)	*p-value*
Adiponectin [ng/ml][a]			
Model 1	4.37 (3.84–4.96)	4.06 (3.57–4.62)	*0.43*
Model 2	4.44 (3.34–5.90)	4.15 (3.17–5.43)	*0.46*
hsCRP [mg/l][a]			
Model 1	0.60 (0.36–0.87)	0.94 (0.65–1.28)	*0.09*
Model 2	0.44 (0.00–1.08)	0.61 (0.14–1.27)	*0.35*
ICAM-1 [ng/ml][a]			
Model 1	531.3 (498.6–566.0)	557.5 (523.3 594.0)	*0.29*
Model 2	600.8 (523.9–689.1)	615.2 (540.3–700.5)	*0.60*
IL-18 [pg/ml][a]			
Model 1	44.4 (29.6–66.4)	55.5 (37.1–82.8)	*0.44*
Model 2	72.6 (28.2–184.6)	95.6 (39.2–231.0)	*0.37*
IL-1 RA [pg/ml][a]			
Model 1	203.0 (165.2–249.4)	199.7 (162.5–245.3)	*0.91*
Model 2	175.5 (107.5–286.5)	170.3 (107.0–270.8)	*0.85*
Omentin-1 [ng/ml][b]			
Model 1	501.4 (449.6–553.3)	505.0 (453.1–556.9)	*0.92*
Model 2	511.7 (396.6–626.8)	507.7 (398.7–616.7)	*0.91*
Resistin [ng/ml][b]			
Model 1	6.85 (6.22–7.47)	7.20 (6.58–7.82)	*0.43*
Model 2	5.87 (4.51–7.23)	6.38 (5.09–7.67)	*0.25*

Table 2. Inflammatory biomarkers according to a vegan or omnivorous diet (n = 72). [a]Expressed as geometric mean (95%-CI), [b]Expressed as mean (95%-CI), Model 1: unadjusted, Model 2: adjusted for age, sex, smoking status, education, waist circumference, physical activity, alcohol consumption.

or analgesic drugs, nor the exclusion of extreme concentrations of the inflammatory biomarkers or the additionally adjustment for type of diet (data not shown).

Discussion

To the best of our knowledge, the present cross-sectional study is the most comprehensive study, investigating a wide spectrum of inflammatory biomarkers in exclusive vegans compared to omnivores. However, we observed no significant differences of the inflammatory biomarkers hsCRP, adiponectin, ICAM-1, IL-18, IL-1 RA, omentin-1 or resistin between vegans and omnivores. Nevertheless, the duration of a vegan diet seemed to have an impact on impaired inflammatory profiles. Interestingly, we detected trends towards differences in inflammatory biomarkers dependent on plasma levels of SFA or PUFA. Further, the present study supports BMI and waist circumference as important influencing factors on the inflammatory state.

Scientific evidence has been suggested that systemic low-grade inflammation is linked to various diseases. Knowing that a vegan or vegetarian diet may be protective against many chronic diseases like type 2 diabetes[4], cardiovascular diseases[5], or cancer[6], recent research hypothesized that plant-based nutritional habits might ameliorate inflammatory processes and accordingly, decrease circulating levels of inflammatory biomarkers and therefore might cause the reduced risk of chronic diseases in these populations[3,12–14]. However, up to date there is restricted evidence, limiting conclusions regarding the effect of a vegan diet on inflammatory biomarkers. Indeed, there are no studies, which perform comprehensive analyses to investigate the association of a vegan diet on the inflammatory profile. Accordingly, until now, only two studies provide data on inflammatory biomarkers in vegans (n = 9)[15] or strict vegetarians (animal products less than once a month, n = 66)[16] as small subgroups, while mainly investigating advanced glycation end products[15] or gut microbiota composition[16] in a vegetarian population. These studies showed conflicting results. Whereas Franco-de-Moraes *et al.*[16] observed higher CRP levels in omnivores compared to vegans, Šebeková *et al.*[15] noticed no differences in CRP levels. The latter is in line with our results. Nevertheless, these studies are mainly focused on CRP as one marker of inflammation, whereas the present study comprise a comprehensive investigation of a wide spectrum of inflammatory biomarkers in exclusive vegans compared to omnivores. Further, knowing that vegetarian patterns on inflammatory biomarkers have been investigated in more studies[3,14], the limitations are similar. In fact, Craddock *et al.*[14] recently mentioned in a systematic review and meta-analysis from 2019, that CRP was explored in seven studies, but significantly lowered concentrations following a vegetarian-based diet was observed in four studies only[14]. Further, the authors haven been noticed that except of CRP, other inflammatory and immune biomarkers of interest were not reported upon or were only explored in single studies, thereby limiting conclusions regarding the effect of vegetarian-based dietary patterns on the inflammatory profile[14]. Interestingly, Haghighatdoost *et al.*[3] emphasize a trend towards lower CRP concentrations in subjects following vegetarian diet of at least 2 years, while no significant effect was found in participants with a duration time of less than 2 years, but at least 6 month[3]. In line with these observations, the present study noticed that 'long-time' vegans (>4.8 years) were more likely to have lower hsCRP level compared to vegans adhering to a vegan diet ≤ 4.8 years. Knowing, that scientific evidence proposed high resistin and IL-18 level to induce insulin resistance and promote inflammatory processes, thereby playing a central role

	Duration vegan diet	Waist circumference	BMI	SFA	MUFA	PUFA	Fruits and vegetables
	Vegans (n = 36)	(n = 72)	(n = 72)	(n = 72)	(n = 72)	(n = 72)	(n = 72)
Adiponectin [ng/ml]							
Model 1	−0.21 (0.21)	−0.40 (0.0005)	−0.26 (0.03)	−0.28 (0.02)	0.15 (0.20)	0.09 (0.45)	0.15 (0.21)
Model 2	−0.13 (0.48)	−0.34 (0.005)	−0.24 (0.06)	−0.26 (0.04)	0.08 (0.51)	0.16 (0.21)	0.17 (0.18)
hsCRP [mg/l]							
Model 1	−0.17 (0.32)	0.44 (0.0001)	0.39 (0.0007)	0.25 (0.03)	−0.04 (0.72)	−0.24 (0.04)	−0.08 (0.51)
Model 2	−0.35 (0.07)	0.37 (0.003)	0.35 (0.005)	0.12 (0.34)	0.07 (0.57)	−0.27 (0.03)	−0.03 (0.79)
ICAM-1 [ng/ml]							
Model 1	0.09 (0.59)	0.39 (0.0006)	0.24 (0.04)	0.12 (0.34)	−0.09 (0.43)	−0.03 (0.78)	−0.007 (0.95)
Model 2	0.11 (0.57)	0.31 (0.01)	0.16 (0.19)	0.04 (0.76)	−0.03 (0.81)	−0.05 (0.69)	−0.09 (0.48)
IL-18 [pg/ml]							
Model 1	0.35 (0.04)	0.04 (0.75)	0.04 (0.77)	0.11 (0.35)	−0.04 (0.74)	−0.07 (0.57)	−0.15 (0.20)
Model 2	0.44 (0.02)	−0.09 (0.49)	0.001 (0.99)	0.10 (0.42)	−0.10 (0.42)	−0.04 (0.78)	−0.07 (0.60)
IL-1 RA [pg/ml]							
Model 1	0.35 (0.03)	0.36 (0.0019)	0.24 (0.04)	0.10 (0.39)	−0.11 (0.38)	0.08 (0.50)	0.06 (0.60)
Model 2	0.34 (0.08)	0.42 (0.0006)	0.24 (0.06)	0.04 (0.75)	0.08 (0.55)	−0.03 (0.84)	0.003 (0.98)
Omentin-1 [ng/ml]							
Model 1	−0.30 (0.08)	−0.29 (0.01)	−0.27 (0.02)	−0.31 (0.008)	0.15 (0.20)	0.08 (0.51)	0.09 (0.44)
Model 2	−0.24 (0.21)	−0.26 (0.04)	−0.29 (0.02)	−0.30 (0.02)	0.08 (0.51)	0.16 (0.20)	−0.006 (0.96)
Resistin [ng/ml]							
Model 1	0.47 (0.004)	−0.13 (0.29)	−0.06 (0.63)	0.17 (0.15)	−0.18 (0.14)	0.07 (0.55)	−0.06 (0.64)
Model 2	0.59 (0.0008)	0.17 (0.19)	0.09 (0.49)	0.30 (0.02)	−0.07 (0.61)	−0.14 (0.27)	−0.15 (0.23)

Table 3. Spearman correlations of inflammatory biomarkers with duration of a vegan diet, waist circumference, BMI, plasma SFA, MUFA, PUFA and consumption of fruits and vegetables. Expressed as Spearman rho (p-value), Model 1: unadjusted, Model 2: mutually adjusted for age, sex, smoking status, education, waist circumference, physical activity, alcohol consumption.

in various metabolic, inflammatory and autoimmune diseases[17,18], it was surprising, that increased resistin and IL-18 concentrations were highly correlated with the duration of a vegan diet, and in some degree, also IL-1 RA. This needs to be verified by further studies, as the effects of short-term or long-term adherence to a vegetarian or vegan diet on low-grade inflammatory state remain underestimated. Taken together, more research is highly warranted to evaluate associations between a vegan or vegetarian diet and inflammatory biomarkers to provide more evidence that a vegan or vegetarian diet may be beneficial to prevent or counteract inflammatory state and might be a nutritional approach to prevent risk of chronic diseases.

An array of nutrients as well as non-nutritive bioactive components of dietary habits may influence the inflammatory profile[14,19]. In this context, the type and quantity of dietary fat may be responsible for changes in inflammatory biomarkers[14,19]. Indeed, scientific evidence linked dietary SFA to an impaired inflammation profile[20], while SFA intake is typically higher in non-vegetarians due to the consumption of animal products[2]. Vegetarian-based populations typically consume a greater proportion of unsaturated fatty acids than non-vegetarians[21], proposed to be inversely associated with inflammation[22]. Interestingly, the present study observed lower plasma levels of SFA in vegans compared to omnivores, while the level of SFA was positive correlated to resistin. Inverse associations have been observed for the anti-inflammatory biomarker adiponectin and omentin-1. As expected, levels of PUFA were higher in vegans compared to omnivores, and corresponding higher plasma PUFA concentrations were inversely correlated with hsCRP concentration. Moreover, also intake of fruit and vegetables has been suggested to attenuation inflammation indicated by a large body of scientific evidence[23,24]. At the level of bioactive compounds occurring in fruits and vegetables, primarily carotenoids and flavonoids seem to modulate inflammatory as well as immunological processes[23,24]. Nevertheless, the present study detected no correlation between consumption of fruits and vegetables and inflammatory biomarkers.

It is important to note that overweight and obesity are associated with increased inflammation biomarkers[25]. In line, BMI and waist circumference were correlated with almost all investigated inflammatory biomarkers, even if obese individuals have been excluded from the study (exclusion criteria BMI $\geq 30\,kg/m^2$). Nevertheless, these findings support higher BMI and increased waist circumference as important influencing factors for impaired inflammatory profiles although in non-obese participants.

The present study has several strengths. Our study covered a comprehensive spectrum of biomarkers that reflect inflammation, including a set of novel markers at the site of adipose-tissue induced inflammatory response. The study provides comprehensive high-quality data as a result of the standardized procedures, including the collection of blood, in combination with extensive information from computer-based questionnaires, dietary assessment by 3-day weighting protocol and anthropometric measurements, enabling us to adjust for the most important potential confounders. However, some limitations of our study deserve to be mentioned. First, the RBVD-study is relatively small, including 36 vegans and 36 omnivores. The study was powered for a primary research question about differences in bone health between vegans and omnivores and therefore it cannot be

ruled out that some findings that were of borderline statistical significance may have been due to insufficient sample size. Second, the cross-sectional design of the present study does not allow for causal inference. Third, the study included middle-aged healthy German men and women, and therefore the results may not be generalized to other populations, such as other ethnic or age.

In conclusion, the RBVD-study is the first study providing a comprehensive spectrum of inflammatory biomarkers in vegans compared to omnivores, observing no significant differences of the inflammatory biomarkers hsCRP, adiponectin, ICAM-1, IL-18, IL-1 RA, omentin-1 or resistin between vegans and omnivores. Additional studies based on larger study populations are required to further evaluate the associations between a vegan diet and inflammatory biomarkers to provide more evidence that a vegan diet could provide means for prevention of chronic disease risk.

Methods

RBVD-Study. *Study population.* Study participants were recruited by announcement and investigated from 01st January 2017 until 31th July 2017 in Berlin. Participants for the present study were individuals who responded to this advertisement, contacted the study center at the German Federal Institute of Risk Assessment (BfR) via phone or mail (n = 161), following by a phone screening consisted of a brief explanation of the study and checking inclusion criteria (age 30–60 years, following the diet at least 1 year) and exclusion criteria (BMI $\geq 30\,kg/m^2$, cardiovascular disease, type 2 diabetes, cancer, pregnancy, breastfeeding, current infection). A omnivorous diet was defined as the consumption of at least three portions of meat per week or 2 portions of meat and 2 portions of processed meat per week, whereas a vegan diet was defined as no consumption of any animal food products. Each participant visited the study center twice. On their first visit, participants gave their written informed consent and received instructions to document their diet by a three-day weighed food protocol. At the second visit, anthropometric measurements and lifestyle characteristics were assessed, and a fasting blood sample was collected. As shown in Fig. 1, the final study population comprises 36 vegans and 36 omnivores, which were matched by sex- and age. The study was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of Charité University Medical Center Berlin (No. EA4/121/16).

Assessment of lifestyle characteristics. Anthropometric measurements (weight, height, and waist circumference) were collected by trained and quality-monitored personnel, while participants wearing only light underwear and no shoes. Body weight was assessed by an electronic digital scale (Omron BF511, Germany) and the height was measured using a flexible anthropometer (SECA, Germany). The formula for BMI comprises weight in kilograms divided by height in meters squared. Waist circumference was defined as in the horizontal plane midway between the lowest ribs and the iliac crest. Information on physical activity, educational level and smoking status was assessed by computer-based questionnaires. The educational level was defined as 'low education' (no degree), 'intermediate education' (vocational school, technical college) or 'high education' (university, university of applied sciences). The amount of time spent on cycling, sports and gardening has been determined for summer and winter separately [hours/week]. Physical activity contains the sum of average hours in summer and winter per week of these activities. Walking comprises the sum of average hours per week during summer and winter. Dietary habits including alcohol consumption, fruit and vegetable consumption were assessed by three-day weighed food records for two week days and one weekend day. Data of the weighing protocols were merged with the German national food code (Bundeslebensmittelschlüssel Version 3.02, BLS) to assign with macro- and micronutrients. In this manuscript the fruit and vegetable consumption (g/d) includes the sum of the intake of fresh fruits, cooked, raw vegetables, as well as potatoes and legumes.

Blood collection and laboratory analysis. About 60 mL of venous blood was collected from fasting participants at the BfR study center. Several routine biomarkers including hsCRP were measured from fresh blood samples at the accredited medical analytics laboratory (Labor28 GmbH, Berlin, Germany) immediately at each study day. About half of blood was fractionated and aliquoted into serum, EDTA-plasma and erythrocytes, and stored in freezers (−80 °C) for conservation until time of analysis.

In 2018, inflammatory biomarkers other than hsCRP were measured in plasma samples at the Institute for Clinical Chemistry and Pathobiochemistry, Otto-von-Guericke University Magdeburg (Magdeburg, Germany). Plasma levels of IL-1 RA were analyzed using a sandwich ELISA from R&D Systems (Minneapolis, Minnesota, USA) with intra-assay coefficients of variation between 3.7% and 7.3%, inter-assay coefficients of variation between 6.7% and 11.0% and a limit of detection of 6.3 pg/ml. Plasma IL-18 was quantified with a sandwich ELISA by IBL (Gunma, Japan) with intra-assay coefficients of variation between 4.9% and 10.8%, inter-assay coefficients of variation between 5.2% and 10.0% and a limit of detection of 12.5 pg/ml. Plasma ICAM-1 concentrations were analyzed using an immunoassay from Life Technologies (Carlsbad, California, USA) with intra-assay coefficients of variation between 2.2% and 7.8%, inter-assay coefficients of variation between 5.1% and 11.1% and a limit of detection of 2.2 ng/ml. Serum concentrations of total adiponectin were also measured with a sandwich ELISA by ALPCO Diagnostics (Salem, New Hampshire, USA) with intra-assay coefficients of variation between 5.3% and 5.4%, inter-assay coefficient of variation between of 5.0% and a limit of detection of 0.019 ng/ml. Serum omentin-1 concentrations were measured with a sandwich ELISA by Biovendor (Brno, Czech Republic) with intra-assay coefficients of variation between 3.2% and 4.1%, inter-assay coefficients of variation between 4.4% and 4.8% and a limit of detection of 0.5 ng/ml. Plasma resistin levels were quantified with an sandwich ELISA from R&D Systems (Minneapolis, Minnesota, USA) with intra-assay coefficients of variation between 3.8% and 5.3%, inter-assay coefficients of variation between 7.8% and 9.2% and a limit of detection of 0.026 ng/ml. Fatty acids in plasma phospholipids were analyzed by gas chromatography by the German Institute of Human Nutrition in Potsdam-Rehbruecke[26]. The total saturated fatty acids (SFA), including myristic acid (C14:0), pentadecanoic acid

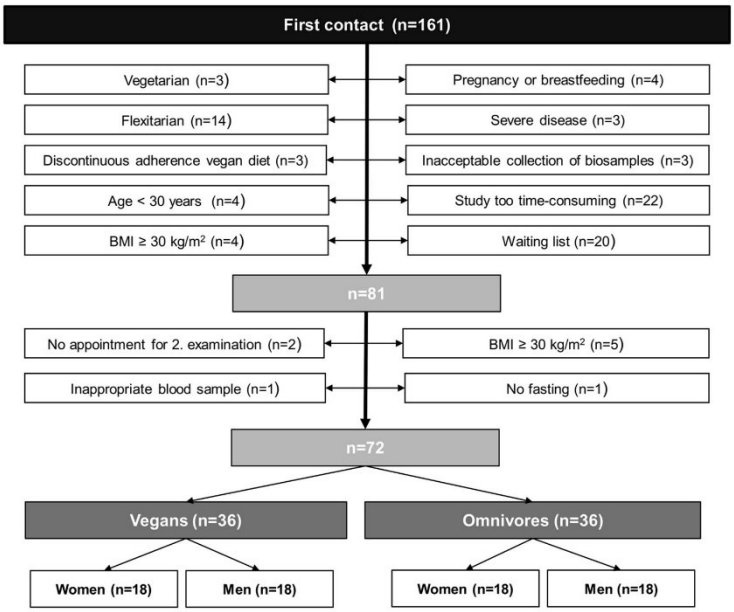

Figure 1. Flowchart.

(C15:0), palmitic acid (C16:0), heptadecanoic acid (C17:0), stearic acid (C18:0) and arachidic acid (C20:0). The total monounsaturated fatty acids (MUFA) containing palmitoleic acid (C16:1n7c), cis-vaccenic acid (C18:1n7c), oleic acid (C18:1n9c), as well as gondoic acid (C20:1n9). The total polyunsaturated fatty acids (PUFA) including alpha-linolenic acid (C18:3n3), eicosapentaenoic acid (C20:5n3), docosapentaenoic acid n-3 (C22:5n3) docosahexaenoic acid n-3 (C22:6n3), linoleic acid (C18:2n6c), gamma-linolenic acid (C18:3n6), docosadienoic acid (C20:2n6), dihomo-¬-linolenic acid (C20:3n6), arachidonic acid (C20:4n6), adrenic acid (C22:4n6), and docosapentaenoic acid n-6 (C22:5n6).

Sample size estimation. As the main research question of the RBVD-study is the investigation of bone health following a vegan diet compared to omnivores, the power calculation was based on the bone health measurement. The sample size was calculated, assuming a clinically relevant difference of at least 5% in bone health (estimated based on broadband ultrasound attenuation measurements) between vegan and omnivores. Along with a level of significance of 5% and a power of 80%, in total 72 participants were required (36 vegans, 36 omnivores) (G*power, t test for independent samples).

Statistical analyses. Normally distributed variables were reported as mean and standard derivation (SD), skewed variables were reported as median and interquartile range (IQR) and log-transformed for analyses. Categorical variables were reported as percentage. For comparison of the characteristics of vegans compared to omnivores, a Chi-Square test for categorical variables and a Student's t test or Mann–Whitney U test for continuous variables were used.

To investigate the association of veganism with inflammatory biomarkers compared to omnivores, an analysis of variance (ANOVA) was performed for model 1 (unadjusted). Additionally, a multivariable adjusted analysis of covariance (ANCOVA) was conducted to detected differences between vegans and omnivores in model 2, adjusted for age, sex, smoking status, education, waist circumference, physical activity and alcohol consumption. Independent of the type of diet, differences in inflammatory biomarkers according to the duration of a vegan diet, waist circumference, BMI, total SFA, total MUFA, total PUFA and fruit and vegetable consumption, have been investigated. Correlations between inflammatory biomarkers and duration of a vegan diet (in vegans only, n = 36), waist circumference, BMI, total SFA, total MUFA, total PUFA and fruit/vegetable consumption were assessed using spearman correlation (unadjusted, Model 1) and spearman partial correlation mutually adjusted for age, sex, smoking status, education, waist circumference, physical activity, alcohol consumption (model 2). For model 2, a multivariable adjusted ANCOVA were performed to investigate differences in inflammatory biomarkers according to the duration of a vegan diet (cut off median: 4.8 years). Skewed variables that were log-transformed before ANOVA or ANOVA were back-transformed and expressed as geometric means and 95%-CI.

Sensitivity analyses were carried out after exclusion of participants taking anti-rheumatic or analgesic drugs. In detail, analyses were performed after exclusion of participants with regular intake of anti-rheumatic and analgesic drugs (at least 3 times within last week before examination) (n = 2), additionally participants with single dose within the last week before examination (n = 7), participants with single dose within in the last two weeks before examination (n = 18), participants with single dose within the last month before examination (n = 27). Further, in sensitivity analyses extreme concentrations of the inflammatory biomarkers have been excluded ($\leq 1^{st}$ or $\geq 99^{th}$ percentile), respectively (n = 2). To eliminate the influence of the type of diet i.e. a vegan or omnivorous diet, the spearman partial correlations have been additionally adjusted for type of diet. All statistical analyses were performed using SAS software, version 9.4 (SAS institute, Cary, N.C., USA). P values of <0.05 were considered statistically significant.

Data availability

The datasets generated during and/or analyzed during the current RBVD-Study are not publicly available due to provisions of the written informed consent.

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References

1. Alles, B. *et al.* Comparison of Sociodemographic and Nutritional Characteristics between Self-Reported Vegetarians, Vegans, and Meat-Eaters from the NutriNet-Sante Study. *Nutrients* **9**, https://doi.org/10.3390/nu9091023 (2017).
2. Craig, W. J. Nutrition concerns and health effects of vegetarian diets. *Nutr Clin Pract* **25**, 613–620, https://doi.org/10.1177/0884533610385707 (2010).
3. Haghighatdoost, F., Bellissimo, N., Totosy de Zepetnek, J. O. & Rouhani, M. H. Association of vegetarian diet with inflammatory biomarkers: a systematic review and meta-analysis of observational studies. *Public Health Nutr* **20**, 2713–2721, https://doi.org/10.1017/S1368980017001768 (2017).
4. Lee, Y. & Park, K. Adherence to a Vegetarian Diet and Diabetes Risk: A Systematic Review and Meta-Analysis of Observational Studies. *Nutrients* **9**, https://doi.org/10.3390/nu9060603 (2017).
5. Kahleova, H., Levin, S. & Barnard, N. D. Vegetarian Dietary Patterns and Cardiovascular Disease. *Prog Cardiovasc Dis* **61**, 54–61, https://doi.org/10.1016/j.pcad.2018.05.002 (2018).
6. Dinu, M., Abbate, R., Gensini, G. F., Casini, A. & Sofi, F. Vegetarian, vegan diets and multiple health outcomes: A systematic review with meta-analysis of observational studies. *Crit Rev Food Sci Nutr* **57**, 3640–3649, https://doi.org/10.1080/10408398.2016.1138447 (2017).
7. Liu, C. *et al.* Adiponectin, TNF-alpha and inflammatory cytokines and risk of type 2 diabetes: A systematic review and meta-analysis. *Cytokine* **86**, 100–109, https://doi.org/10.1016/j.cyto.2016.06.028 (2016).
8. Jefferis, B. J. *et al.* Interleukin 18 and coronary heart disease: prospective study and systematic review. *Atherosclerosis* **217**, 227–233, https://doi.org/10.1016/j.atherosclerosis.2011.03.015 (2011).
9. Pearson, T. A. *et al.* Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* **107**, 499–511 (2003).
10. Shibata, R., Ouchi, N. & Murohara, T. Adiponectin and cardiovascular disease. *Circ J* **73**, 608–614 (2009).
11. Li, S., Shin, H. J., Ding, E. L. & van Dam, R. M. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA* **302**, 179–188, https://doi.org/10.1001/jama.2009.976 (2009).
12. Eichelmann, F., Schwingshackl, L., Fedirko, V. & Aleksandrova, K. Effect of plant-based diets on obesity-related inflammatory profiles: a systematic review and meta-analysis of intervention trials. *Obes Rev* **17**, 1067–1079, https://doi.org/10.1111/obr.12439 (2016).
13. Barbaresko, J., Koch, M., Schulze, M. B. & Nothlings, U. Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. *Nutr Rev* **71**, 511–527, https://doi.org/10.1111/nure.12035 (2013).
14. Craddock, J. C., Neale, E. P., Peoples, G. E. & Probst, Y. C. Vegetarian-Based Dietary Patterns and their Relation with Inflammatory and Immune Biomarkers: A Systematic Review and Meta-Analysis. *Adv Nutr*, https://doi.org/10.1093/advances/nmy103 (2019).
15. Sebeková, K. *et al.* Plasma levels of advanced glycation end products in healthy, long-term vegetarians and subjects on a western mixed diet. *Eur J Nutr* **40**, 275–281 (2001).
16. Franco-de-Moraes, A. C. *et al.* Worse inflammatory profile in omnivores than in vegetarians associates with the gut microbiota composition. *Diabetol Metab Syndr* **9**, 62, https://doi.org/10.1186/s13098-017-0261-x (2017).
17. Acquarone, E., Monacelli, F., Borghi, R., Nencioni, A. & Odetti, P. Resistin: A reappraisal. *Mech Ageing Dev* **178**, 46–63, https://doi.org/10.1016/j.mad.2019.01.004 (2019).
18. Dinarello, C. A., Novick, D., Kim, S. & Kaplanski, G. Interleukin-18 and IL-18 binding protein. *Front Immunol* **4**, 289, https://doi.org/10.3389/fimmu.2013.00289 (2013).
19. Galland, L. Diet and inflammation. *Nutr Clin Pract* **25**, 634–640, https://doi.org/10.1177/0884533610385703 (2010).
20. Ruiz-Nunez, B., Dijck-Brouwer, D. A. & Muskiet, F. A. The relation of saturated fatty acids with low-grade inflammation and cardiovascular disease. *J Nutr Biochem* **36**, 1–20, https://doi.org/10.1016/j.jnutbio.2015.12.007 (2016).
21. Davis, B. C. & Kris-Etherton, P. M. Achieving optimal essential fatty acid status in vegetarians: current knowledge and practical implications. *Am J Clin Nutr* **78**, 640S–646S, https://doi.org/10.1093/ajcn/78.3.640S (2003).
22. Kalogeropoulos, N. *et al.* Unsaturated fatty acids are inversely associated and n-6/n-3 ratios are positively related to inflammation and coagulation markers in plasma of apparently healthy adults. *Clin Chim Acta* **411**, 584–591, https://doi.org/10.1016/j.cca.2010.01.023 (2010).
23. Watzl, B. Anti-inflammatory effects of plant-based foods and of their constituents. *Int J Vitam Nutr Res* **78**, 293–298, https://doi.org/10.1024/0300-9831.78.6.293 (2008).
24. Lapuente, M., Estruch, R., Shahbaz, M. & Casas, R. Relation of Fruits and Vegetables with Major Cardiometabolic Risk Factors, Markers of Oxidation, and Inflammation. *Nutrients* **11**, https://doi.org/10.3390/nu11102381 (2019).
25. Ellulu, M. S., Patimah, I., Khaza'ai, H., Rahmat, A. & Abed, Y. Obesity and inflammation: the linking mechanism and the complications. *Arch Med Sci* **13**, 851–863, https://doi.org/10.5114/aoms.2016.58928 (2017).
26. Weitkunat, K. *et al.* Odd-chain fatty acids as a biomarker for dietary fiber intake: a novel pathway for endogenous production from propionate. *Am J Clin Nutr* **105**, 1544–1551, https://doi.org/10.3945/ajcn.117.152702 (2017).

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Author contributions
Conceived and designed the study: J.M., C.W.; Biomarker measurements: R.B., MB.S.; Statistical analyses: J.M.; Supervision of the project: C.W., K.A.; Draft of the manuscript: J.M.; Intellectually contribution to the manuscript: J.M., R.B., A.L., B.I., K.M., M.B.S., K.A. and C.W. All authors reviewed the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
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2.1.2. Unterschiede in den Konzentrationen von verschiedenen inflammatorischen Biomarkern zwischen Veganer:innen/Vegetarier:innen und Mischköstler:innen – Systematisches Review mit Metaanalyse

***Menzel J**, Jabakhanji A, Biemann R, Mai K, Abraham K, Weikert C, Systematic review and meta-analysis of the associations of vegan and vegetarian diets with inflammatory biomarkers, Scientific Reports, 2020 Dec 10;10(1):21736. doi: 10.1038/s41598-020-78426-8.*

Die Ergebnisse der Originalpublikation werden im Folgenden zusammengefasst und können dem Abstract der Publikation ähneln. Die deutsche Übersetzung erfolgte durch die Autorin.

In bereits existierenden systematischen Übersichtsarbeiten wird der Vegetarismus und Veganismus bisher zusammen mit anderen pflanzenbasierten Ernährungsformen betrachtet [73]. Um Unterschiede von Konzentrationen inflammatorischer Biomarkern explizit zwischen Vegetarier:innen und Veganer:innen in Vergleich zur Mischkost getrennt zu untersuchen, wurde in dieser Originalarbeit ein systematisches Review mit Metaanalyse erarbeitet [73], dabei wurden auch die Ergebnisse der Originalarbeit 1 aufgenommen.

Die systematische Literatursuche wurde in den Datenbanken Pubmed und EMBASE durchgeführt, dabei wurden Publikationen bis April 2020 betrachtet [73]. Es wurden folgende Biomarker untersucht: CRP, IL-6, IL-18, IL-1 RA, TNF-α, E-Selektin, interzelluläres Adhäsionsmolekül-1 (ICAM-1), monozytisches chemoattraktives Protein-1 (MCP-1), Adiponektin, Omentin-1 und Resistin [73]. Von den 1073 identifizierten wissenschaftlichen Publikationen zu diesem Thema, erfüllten 21 Querschnittsstudien die Einschlusskriterien und wurden in das systematische Review (und Metaanalyse) einbezogen [73].

Bezüglich der veganen Ernährung zeigte sich, dass insgesamt nur 3 von 21 Studien den Zusammenhang zwischen einer veganen Ernährungsweise im Vergleich zu einer Mischkost in Bezug auf CRP in gesunden Populationen untersuchten [73]. Die Originalpublikation 1 ist dabei eine dieser Publikation. Eine durchgeführte Metaanalyse zeigte, dass eine vegane Ernährungsweise mit niedrigeren CRP-Werten im Vergleich zu Mischköstler:innen assoziiert waren (Mittelwertdifferenz: -0.54 mg/l, 95%-KI: -0.79 bis -0.28, $p<0.0001$) [73]. Andere inflammatorische Biomarker z.B. TNF-α, IL-18, Adiponektin u.a. wurden nur von zwei weiteren Studien untersucht [73].

Hinsichtlich der vegetarischen Ernährung untersuchten 19 von 21 Studien die Konzentrationen von CRP zwischen Vegetarismus im Vergleich zur Mischkost [73]. Vierzehn Studien schlossen gesunde Teilnehmer:innen ein, sechs Studien untersuchten vorerkrankte Teilnehmer:innen [73]. Die Metaanalyse zeigte, dass die Assoziationen hinsichtlich CRP bei Vegetarier:innen weniger stark ausgeprägt war (Mittelwertdifferenz: -0.25 mg/l, 95%-KI: -0.49 bis 0.00, $p=0.05$)

[73]. Zudem war bei vorerkrankten Patient:innen mit eingeschränkter Nierenfunktion die Assoziation für CRP bei vegetarischer Ernährungsweise mit -3.91 mg/l (95%-KI: -5.23 bis -2.60; p<0.0001) deutlich stärker ausgeprägt [73]. Für alle anderen inflammatorischen Biomarker wurden keine wesentlichen Assoziationen beobachtet [73].

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Systematic review and meta-analysis of the associations of vegan and vegetarian diets with inflammatory biomarkers

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Plant-based diets like vegetarian or vegan diets might influence circulating levels of inflammatory biomarkers, thereby reducing the risk of chronic diseases. This systematic review and meta-analysis aimed to investigate the associations of veganism and vegetarianism with circulating inflammatory biomarkers in comparison to omnivores. Literature search was conducted in Pubmed and EMBASE until April 2020 and mean differences of biomarkers were assessed for: C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-18 (IL-18), interleukin-1 receptor antagonist (IL-1 RA), tumor necrosis factor-alpha (TNF-α), E-selectin, intercellular adhesion molecule-1 (ICAM-1), monocyte chemoattractant protein-1 (MCP-1), adiponectin, omentin-1 and resistin. Of initially identified 1073 publications, 21 cross-sectional studies met the inclusion criteria and were included in the systematic review and meta-analysis. Vegan diet was associated with lower levels of CRP compared to omnivores [mean difference − 0.54 mg/l, 95%-CI: − 0.79 to − 0.28, $p < 0.0001$]. This association was less pronounced in vegetarians [mean difference − 0.25 mg/l, 95%-CI: − 0.49 to 0.00, $p = 0.05$]. In patients with impaired kidney function, the association between vegetarian nutrition and CRP was much stronger with − 3.91 mg/l (95%-CI: − 5.23 to − 2.60; $p < 0.0001$). No substantial effects were observed for all other inflammatory biomarkers. Despite strong associations between CRP and a vegan or vegetarian diet were seen, further research is needed, as most inflammatory biomarkers were investigated only in single studies so far.

Since recent years, a growing trend for vegetarian and vegan diets can be recognized in Germany and other Western countries[1]. These plant-based diets were typically characterized by a higher consumption of fruits, vegetables, legumes, whole-grains, nuts, and various soy products[2], corresponding to a lower intake of saturated fat and cholesterol[2], as well as a larger amounts of antioxidant micronutrients like vitamins C and E, dietary fibre and phytochemicals[2,3]. Due to the increased awareness of environmental problems and compassion for animals, a growing trend toward veganism has remarkably emerged in the past few years. Another decisive factor for people turning to a vegan diet is the potential of health benefits. As a matter of fact, scientific evidence leads to the assumption that a vegetarian or vegan nutrition may be protective against many chronic inflammatory diseases like type 2 diabetes[4], cardiovascular diseases[5], or cancer[6]. Interestingly, recent research suggested associations between low-grade inflammation and increased risk of various chronic diseases. Hence, the development of chronic diseases could be influenced by inflammatory biomarkers acting as intermediate risk factors. In fact, it has been found that elevated concentrations of inflammatory markers like high-sensitivity C-reactive protein

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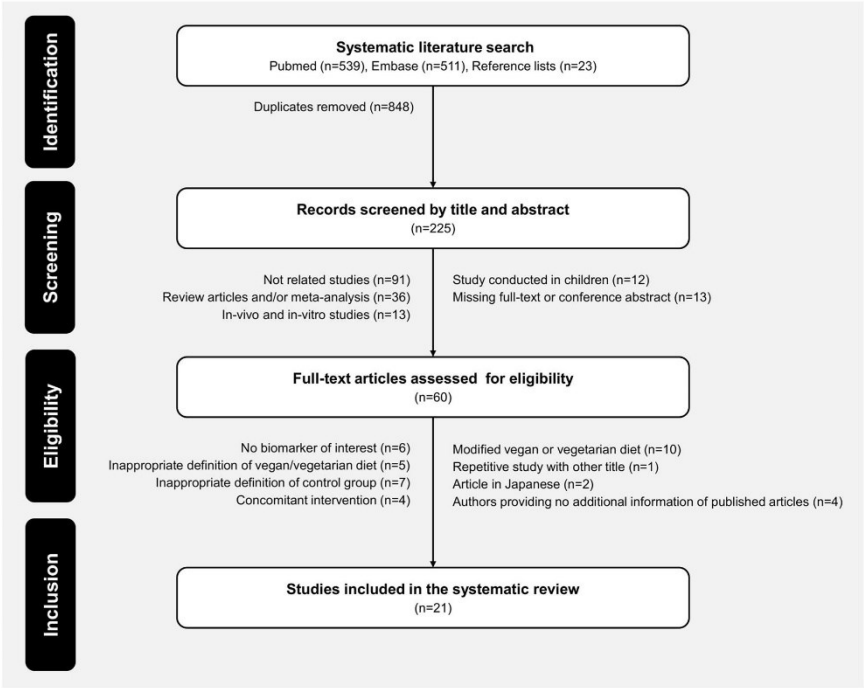

Figure 1. Flowchart. Selection process from initial search to final number of the included studies.

(hs-CRP), interleukin 6 (IL-6) or tumor necrosis factor-α (TNF-α) were associated with pathogenetic mechanisms of numerous chronic diseases in type 2 diabetes[7], cardiovascular disease[8] or selected cancer types[9]. On the contrary, concentrations of adiponectin were inversely associated with these diseases[10–12].

Recent scientific evidence suggested that plant-based diets may modulate inflammatory biomarker profiles, showing an attenuation of inflammation markers as for instance CRP, IL-6 and soluble intercellular adhesion molecule 1 (sICAM-1)[13–15]. Up to date, meta-analyses noticed that vegetarian nutrition is associated with lower CRP concentrations[3,16], while the impact of an exclusive vegan diet on inflammatory biomarkers was only examined by few studies. Therefore, we aimed to conduct a systematical review and meta-analysis to investigate the effects of a vegetarian or vegan diet on a comprehensive spectrum of inflammatory biomarkers, i.e. C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-18 (IL-18), interleukin-1 receptor antagonist (IL-1 RA), tumor necrosis factor-alpha (TNF-α), E-selectin, intercellular adhesion molecule-1 (ICAM-1), monocyte chemoattractant protein-1 (MCP-1), adiponectin, omentin-1 and resistin, in healthy and diseased population, separately.

Results

The systematic literature search produced a total of 1073 citations through database searching. In detail, we identified 511 publications from Embase, 539 from PubMed and 23 additional publications from manual searching the reference lists. After exclusion of duplicates from different databases (n = 848), publications reduced to 225 articles. After initial screening by titles and abstracts, 165 publications did not meet the study inclusion criteria and were excluded. From the remaining 60 citations, further 39 records were excluded following full-text assessment. In total, 21 studies have been included in the present systematic review. Detailed processes of study selection are shown in Fig. 1[17–37].

Main characteristics of the included 21 studies are summarized in Table 1. Studies were conducted across 3 continents, most studies were conducted in Asia (12 studies[19,22–27,29,32,35,36]), followed by Europe (6 studies[18,20,21,28,30,37]) and South America (3 studies[17,33,34]). All studies, with exception of one prospective study[29], used a cross-sectional design. However, the study with a prospective design has been considered as cross-sectional study, because we only used cross-sectional data from the baseline characteristics.

Twenty studies investigated the association between a vegetarian diet and inflammatory biomarkers compared to omnivores[17–36], two studies of them examined also the association between a vegan diet and inflammatory biomarkers, in parallel to a vegetarian diet[18,34]. The study by Menzel et al.[37] investigated the association between a vegan diet and inflammatory biomarkers compared to an omnivorous diet only. In total, the duration time following a vegan diet ranged from at least one year up to 20 years, and for vegetarian diet from one year up to 25 years.

Study characteristics			Diet group characteristics			Studied biomarkers	SQS
			Vegetarians	Vegans	Omnivores		
Mezzano et al. (1999)[17]							
Country	Chile	n	(n = 26)		(n = 26)	CRP	5
Study design	Cross-sectional	Age[a]	47.0 ± 12.7[c]		39.5 ± 12.2[c]		
Study population (n)	Apparently healthy (n = 52)	BMI[b]	23.0 ± 3.3[ci]		23.0 ± 3.3[ci]		
Male % (n)	46.1% (n = 24)	Duration[a]	≥ 1				
Šebeková et al. (2001)[18]							
Country	Slovak Republic	n	(n = 19)	(n = 9)	(n = 19)	CRP	5
Study design	Cross-sectional	Age[a]	36.1 ± 2.5[d]	36.6 ± 3.0[d]	30.5 ± 1.6[d]		
Study population (n)	Apparently healthy (n = 61)	BMI[b]	22.0 ± 0.05[d]	20.6 ± 0.8[d]	23.8 ± 0.04[d]		
Male % (n)	38% (n = 23)	Duration[a]	8.2 ± 0.8[c]	7.2 ± 1.0[c]			
Szeto et al. (2004)[19]							
Country	China	n	(n = 30)		(n = 30)	CRP	3
Study design	Cross-sectional	Age[a]	44.2 ± 9.0[c]		44.0 ± 9.2[c]		
Study population (n)	Apparently healthy (n = 60)	BMI[b]	NR		NR		
Male % (n)	10% (n = 6)	Duration[a]	21.8 ± 12.2[c]				
Krajcovicova-Kudlackova et al. (2005)[20]							
Country	Slovak Republic	n	(n = 133)		(n = 137)	hs-CRP	6
Study design	Cross-sectional	Age[a]	46.2 ± 1.4[d]		47.2 ± 1.4[d]		
Study population (n)	Apparently healthy (n = 270)	BMI[b]	22.9 ± 0.2[e]		24.7 ± 0.3[e]		
Male % (n)	35.5% (n = 96)	Duration[a]	10.4 ± 0.4[c]				
Šebeková et al. (2006)[21]							
Country	Slovak Republic	n	(n = 90)		(n = 46)	hs-CRP	5
Study design	Cross-sectional	Age[a]	37.7 (35.1–40.3)[f]		37.7 (33.5–40.7)[f]		
Study population (n)	Apparently healthy (n = 136)	BMI[b]	22.7 (22.1–23.3)[f]		23.8 (22.7–24.9)[f]		
Male % (n)	36% (n = 49)	Duration[a]	2–25				
Chen et al. (2008)[22]							
Country	Taiwan	n	(n = 99)		(n = 99)	hs-CRP	5
Study design	Cross-sectional	Age[a]	51.2 ± 8.9[c]		49.4 ± 9.6[c]		
Study population (n)	Apparently healthy (n = 198)	BMI[b]	22.9 ± 2.8[c]		23.8 ± 3.6[c]		
Male % (n)	43.9% (n = 87)	Duration[a]	≥ 1				
Hung et al. (2008)[23]							
Country	Taiwan	n	(n = 71)		(n = 388)	CRP	5
Study design	Cross-sectional	Age[a]	49.1 ± 11.2[c]		50.6 ± 9.5[c]		
Study population (n)	Apparently healthy or metabolic syndrome (n = 459)	BMI[b]	22.6 ± 2.7[c]		23.9 ± 3.1[c]		
Male % (n)	57.7% (n = 265)	Duration[a]	NR				
Chen et al. (2011)[24]							
Country	Taiwan	n	(n = 173)		(n = 190)	hs-CRP	6
Study design	Cross-sectional	Age[a]	54.0 ± 9.7[c]		49.9 ± 9.8[c]		
Study population (n)	Apparently healthy (n = 363)	BMI[b]	22.9 ± 2.9[c]		23.3 ± 3.5[c]		
Male % (n)	0% (n = 0)	Duration[a]	≥ 1				
Su et al. (2011)[25]							
Country	Taiwan	n	(n = 49)		(n = 41)	hs-CRP	3
Study design	Cross-sectional	Age[a]	58.6 ± 6.0[c]		57.2 ± 5.4[c]		
Study population (n)	Apparently healthy (n = 90)	BMI[b]	23.2 ± 2.7[c]		23.1 ± 2.9[c]		
Male % (n)	0% (n = 0)	Duration[a]	10.8 ± 7.5[c]				
Wu et al. (2011)[26]							
Country	Taiwan	n	(n = 19)		(n = 299)	hs-CRP	5
Study design	Cross-sectional	Age[a]	63.3 ± 2.6[c]		57.5 ± 1.2[c]		
Continued							

Study characteristics			Diet group characteristics			Studied biomarkers	SQS
			Vegetarians	Vegans	Omnivores		
Study population (n)	Haemodialysis patients (n = 318)	BMI[b]	22.7 ± 0.3[c]		20.2 ± 0.8[c]		
Male % (n)	46.9% (n = 149)	Duration[a]	NR				
Lee et al. (2014)[27]							
Country	Republic of Korea	n	(n = 357)		(n = 357)	hs-CRP	6
Study design	Cross-sectional	Age[a]	52.8 ± 8.5[c]		53.8 ± 8.7[c]		
Study population (n)	Apparently healthy or metabolic syndrome (n = 714)	BMI[b]	24.9 ± 2.9[c]		23.7 ± 3.3[c]		
Male % (n)	42.9% (n = 306)	Duration[a]	NR				
Montalcini et al. (2015)[28]							
Country	Italy	n	(n = 26)		(n = 26)	IL-6	5
Study design	Cross-sectional	Age[a]	32.6 ± 8.4[c]		30.5 ± 6.7[c]	TNF-α	
Study population (n)	Apparently healthy (n = 52)	BMI[b]	21.9 ± 2.0[c]		21.8 ± 2.0[c]	MCP-1	
Male % (n)	50% (n = 26)	Duration[a]	≥ 3				
Chuang et al. (2016)[29]							
Country	Taiwan	n	(n = 686)		(n = 3423)	CRP	7
Study design	Cross-sectional[h]	Age[a]	42.2 ± 12.3[c]		45.1 ± 12.2[c]		
Study population (n)	Apparently healthy (n = 4109)	BMI[b]	22.1 ± 3.1[c]		22.9 ± 3.2[c]		
Male % (n)	27.1% (n = 1115)	Duration[a]	≥ 3				
Kandouz et al. (2016)[30]							
Country	United Kingdom	n	(n = 16)		(n = 122)	CRP	5
Study design	Cross-sectional	Age[a]	71.7 ± 11.6[c]		63.7 ± 16.9[c]		
Study population (n)	Kidney failure (n = 138)	BMI[b]	25.4 ± 5.2[c]		26.5 ± 6.0[c]		
Male % (n)	63.9% (n = 88)	Duration[a]	early adult hood				
Lee et al. (2016)[31]							
Country	Taiwan	n	(n = 54)		(n = 100)	hs-CRP	4
Study design	Cross-sectional	Age[a]	65.1 ± 11.3[c]		57.7 ± 10.5[c]	IL-6	
Study population (n)	Type 2 diabetes (n = 154)	BMI[b]	24.9 ± 6.2[c]		26.5 ± 5.7[c]		
Male % (n)	41.5% (n = 64)	Duration[a]	≥ 1				
Ou et al. (2016)[32]							
Country	Taiwan	n	(n = 21)		(n = 42)	hs-CRP	4
Study design	Cross-sectional	Age[a]	56.2 ± 13.7[c]		56.3 ± 11.7[c]		
Study population (n)	Dialysis Patients (n = 63)	BMI[b]	20.4 ± 2.1[c]		22.5 ± 3.4[c]		
Male % (n)	22.6% (n = 12)	Duration[a]	≥ 1.5				
Acosta-Navarro et al. (2017)[33]							
Country	Brazil	n	(n = 44)		(n = 44)	hs-CRP	6
Study design	Cross-sectional	Age[a]	45.5 ± 7.8[c]		46.8 ± 9.6[c]		
Study population (n)	Apparently healthy (n = 88)	BMI[b]	23.1 ± 2.9[c]		27.2 ± 4.8[c]		
Male % (n)	100% (n = 88)	Duration[a]	17.8 ± 12.5[c]				
Franco-De-Moraes et al. (2017)[34]							
Country	Brazil	n	(n = 102)	(n = 66)	(n = 100)	CRP	5
Study design	Cross-sectional	Age[a]	49.6 ± 8.6[c]	49.6 ± 8.5[c]	49.1 ± 8.2[c]	E-selectin	
Study population (n)	Apparently healthy (n = 268)	BMI[b]	24.2 ± 3.9[c]	23.2 ± 4.1[c]	26.4 ± 4.7[c]	TNF-α	
Male % (n)	45.8% (n = 123)	Duration[a]	≥ 1	≥ 1			
Tseng et al. (2018)[35]							
Country	Taiwan	n	(n = 15)		(n = 140)	hs-CRP	5
Study design	Cross-sectional	Age[a]	63.2 ± 2.5[c]		58.5 ± 1.3[c]		
Study population (n)	Haemodialysis patients (n = 155)	BMI[b]	20.2 ± 0.5[c]		22.4 ± 0.2[c]		
Male % (n)	45.1% (n = 70)	Duration[a]	NR				
Ganie et al. (2019)[36]							

Continued

Study characteristics			Diet group characteristics			Studied biomarkers	SQS
			Vegetarians	Vegans	Omnivores		
Country	India	n	(n = 179)		(n = 141)	hs-CRP	7
Study design	Cross-sectional	Age[a]	26.5 ± 6.0[c]		26.6 ± 4.1[c]	IL-6	
Study population (n)	Apparently healthy (n = 320)	BMI[b]	24.0 ± 4.2[c]		24.0 ± 3.6[c]	TNF-α	
Male % (n)	0% (n = 0)	Duration[a]	NR			Adiponectin	
Study population (n)	PCOS (n = 144)	n	(n = 82)		(n = 62)	Resistin	
Male % (n)	0% (n = 0)	Age[a]	25.7 ± 3.8[c]		26.1 ± 4.4[c]		
		BMI[b]	24.9 ± 3.6[c]		24.6 ± 3.5[c]		
		Duration[a]	NR				
Menzel et al.(2020) [37]							
Country	Germany	n		(n = 36)	(n = 36)	hs-CRP	6
Study design	Cross-sectional	Age[a]		37.5 (32.5–44.0)[g]	38.5 (32.0–46.0)[g]	IL-18	
Study population (n)	Apparently healthy (n = 72)	BMI[b]		22.9 ± 3.2[c]	24.0 ± 2.1[c]	IL-1 RA	
Male % (n)	50% (n = 36)	Duration[a]		4.8 (3.1–8.7)[g]		ICAM-1	
						Adiponectin	
						Omentin-1	
						Resistin	

Table 1. Characteristics of reviewed studies on the association of vegan or vegetarian diet with inflammatory biomarkers. [a]Reported in years; [b] reported in kg/m^2; [c]expressed as mean ± SD; [d]mean ± SE; [e]mean ± SEM; [f] mean (95%-CI); [g] median (IQR); [h] using baseline values; [i] mean across both diet groups; SQS: Study Quality Score (using Newcastle—Ottawa Quality Assessment Scale adapted for cross-sectional studies); NR: not reported.

Selected studies were published between 1999[17] to 2020[37]. Numbers of study participants varied between n = 52[17,28] and n = 4109[29]. Overall, analysis comprised a total number of 2291 vegetarians, 111 vegans and 5868 omnivores, with a mean age of 46.2 years (vegans: 41.7 years; vegetarians: 49.6 years; omnivores: 47.2 years). The BMI was on average 23.1 kg/m^2 of all studies (not reported for Szeto et al.[19]) (vegans: 22.2 kg/m^2; vegetarians: 23.0 kg/m^2; omnivores: 23.9 kg/m^2). Of all participants, 89.8% had a healthy mean BMI of < 25.0 kg/m^2 and 10.2% had a mean BMI between 25.0 and 29.9 kg/m^2. No study included participants with mean BMI ≥ 30 kg/m^2.

The majority of the studies were conducted in apparently healthy participants comprising 88.2% of the participants (16 out of 21 studies, Table 1). Of note, Ganie et al.[36] investigated 320 healthy participants and 144 women with polycystic ovary syndrome (PCOS), both groups separated by diet (vegetarian vs. non-vegetarian). Hung et al.[23] and Lee et al.[27] conducted the study in a mixed population of apparently healthy participants and patients with metabolic syndrome (less than 20% of the study population). Moreover, six studies were conducted in participants diagnosed with impaired kidney function[26,30,32,35] , type 2 diabetes[31] or PCOS[36]. Seventeen studies used populations consisting of men and women, only Acosta-Navarra et al.[33] included exclusively male individuals, and three other studies included only female subjects[24,25,36]. In total, 38.2% of all involved participants were male. With regard to the outcome assessment, most of the studies focused on CRP (n = 20[17–27,29–37]), whereas a restricted number of studies evaluated IL-6 (n = 3[28,31,36]), TNF-α (n = 3[28,34,36]), adiponectin (n = 2[36,37]) or resistin (n = 2[36,37]). The other inflammatory biomarkers E-selectin (n = 1[34]), MCP-1 (n = 1[28]), IL-18, IL-1 RA, ICAM-1 and omentin-1 were only analyzed in one study[37].

Association of vegan diet and inflammatory biomarkers. In total, only 3 out of 21 studies investigated the association between a vegan diet and an omnivorous diet in respect to circulating CRP levels in apparently healthy individuals (Fig. 2A, Supplemental Table S1)[18,34,37]. Franco-De-Moraes et al. also investigated the association between a vegan diet and the inflammatory biomarker E-selectin and TNF-α[34]. A recent study provided new data regarding IL-18, IL-1 RA, ICAM-1, adiponectin, omentin-1 and resistin[37].

When results of three or more studies were available, a meta-analysis has been performed. Hence, in vegans a meta-analysis was only possible for CRP. As depicted in Fig. 2A, the present study observed lower CRP levels in vegans compared to omnivores, showing a mean difference (MD) between vegans and omnivores of − 0.54 mg/l, 95%-CI: − 0.79 to − 0.28 mg/l, $p < 0.0001$). Regarding E-selectin and TNF-α, Franco-De-Moraes et al.[34] observed no differences between vegans and omnivores (Table 2). Furthermore, Menzel et al.[37] observed no differences between vegans and omnivores with respect to IL-18, IL-1 RA, ICAM-1, adiponectin, omentin-1 and resistin (Table 2).

Association of vegetarian diet and inflammatory biomarkers. 19 out of 21 studies[17–27,29–36] investigated the association between vegetarian diet and CRP, compared to omnivores. In detail, 14 studies[17–25,27,29,33,34,36] were conducted in apparently healthy participants and six in diseased individuals, i.e. patients with impaired kidney function[26,30,32,35] , type 2 diabetes[31] or PCOS[36]. Moreover, in total four studies[28,31,34,36] investigated dif-

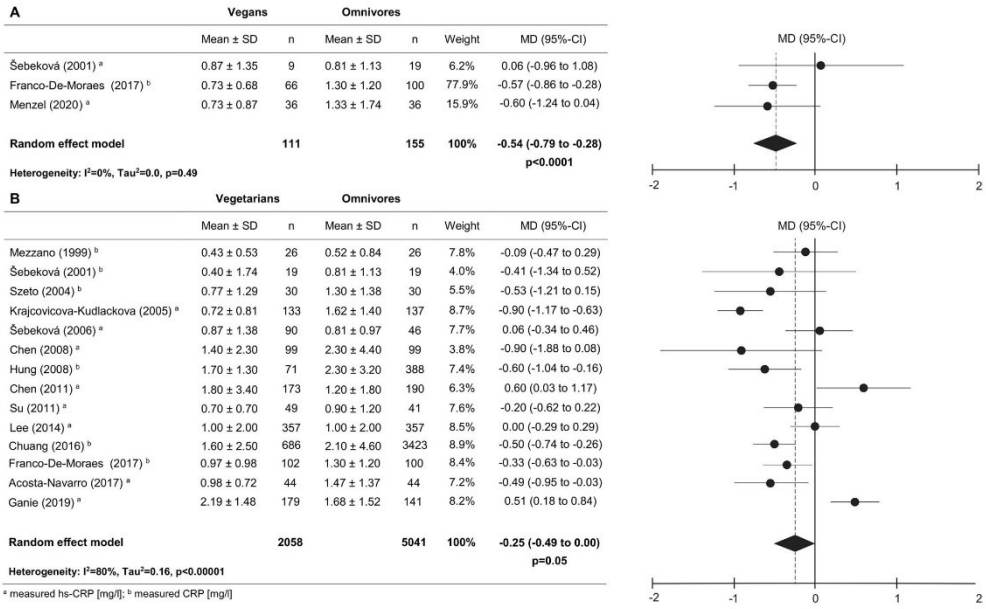

Figure 2. Forest plots of the effect of a vegan and vegetarian diet on CRP concentrations compared to omnivorous diet in apparently healthy participants. Forest plot showing the overall effect of a vegan diet (**A**) or a vegetarian diet (**B**) on CRP concentrations compared to omnivorous diet in apparently healthy participants. Results are presented as mean difference (MD) (95%-CI). The study-specific MD and 95%-CI are represented by the black dot and horizontal line, respectively. The center of the diamond and the vertical dashed line represent the overall effect size of all studies; the width of the diamond represents the overall pooled 95%-CI.

ferences on E-selectin, TNF-α, IL-6, MCP-1, restistin and adiponectin in vegetarians compared to omnivores (Table 2).

Among the different biomarkers, a meta-analysis was possible for CRP and TNF-α in apparently healthy individuals. Regarding TNF-α no significant differences between vegetarians and omnivores have been observed [mean difference 0.02 pg/ml, 95%-CI: − 0.39 to 0.43 pg/ml, $p = 0.93$]. However, meta-analysis revealed lower CRP levels in vegetarians compared to omnivores, showing a mean difference between vegetarians and omnivores of − 0.25 mg/l (95%-CI: − 0.49 to 0.00 mg/l; $p = 0.05$) (Fig. 2B). The generated funnel plot for CRP showed no evidence for publication bias (Supplemental Figure S1). In addition, also different statistical methods e.g. Egger regression or Funnel plot regression showed no evidence for publication bias (Supplemental Table S2). Interestingly, the association between vegetarian diet and CRP is much more pronounced in pre-diseased individuals with impaired kidney function − 3.91 mg/l (95%-CI: − 5.23 to − 2.60 mg/l; $p < 0.0001$) (Table 3, Supplemental Figure S2). With regard to E-selectin and MCP-1, no significant differences between apparently healthy vegetarians and omnivores have been observed (Table 2). Lower levels of IL-6 have been observed by Ganie et al. ($p < 0.01$)[36], and in some degree by Montalcini et al. ($p = 0.50$)[28] in apparently healthy vegetarians compared to omnivores.

Regarding pre-diseased participants, Lee et al. observed higher IL-6 levels in participants with type 2 diabetes following a vegetarian diet ($p = 0.01$)[31]. In participants with PCOS no differences were observed ($p = 0.91$)[36]. Furthermore, Ganie et al.[36] observed lower adiponectin levels in vegetarians compared to omnivores, however, less pronounced in healthy participants ($p = 0.05$) compared to participants with PCOS ($p < 0.001$). With regard to resistin, it has been observed that vegetarians have higher levels compared to omnivores, however significant in patients with PCOS only ($p < 0.001$), not in apparently healthy participants ($p = 0.16$)[36].

Sensitivity analysis. Mean difference (95%-CI) of CRP concentrations were performed, according to prespecified potential sources of heterogeneity of study quality, continent or duration of vegetarian diet. Studies were categorized into high-quality or low-quality studies using a cut-off point of 6 stars. Accordingly, with respect to vegetarian diet, eight studies scored < 6 stars; six studies scored ≥ 6 stars (Table 1, Supplemental Table S3). No relevant changes in results have been observed after stratifying by study quality (p for subgroup differences = 0.61, Supplemental Figure S3) or by continent (p for subgroup differences = 0.73, Supplemental Figure S4). Regarding duration of vegetarian diet (< 10 or ≥ 10 years), we observed no difference between both subgroups (p for subgroup differences = 0.35, Supplemental Figure S4). However, we observed lower CRP levels in vegetarians compared to omnivores when the duration of the diet was ≥10 years (-0.42 mg/l (95%-CI: -0.81 to -0.02 mg/l;

References	Study population (n)	*Biomarker values*			*p-value* [a]	*p-value* [b]
		Vegetarians	Vegans	Omnivores		
Adiponectin [ng/ml]						
Ganie et al. (2019)[36]	Apparently healthy (n = 320)	6.75 ± 4.45		7.20 ± 5.38	*0.05*	
Ganie et al. (2019)[36]	PCOS (n = 144)	3.15 ± 2.01		6.33 ± 2.81	*< 0.001*	
Menzel et al. (2020)[37]	Apparently healthy (n = 72)		4.76 ± 2.23	4.32 ± 1.57		*0.52*
E-selectin [pg/ml]						
Franco-De-Moraes et al. (2017)[34]	Apparently healthy (n = 268)	34.5 ± 20.6	33.2 ± 27.3	39.2 ± 23.5	*0.06*	*0.09*
ICAM-1 [ng/ml]						
Menzel et al. (2020)[37]	Apparently healthy (n = 72)		538.9 ± 97.2	569.9 ± 125.0		*0.36*
IL-6 [pg/ml]						
Montalcini et al. (2015)[28]	Apparently healthy (n = 52)	1.97 ± 2.80		1.52 ± 1.40	*0.50*	
Lee et al. (2016)[31]	Type 2 diabetes (n = 154)	2.50 ± 1.90		2.00 ± 1.70	*0.04*	
Ganie et al. (2019)[36]	Apparently healthy (n = 320)	8.44 ± 5.64		4.39 ± 3.94	*< 0.01*	
Ganie et al. (2019)[36]	PCOS (n = 144)	22.95 ± 14.40		19.86 ± 7.85	*0.91*	
IL-18 [pg/ml]						
Menzel et al. (2020)[37]	Apparently healthy (n = 72)		66.1 ± 55.3	89.4 ± 76.3		*0.20*
IL-1 RA [pg/ml]						
Menzel et al. (2020)[37]	Apparently healthy (n = 72)		268.5 ± 317.9	231.4 ± 133.3		*0.90*
MCP-1 [pg/ml]						
Montalcini et al. (2015)[28]	Apparently healthy (n = 52)	376.6 ± 138.2		320.9 ± 133.7	*0.17*	
Omentin-1 [ng/ml]						
Menzel et al.(2020)[37]	Apparently healthy (n = 72)		501.4 ± 163.5	505.0 ± 148.3		*0.92*
Resistin [ng/ml]						
Ganie et al. (2019)[36]	Apparently healthy (n = 320)	6.18 ± 3.69		5.48 ± 3.10	*0.16*	
Ganie et al. (2019)[36]	PCOS (n = 144)	10.84 ± 4.54		6.27 ± 2.28	*< 0.001*	
Menzel et al. (2020)[37]	Apparently healthy (n = 72)		6.85 ± 1.99	7.20 ± 1.75		*0.43*
TNF-α [pg/ml]						
Montalcini et al. (2015)[28]	Apparently healthy (n = 52)	2.41 ± 0.90		2.42 ± 1.10	*0.97*	
Franco-De-Moraes et al. (2017)[34]	Apparently healthy (n = 268)	3.13 ± 2.63	2.67 ± 1.44	3.10 ± 1.96	*0.48*	*0.17*
Ganie et al. (2019)[36]	Apparently healthy (n = 320)	24.18 ± 20.18		22.98 ± 16.42	*0.21*	
Ganie et al. (2019)[36]	PCOS (n = 144)	45.13 ± 35.74		35.47 ± 23.68	*0.91*	

Table 2. Summary of the concentrations of inflammatory biomarkers in vegetarians, vegan in comparison control group. [a]Vegetarian versus omnivore; [b]Vegan versus omnivore; Values expressed as mean ± SD.

	Vegetarians		Omnivores		Weight	MD (95%-CI)
	Mean ± SD	n	Mean ± SD	n		
A Impaired kidney function						
Wu et al. (2011)[26 b]	4.00 ± 0.30	19	8.80 ± 0.40	299	50.4%	− 4.80 (− 4.94 to − 4.66)
Ou et al. (2016)[32b]	6.70 ± 9.80	21	6.60 ± 11.2	42	5.3%	0.10 (− 5.29 to 5.49)
Kandouz et al. (2016)[30b]	4.53 ± 5.69	16	7.17 ± 8.25	122	13.0%	− 2.64 (− 5.79 to 0.51)
Tseng et al. (2018)[35a]	4.00 ± 1.64	15	7.70 ± 7.19	140	31.3%	− 3.70 (− 5.15 to − 2.25)
Random effect model		**71**		**603**	**100%**	**− 3.91 (− 5.23 to − 2.60)**
Heterogeneity: $I^2 = 58\%$, $Tau^2 = 0.89$, $p = 0.07$						*p < 0.0001*
B Type 2 diabetes						
Lee et al. (2016)[31a]	2.10 ± 2.60	54	1.50 ± 1.90	100		**0.60 (− 0.19 to 1.39)**
						p = 0.14
C Polycystic Ovary Syndrome						
Ganie et al. (2019)[36 a]	3.83 ± 1.68	82	2.38 ± 0.88	62		**1.45 (1.03 to 1.87)**
						p < 0.0001

Table 3. Summarized mean differences showing the overall effect of vegetarian diet on CRP concentrations compared to omnivorous diet in patients with impaired kidney function (A), type 2 diabetes (B) and PCOS (C). [a] measured hs-CRP [mg/l] ; [b]measured CRP [mg/l]; Results are presented as mean difference (MD) (95%-CI).

p=0.04). In contrast, the effect was less pronounced following the diet <10 years (-0.19 mg/l (95%-CI: -0.46 to 0.09 mg/l; p=0.18). In addition, meta-regression reveled no effect modification by BMI (all models $p > 0.05$).

Discussion

To the best of our knowledge, the present systematic review/ meta-analysis of cross-sectional studies is the most comprehensive evaluation, covering a wide spectrum of inflammatory biomarkers in vegans and vegetarians compared to omnivores, respectively. Accordingly, the present systematic review provides evidence that vegan and vegetarian diets are associated with lower CRP levels, a major marker of inflammation and a mediator of inflammatory processes. Of note, the association was stronger in pre-diseased vegetarians with impaired kidney function. No substantial effects were observed for IL-6, IL-18, IL-1 RA, TNF-α, E-selectin, ICAM-1, MCP-1, adiponectin, omentin-1 and resistin. However, with exception of CRP the most inflammatory biomarkers of interest were investigated only in single studies so far.

Given that CRP is an established biomarker of systemic low-grade inflammation linked to various diseases, e.g. atherosclerotic cardiovascular disease[38], the results of this review support the suggestion that vegetarian or vegan nutrition habits might ameliorate inflammatory processes and decrease circulating levels of inflammatory biomarkers. These anti-inflammatory properties might reduce risk of chronic inflammatory diseases in vegan or vegetarian populations. Our results are in line with other studies, suggesting an improvement in inflammatory profiles of plant-based/vegetarian-based diets indicated by decreases in CRP levels[14,16]. Moreover, Haghighatdoost et al. found a trend towards lower CRP concentrations in subjects following a vegetarian diet for at least 2 years[3], while no significant effect was found in studies using a minimum duration of 6 months of vegetarianism. In line with these observations, the present study revealed that the association might depend on the duration of the diet. According to the sensitivity analysis, the effect was more obvious in participants following a vegetarian diet of at least 10 years. Taken together, our findings provide evidence that a vegan or vegetarian diet may be beneficial to prevent or counteract inflammatory state underlying numerous chronic diseases and therefore might be a nutritional approach to reduce risk of chronic diseases. Moreover, adoption of a vegan or vegetarian diet may also have beneficial effects in pre-diseased populations. Next to the decreased level of CRP in vegetarian patients with impaired kidney function, a plant-based diet may hamper the development or progression of some complications of chronic kidney disease, due to the associated cardioprotective, anti-oxidant, and lipid-lowering properties[39]. Current evidence proposed this also for other cardiometabolic diseases, as vegetarian and vegan diets present potential advantages in managing type 2 diabetes offering additional benefits for the comorbidities of cardiovascular disease, kidney disease, and neuropathy[40].

Up to date, the mechanisms by which vegan or vegetarian diets might reduce the low-grade inflammatory state remain underestimated, but research holds great promise in revealing the mechanisms linking dietary patterns with inflammation. Accordingly, it has been suggested that exposure to animal foods may favor an intestinal environment which could trigger systemic inflammation[34]. Indeed some studies noticed differences in gut microbiota composition of vegans/vegetarian compared to omnivores, who differ according to their inflammatory and metabolic profiles[34,41]. However, a recent systematic review from 2019 noticed no consistent association between a vegan or vegetarian diet and microbiota composition compared to omnivores[42]. Interestingly, recent studies revealed the role in regulating of gut microbiota and gut homeostasis by inflammasomes[43]. These represent a group of protein complexes that recognize a diverse set of inflammation-inducing stimuli and promote the secretion of pro-inflammatory cytokines[44]. Those mechanisms may affect the immune homeostasis related to coinciding decrease in the future-risk for metabolic diseases, e.g. metabolic syndrome or atherosclerosis[44]. Although further research is clearly required, the role of inflammasomes in regulating of gut flora represents a new promising research field which may help elucidate the mechanisms by which diet impacts gut microflora, inflammation and health[41].

Despite 21 studies have been identified for inclusion in this review, many inflammatory biomarkers of interest were not investigated upon or were only explored in single studies. Thus, the present meta-analysis could not provide comprehensive conclusion about the associations between a vegan or vegetarian diet and each inflammatory marker. Therefore, more research is highly warranted to evaluate associations between a vegan or vegetarian diet and inflammatory biomarkers.

The present systematic review/meta-analysis has several strengths. Our study covered a comprehensive spectrum of biomarkers that reflect chronic inflammation and immune reactions, including a set of novel molecules at the site of adipose-tissue induced inflammatory response. Importantly, we focused on comprehensive nutritional vegan or vegetarian habits rather than on the use of single dietary supplements or artificial dietary approaches, which allows translation of the findings to general populations. In comparison to other meta-analyses[3,16], our study comprises no mixture of vegetarian and vegan diet, but performed strict separate analyses regarding vegans and vegetarians. Moreover, we investigated the association between biomarker profiles in apparently healthy and diseased patients, respectively.

Some limitations of our study deserve to be mentioned. RCTs are considered as the gold standard for establishing causal conclusions, however, published RCTs of vegan or vegetarian diets on inflammatory biomarkers based on our inclusion criteria are missing. Therefore, the present systematic review/ meta-analysis could include cross-sectional studies only, which does not allow for causal inference. The majority of the studies included a low number of participants. Furthermore, high heterogeneity among the studies in vegetarians was detected. It should be noted that our analysis is restricted by the data provided within the available studies each having its own methodological characteristics and potential drawbacks. In this respect, we should acknowledge the differences in the assay quality measurements and selection of investigated inflammatory biomarkers.

In conclusion, this systematic review and meta-analysis provide evidence that either vegan or vegetarian diet is associated with lower CRP concentrations compared to omnivores in apparently healthy participants and

metabolically afflicted patients. Further research is highly warranted, as several biomarkers of interest were only investigated in single studies so far.

Methods

Study protocol. The study was planned, conducted and reported according to the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. The study protocol was prospectively registered with the International Prospective Register of Systematic Reviews (PROSPERO; registration number CRD42018079220).

Data sources and search strategy. A systematic literature search was conducted to identify relevant articles using PubMed and Embase databases until 15th of April 2020. No restriction was made in terms of language or the date of study publications. The following search terms were used (MeSH term or text words): ('vegan', 'vegans', 'veganism' OR 'vegetarian', 'vegetarians', 'vegetarianism') AND ('adiponectin' OR 'c-reactive protein', 'CRP', 'hscrp', OR 'e-selectin' OR 'intercellular adhesion molecule-1', 'ICAM-1' OR 'interleukin-1 receptor antagonist', 'IL-1RA' OR 'interleukin-6', 'IL-6' OR 'interleukin-18', 'IL-18' OR 'monocyte chemoattractant protein-1', 'MCP-1' OR 'intelectin-1', 'omentin-1' OR 'resistin' OR 'TNF-alpha', 'TNF-α'). Additionally, reference lists of related original articles, review articles and meta-analyses were further screened for potentially eligible publications using a manual approach. When necessary, the relevant authors were contacted by the investigators to acquire important missing information; in case of non-respondents, studies were excluded.

Study selection and eligibility criteria. Studies were eligible for inclusion in the review if they reported results of vegetarianism or veganism, in comparison to a control group on circulating levels of inflammatory biomarkers in adults (aged ≥ 18 years). In detail, vegetarian diets were defined as meat, poultry, fish abstinence, and the partial exclusion of animal products such as eggs, and/or dairy products. Vegan diet implies the complete exclusion of any animal products (consumption less than one meal per month). Of note, one study mentioned participants as "strict vegetarian" which are considered as vegans in the present study[34]. Participants of the control groups were considered if they eat meat products (omnivorous western diet). The present review included all study designs, i.e. cross sectional studies, prospective cohort studies and RCT. Moreover, no specific criterion was considered for the duration of being on a vegan/vegetarian diet, except for RCT (an intervention time of at least 4 weeks was considered). Only studies published in English or German have been included in the present study. With regards to outcome assessment, our initial approach was to evaluate the existing literature on the well-known biomarkers of inflammation CRP, IL-6, IL-18, IL-1 RA, TNF-α, E-selectin, ICAM-1, MCP-1, adiponectin, omentin-1 and resistin. Studies were excluded if they assessed the effects of a general healthy lifestyle that included a vegan/vegetarian diet only as one component. Further, studies investigating a modified vegan or vegetarian diet (eg. raw, low-fat, low-carbohydrate, low-calorie, low-protein, gluten-free) were excluded. Two reviewers (JM and AJ) independently reviewed the titles and abstracts of all potential studies, followed by full-text selection. Disagreement was resolved by discussion and consensus, and if needed the opinion of a third author (CW) was decisive.

Data extraction and quality assessment. For each of the selected studies, the following information was recorded in the data extraction sheets. Study characteristics were extracted including first author's last name, publication year, country, study design, sample size, mean age, gender distribution (proportion of males), duration of being on a vegan/vegetarian diet, and inflammatory biomarker values for vegans/vegetarians and omnivores (mean ± SD). Proportion of males was calculated when not provided. If necessary, biomarker values have been transformed to mean and standard deviation (SD). For studies conducted in a diseased population, the disease was also extracted. If studies introduced additional interventions after a certain period of time, only values from the dietary intervention were extracted. Extracted data were converted to international units. As the inflammatory biomarker CRP has been measured as CRP or hsCRP in different studies, the manuscript refer both terms as CRP. Two reviewers evaluated study quality independently using the Newcastle—Ottawa Quality Assessment Scale adapted for cross-sectional studies[45]; if needed, the opinion of a third author (CW) was decisive.

Meta-analysis. To estimate the pooled effect of vegan and vegetarian diets compared to omnivorous diet on each respective inflammatory biomarker, the effect size was calculated using the differences between means (95%-CI) of inflammatory biomarkers concentrations (vegans vs. omnivores; vegetarians vs. omnivores), separated by health status (apparently healthy or diseased). Meta-analysis have been planned to perform when at least results of 3 studies were available. Summary estimates and corresponding 95%-CI were derived using a random-effects model, which assigns a weight to each study on the basis of an individual study's inverse variance. Consistency of results was evaluated based on calculation of I^2 index, also known as a 'heterogeneity index', which examines the null hypothesis that all studies are evaluating the same effect. To identify publication bias, a funnel plot was generated and examined; asymmetry was assessed by visual inspection. Further, publication bias was analyzed through Egger's regression, Begg rank correlation, funnel plot regression, and trim-and-fill tests using an available macro PubBias for SAS[46]. This was exclusively applied to meta-analyses with ≥ 10 studies. Last, sensitivity analysis for study quality was performed to investigate single studies as potential sources of heterogeneity including study quality score (< 6 or ≥ 6 stars), continents (Asia, Europe, South America) and duration of vegetarian diet (< 10 or ≥ 10 years). Of note, for Hung et al.[23] and Ganie et al.[36] the last classification was not possible, due to missing information on duration of vegetarian diet. Meta-regression analyses were performed examine whether there were differences in inflammatory biomarkers between dietary groups, controlling for

BMI (mean BMI or difference of BMI between diet groups). These analyses were performed whenever the number of studies was sufficient (n > 10). These analyses were performed using statistical software R (version 4.0.2) with the Meta-package. Other statistical analyses were performed using statistical software Review Manager (RevMan), Version 5.3. (Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboration, 2014) and SAS software, version 9.4 (SAS institute, Cary, N.C., USA). *p* values of < 0.05 were considered statistically significant. All modifications of figures were performed using Adobe Illustrator CC 2019.

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References

1. Alles, B. *et al.* Comparison of Sociodemographic and Nutritional Characteristics between Self-Reported Vegetarians, Vegans, and Meat-Eaters from the NutriNet-Sante Study. *Nutrients* **9**, doi:https://doi.org/10.3390/nu9091023 (2017).
2. Craig, W. J. Nutrition concerns and health effects of vegetarian diets. *Nutr. Clin. Pract.* **25**, 613–620. https://doi.org/10.1177/0884533610385707 (2010).
3. Haghighatdoost, F., Bellissimo, N., Totosy de Zepetnek, J. O. & Rouhani, M. H. Association of vegetarian diet with inflammatory biomarkers: a systematic review and meta-analysis of observational studies. *Public Health Nutr.* **20**, 2713–2721. https://doi.org/10.1017/S1368980017001768 (2017).
4. Lee, Y. & Park, K. Adherence to a vegetarian diet and diabetes risk: a systematic review and meta-analysis of observational studies. *Nutrients* https://doi.org/10.3390/nu9060603 (2017).
5. Kahleova, H., Levin, S. & Barnard, N. D. Vegetarian dietary patterns and cardiovascular disease. *Prog. Cardiovasc. Dis.* **61**, 54–61. https://doi.org/10.1016/j.pcad.2018.05.002 (2018).
6. Dinu, M., Abbate, R., Gensini, G. F., Casini, A. & Sofi, F. Vegetarian, vegan diets and multiple health outcomes: a systematic review with meta-analysis of observational studies. *Crit. Rev. Food Sci. Nutr.* **57**, 3640–3649. https://doi.org/10.1080/10408398.2016.1138447 (2017).
7. Liu, C. *et al.* Adiponectin, TNF-alpha and inflammatory cytokines and risk of type 2 diabetes: a systematic review and meta-analysis. *Cytokine* **86**, 100–109. https://doi.org/10.1016/j.cyto.2016.06.028 (2016).
8. Pearson, T. A. *et al.* Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* **107**, 499–511. https://doi.org/10.1161/01.cir.0000052939.59093.45 (2003).
9. Il'yasova, D. *et al.* Circulating levels of inflammatory markers and cancer risk in the health aging and body composition cohort. *Cancer Epidemiol. Biomarkers Prev.* **14**, 2413–2418. https://doi.org/10.1158/1055-9965.EPI-05-0316 (2005).
10. Li, S., Shin, H. J., Ding, E. L. & van Dam, R. M. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA* **302**, 179–188. https://doi.org/10.1001/jama.2009.976 (2009).
11. Shibata, R., Murohara, T. & Ouchi, N. Protective role of adiponectin in cardiovascular disease. *Curr. Med. Chem.* **19**, 5459–5466. https://doi.org/10.2174/092986712803833164 (2012).
12. Wei, T., Ye, P., Peng, X., Wu, L. L. & Yu, G. Y. Circulating adiponectin levels in various malignancies: an updated meta-analysis of 107 studies. *Oncotarget* **7**, 48671–48691. https://doi.org/10.18632/oncotarget.8932 (2016).
13. Barbaresko, J., Koch, M., Schulze, M. B. & Nothlings, U. Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. *Nutr. Rev.* **71**, 511–527. https://doi.org/10.1111/nure.12035 (2013).
14. Eichelmann, F., Schwingshackl, L., Fedirko, V. & Aleksandrova, K. Effect of plant-based diets on obesity-related inflammatory profiles: a systematic review and meta-analysis of intervention trials. *Obes. Rev.* **17**, 1067–1079. https://doi.org/10.1111/obr.12439 (2016).
15. Neale, E. P., Batterham, M. J. & Tapsell, L. C. Consumption of a healthy dietary pattern results in significant reductions in C-reactive protein levels in adults: a meta-analysis. *Nutr. Res.* **36**, 391–401. https://doi.org/10.1016/j.nutres.2016.02.009 (2016).
16. Craddock, J. C., Neale, E. P., Peoples, G. E. & Probst, Y. C. Vegetarian-based dietary patterns and their relation with inflammatory and immune biomarkers: a systematic review and meta-analysis. *Adv. Nutr.* **10**, 433–451. https://doi.org/10.1093/advances/nmy103 (2019).
17. Mezzano, D. *et al.* Vegetarians and cardiovascular risk factors: hemostasis, inflammatory markers and plasma homocysteine. *Thromb. Haemost.* **81**, 913–917 (1999).
18. Sebekova, K. *et al.* Plasma levels of advanced glycation end products in healthy, long-term vegetarians and subjects on a western mixed diet. *Eur. J. Nutr.* **40**, 275–281. https://doi.org/10.1007/s394-001-8356-3 (2001).
19. Szeto, Y. T., Kwok, T. C. & Benzie, I. F. Effects of a long-term vegetarian diet on biomarkers of antioxidant status and cardiovascular disease risk. *Nutrition* **20**, 863–866. https://doi.org/10.1016/j.nut.2004.06.006 (2004).
20. Krajcovicova-Kudlackova, M. & Blazicek, P. C-reactive protein and nutrition. *Bratisl Lek Listy* **106**, 345–347 (2005).
21. Sebekova, K. *et al.* Association of metabolic syndrome risk factors with selected markers of oxidative status and microinflammation in healthy omnivores and vegetarians. *Mol. Nutr. Food Res.* **50**, 858–868. https://doi.org/10.1002/mnfr.200500170 (2006).
22. Chen, C. W. *et al.* Total cardiovascular risk profile of Taiwanese vegetarians. *Eur. J. Clin. Nutr.* **62**, 138–144. https://doi.org/10.1038/sj.ejcn.1602689 (2008).
23. Hung, K. C. *et al.* The comparison of the metabolic syndrome between Chinese vegetarians and omnivores. *Diabetes Metab. Syndr. Clin. Res. Rev.* **2**, 99–104. https://doi.org/10.1016/j.dsx.2008.02.002 (2008).
24. Chen, C. W., Lin, C. T., Lin, Y. L., Lin, T. K. & Lin, C. L. Taiwanese female vegetarians have lower lipoprotein-associated phospholipase A2 compared with omnivores. *Yonsei Med. J.* **52**, 13–19. https://doi.org/10.3349/ymj.2011.52.1.13 (2011).
25. Su, T. C., Torng, P. L., Jeng, J. S., Chen, M. F. & Liau, C. S. Arterial function of carotid and brachial arteries in postmenopausal vegetarians. *Vasc. Health Risk Manag.* **7**, 517–523. https://doi.org/10.2147/VHRM.S18881 (2011).
26. Wu, T. T. *et al.* Nutritional status of vegetarians on maintenance haemodialysis. *Nephrology (Carlton)* **16**, 582–587. https://doi.org/10.1111/j.1440-1797.2011.01464.x (2011).
27. Lee, C. G. *et al.* Vegetarianism as a protective factor for colorectal adenoma and advanced adenoma in Asians. *Dig. Dis. Sci.* **59**, 1025–1035. https://doi.org/10.1007/s10620-013-2974-5 (2014).
28. Montalcini, T. *et al.* High vegetable fats intake is associated with high resting energy expenditure in vegetarians. *Nutrients* **7**, 5933–5947. https://doi.org/10.3390/nu7075259 (2015).
29. Chuang, S. Y. *et al.* Vegetarian diet reduces the risk of hypertension independent of abdominal obesity and inflammation: a prospective study. *J. Hypertens.* **34**, 2164–2171. https://doi.org/10.1097/HJH.0000000000001068 (2016).
30. Kandouz, S., Mohamed, A. S., Zheng, Y., Sandeman, S. & Davenport, A. Reduced protein bound uraemic toxins in vegetarian kidney failure patients treated by haemodiafiltration. *Hemodial. Int.* **20**, 610–617. https://doi.org/10.1111/hdi.12414 (2016).

31. Lee, Y. J., Wang, M. Y., Lin, M. C. & Lin, P. T. Associations between Vitamin B-12 status and oxidative stress and inflammation in diabetic vegetarians and omnivores. *Nutrients* **8**, 118. https://doi.org/10.3390/nu8030118 (2016).
32. Ou, S. H. *et al.* Potential role of vegetarianism on nutritional and cardiovascular status in Taiwanese dialysis patients: a case-control study. *PLoS ONE* **11**, e0156297. https://doi.org/10.1371/journal.pone.0156297 (2016).
33. Acosta-Navarro, J. *et al.* Reduced subclinical carotid vascular disease and arterial stiffness in vegetarian men: the CARVOS study. *Int. J. Cardiol.* **230**, 562–566. https://doi.org/10.1016/j.ijcard.2016.12.058 (2017).
34. Franco-de-Moraes, A. C. *et al.* Worse inflammatory profile in omnivores than in vegetarians associates with the gut microbiota composition. *Diabetol. Metab. Syndr.* **9**, 62. https://doi.org/10.1186/s13098-017-0261-x (2017).
35. Tseng, C. Y. *et al.* Vegetarian diet may ameliorate uremic pruritus in hemodialysis patients. *Ren. Fail.* **40**, 514–519. https://doi.org/10.1080/0886022x.2018.1512871 (2018).
36. Ganie, M. A. *et al.* Comparative evaluation of biomarkers of inflammation among indian women with polycystic ovary syndrome (PCOS) consuming vegetarian vs. non-vegetarian diet. *Front. Endocrinol. (Lausanne)* **10**, 699. https://doi.org/10.3389/fendo.2019.00699 (2019).
37. Menzel, J. *et al.* Associations of a vegan diet with inflammatory biomarkers. *Sci. Rep.* **10**, 1933. https://doi.org/10.1038/s41598-020-58875-x (2020).
38. Nakou, E. S., Liberopoulos, E. N., Milionis, H. J. & Elisaf, M. S. The role of C-reactive protein in atherosclerotic cardiovascular disease: an overview. *Curr. Vasc. Pharmacol.* **6**, 258–270. https://doi.org/10.2174/157016108785909733 (2008).
39. Gluba-Brzozka, A., Franczyk, B. & Rysz, J. Vegetarian diet in chronic kidney disease-a friend or foe. *Nutrients* https://doi.org/10.3390/nu9040374 (2017).
40. Trapp, C. B. & Barnard, N. D. Usefulness of vegetarian and vegan diets for treating type 2 diabetes. *Curr. Diab. Rep.* **10**, 152–158. https://doi.org/10.1007/s11892-010-0093-7 (2010).
41. Glick-Bauer, M. & Yeh, M. C. The health advantage of a vegan diet: exploring the gut microbiota connection. *Nutrients* **6**, 4822–4838. https://doi.org/10.3390/nu6114822 (2014).
42. Trefflich, I. *et al.* Is a vegan or a vegetarian diet associated with the microbiota composition in the gut? Results of a new cross-sectional study and systematic review. *Crit. Rev. Food Sci. Nutr.* https://doi.org/10.1080/10408398.2019.1676697 (2019).
43. Chen, G. Y. Regulation of the gut microbiome by inflammasomes. *Free Radic. Biol. Med.* **105**, 35–40. https://doi.org/10.1016/j.freeradbiomed.2016.11.011 (2017).
44. Strowig, T., Henao-Mejia, J., Elinav, E. & Flavell, R. Inflammasomes in health and disease. *Nature* **481**, 278–286. https://doi.org/10.1038/nature10759 (2012).
45. Herzog, R. *et al.* Are healthcare workers' intentions to vaccinate related to their knowledge, beliefs and attitudes? A systematic review. *BMC Public Health* **13**, 154. https://doi.org/10.1186/1471-2458-13-154 (2013).
46. Rendina-Gobioff, G. & Kromrey, J. PUB_BIAS: A SAS® Macro for Detecting Publication Bias in Meta-Analysis. University of South Florida, Tampa, FL. https://analytics.ncsu.edu/sesug/2006/PO2004_2006.PDF (Accessed September 2004, 2020) (2006).

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Conceived and designed the study: J.M., C.W.; Review study selection: J.M., A.J., C.W.; Interpretation of data: J.M., A.J., R.B., K.M., K.A., C.W.; Statistical analyses: J.M.; Supervision of the project: C.W., K.A.; Draft of the manuscript: J.M., A.J.; Intellectually contribution to the manuscript: J.M., A.J., R.B., K.M., K.A., and C.W. All authors reviewed the manuscript.

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2.2. Vegane Ernährung und Knochengesundheit

2.2.1 Unterschiede in der Knochengesundheit zwischen Veganer:innen und Mischköstler:innen und Ableitung eines explorativen Biomarkermusters mit Einfluss auf die Knochengesundheit

***Menzel J**, Abraham K, Stangl GI, Ueland PM, Obeid R, Schulze MB, Herter-Aeberli I, Schwerdtle T, Weikert C, Vegan Diet and Bone Health—Results from the Cross-Sectional RBVD Study, Nutrients, 2021 Feb 21;13(2):685. doi: 10.3390/nu13020685.*

Die Ergebnisse der Originalpublikation werden im Folgenden zusammengefasst und können dem Abstract der Publikation ähneln. Die deutsche Übersetzung erfolgte durch die Autorin.

Neben den beschriebenen vorteilhaften Auswirkungen einer veganen Ernährung auf die Gesundheit, wird auch berichtet, dass eine vegane Ernährungsform in Hinblick auf die Knochengesundheit negative Auswirkungen haben könnte [46].

In der vorliegenden Originalarbeit wurden daher die Unterschiede der Knochengesundheit zwischen Veganer:innen und Mischköstler:innen in der RBVD-Studie untersucht [46]. Als Ergebnis zeigte sich, dass Veganer:innen im Vergleich zur Mischkost, in allen Ultraschall-Parametern niedrigere Werte hatten [46]. Dabei erreichte jedoch nur der wichtigste untersuchte Parameter (BUA) statistische Signifikanz (Vegan (Mittelwert (MW) ± Standardabweichung (SD): 111.8 ± 10.7 dB/MHz, Mischkost (MW ± SD): 118.0 ± 10.8 dB/MHz, p=0.02) [46].

Zudem zeigten sich Unterschiede zwischen Veganer:innen und Mischköstler:innen in wichtigen ernährungsbedingten und knochenrelevanten Biomarkern (Blut und Urin) [46]. So hatten Veganer:innen im Vergleich zur Mischkostgruppe höhere Konzentrationen des Knochenresorptionsmarker C-terminale Crosslinks (CTX), α-Klotho, der Vitamine K1 und Folat, der Aminosäure Glutamin, sowie eine Tendenz zu höheren Parathormon (PTH) Konzentrationen [46]. Die Konzentrationen der Vitamine A und B2, der Aminosäure Lysin, Zink, Selenoprotein P und Omega-3-Fettsäuren, sowie die Calcium- und Jodausscheidung waren hingegen niedriger [46].

Es wurde durch die Anwendung der RRR ein exploratives Biomarkermuster mit ernährungsbedingten und knochenrelevanten Biomarkern generiert, welches die Varianz von BUA und SOS maximal erklärt [46]. Nach Anwendung einer RRR mit insgesamt 28 Biomarkern, konnte festgestellt werden, dass 12 Biomarker in Kombination am stärksten die Varianz für BUA und SOS erklären [46]. Der berechnete Biomarkermuster-Score erklärt dabei insgesamt 34.4% der Gesamtvarianz für BUA und SOS [46]. Das Muster beinhaltet dabei die Mitwirkung folgender Biomarker mit positiven Faktorladungen für Lysin, Jod im Urin,

schilddrüsenstimulierendes Hormon (TSH), Selenoprotein P, Vitamin A, Leucin, α-klotho, Omega-3-Fettsäuren, Calcium und Magnesium im Urin, Vitamin B6 und negative Faktorladung für FGF23 [46]. Eine Varianzanalyse über Tertile des Biomarkermuster-Scores zeigte dabei, dass die Teilnehmer:innen in der dritten Tertile (T3) im Mittel 11% höhere BUA-Werte hatten, als im Vergleich zur ersten Tertile (T1) (p für Trend<0.0001) [46]. Außerdem zeigte sich auch ein Anstieg über die Tertile für SOS (2.6%) sowie SI (18.5%) [46]. Der prozentuale Anteil der Veganer:innen nahm hingegen mit steigender Tertile des Biomarkermuster-Scores ab (T1: 70%, T2: 61%, T3: 26%) [46].

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Vegan Diet and Bone Health—Results from the Cross-Sectional RBVD Study

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Abstract: Scientific evidence suggests that a vegan diet might be associated with impaired bone health. Therefore, a cross-sectional study ($n = 36$ vegans, $n = 36$ omnivores) was used to investigate the associations of veganism with calcaneal quantitative ultrasound (QUS) measurements, along with the investigation of differences in the concentrations of nutrition- and bone-related biomarkers between vegans and omnivores. This study revealed lower levels in the QUS parameters in vegans compared to omnivores, e.g., broadband ultrasound attenuation (vegans: 111.8 ± 10.7 dB/MHz, omnivores: 118.0 ± 10.8 dB/MHz, $p = 0.02$). Vegans had lower levels of vitamin A, B2, lysine, zinc, selenoprotein P, n-3 fatty acids, urinary iodine, and calcium levels, while the concentrations of vitamin K1, folate, and glutamine were higher in vegans compared to omnivores. Applying a reduced rank regression, 12 out of the 28 biomarkers were identified to contribute most to bone health, i.e., lysine, urinary iodine, thyroid-stimulating hormone, selenoprotein P, vitamin A, leucine, α-klotho, n-3 fatty acids, urinary calcium/magnesium, vitamin B6, and FGF23. All QUS parameters increased across the tertiles of the pattern score. The study provides evidence of lower bone health in vegans compared to omnivores, additionally revealing a combination of nutrition-related biomarkers, which may contribute to bone health. Further studies are needed to confirm these findings.

Keywords: bone health; BUA; SOS; QUS; vegan; diet; biomarker; reduced rank regression; RRR

1. Introduction

In recent years, plant-based diets have become increasingly popular in Germany and many other Western countries [1,2]. In particular, a growing trend toward a vegan diet has been observed, referring to a diet without consumption of any animal products. People are turning to a vegan diet not only due to compassion for animals and awareness of environmental problems but also for health benefits [1]. Indeed, scientific evidence suggests that a vegan or vegetarian diet may protect against many chronic diseases, e.g., diabetes [3],

cardiovascular diseases [4], or cancer [5]. However, a vegan diet was found to be associated with lower bone mineral density (BMD), which is associated with higher fracture risk, compared to omnivores [6]. The skeleton is a dynamic and metabolically active tissue [7] and is exquisitely sensitive to its microenvironment [8]. Accordingly, nutritional habits have been considered an important modifiable factor influencing BMD [8,9]. Consuming a vegan diet arises concern about an inadequate supply of some nutrients [10], possibly contributing to an impaired BMD in vegans. For instance, calcium and vitamin D are well known as major determinants of bone health [9], but they are considered as potential critical nutrients in vegans [10]. Other critical nutrients for a vegan diet are long-chain n-3 fatty acids [10], vitamins (B12, A) [10], or minerals (zinc, selenium, iodine) [10], which are also related to bone health [8]. On the other hand, vegetarian and vegan diets provide important nutrients that protect bone, e.g., vitamin K [7,8,11] and folate [7,12–14].

Therefore, the present study aimed to investigate the differences in bone health between vegans and omnivores, as measured using quantitative ultrasound. Furthermore, the study aimed to detect differences in nutritional biomarkers that are related to bone health (selected vitamins, minerals, fatty acids, and amino acids), along with differences in biomarkers of bone turnover, calcium homeostasis, inflammation, and the fibroblast growth factor 23 (FGF23)–α-klotho axis. In addition, via the application of reduced rank regression (RRR), the study aimed to detect an exploratory biomarker pattern that may reveal a combination of biomarkers that contribute to bone health and thereby may explain the suggested reduced bone health in vegans. As is known, classic endocrine feedback loops ensure the regulation of blood calcium alongside the involvement of parathyroid hormone (PTH), vitamin D, and FGF23 [15] having their own impacts on bone health; thus, the complexity of the homeostatic regulatory biomarkers of bones should be considered, too. Therefore, the RRR included not only classical nutritional biomarkers but also other important nutrition-associated bone-related biomarkers.

2. Materials and Methods

2.1. Study Population

The study participants were investigated between January 2017 and July 2017. Participants of the present "Risks and Benefits of a Vegan Diet" (RBVD) study were individuals who responded to an advertisement by contacting the study center at the German Federal Institute for Risk Assessment (BfR) via phone or mail (n = 161). A phone screening followed, including a brief explanation of the study and checking the inclusion criteria (age 30–60 years, following the diet for at least 1 year) and exclusion criteria (body mass index (BMI) $\geq$ 30, cardiovascular disease, type 2 diabetes, cancer, pregnancy, breastfeeding, current infection) [16]. An omnivorous diet was defined as the consumption of at least three portions of meat per week or two portions of meat and two portions of processed meat per week, whereas a vegan diet was defined as no consumption of any animal food products [16]. The cross-sectional study was conducted at the BfR in Berlin, Germany. Each participant visited the study center twice [16]. On their first visit, participants gave their written informed consent and received instructions to collect 24 h urine and to document their diet using a three-day weighed food protocol. At the second visit, anthropometric measurements, a quantitative ultrasound measurement, and their lifestyle characteristics were assessed, and a fasting blood sample was collected [16]. In total, the final study population comprised 36 vegans and 36 omnivores that were sex- and age-matched. A flowchart was published previously [16]. The study was approved by the Ethics Committee of Charité University Medical Center Berlin (no. EA4/121/16) and was conducted in accordance with the Declaration of Helsinki.

2.2. Quantitative Ultrasound Measurement

In our study, bone health was assessed using quantitative ultrasound (QUS) measurements. According to the manufacturer's instructions, QUS measurements were performed by trained personnel on the right and left os calcis using the Achilles EXPII bone ultra-

sonometer (General Electric Healthcare, Little Chalfont, UK). In the case of unilateral foot pathology (ankle edema, trauma, or fracture) of a heel, only the opposite heel was measured. The instrument measures the frequency-dependent broadband ultrasound attenuation (BUA) (dB/MHz) and the speed of sound (SOS) (m/s). The stiffness index (SI) was automatically calculated from the BUA and SOS using the Achilles EXPII system via the following equation: stiffness index = (0.67 × BUA + 0.28 × SOS) − 420 [17]. The mean values of quantitative ultrasound measurements were calculated from the left and right heel measurements, except in four participants, where only one heel site measurement was available. Due to the anatomical conditions of the feet, the measurement for one participant was not possible.

2.3. Assessment of Lifestyle Characteristics

Anthropometric measurements (weight, height, and waist circumference) were performed by trained and quality-monitored personnel while the participants wore only light underwear and had no shoes on. Body weight was assessed using an electronic digital scale (Omron BF511 Omron Healthcare Ltd., Kyoto, Japan) and the height was measured using a flexible anthropometer (SECA 213, Hamburg, Germany). Waist circumference was defined using the horizontal plane midway between the lowest ribs and the iliac crest. Information on educational level, smoking habits, and supplement intake was assessed using computer-based questionnaires. The educational levels were defined as high education (university, university of applied sciences), intermediate education (vocational school, technical college), or low education (no degree). Physical activity was determined using a validated physical activity questionnaire [18]. Physical activity comprised the sum of the average hours per week spent in cycling, sports, and gardening during summer and winter. Walking included the sum of the average hours per week during summer and winter.

2.4. Blood and Urine Collection and Laboratory Analysis

About 60 mL of venous blood was collected from each participant at the BfR study center. Several routine biomarkers, including serum concentration of alkaline phosphatase, high-sensitivity C-reactive protein (hsCRP), thyroid-stimulating hormone (TSH), zinc, and total homocysteine (using NaF blood), were measured at an accredited medical analytics laboratory (Labor 28 GmbH, Berlin, Germany) on the same day. About half of the blood was fractionated into serum/plasma and erythrocytes and stored at −80 °C until further analysis. In 2018, Labor 28 determined the bone turnover biomarker b-CrossLaps (CTX) and osteocalcin in the serum. Due to an implausibly high value of CTX in a participant, one measurement was not considered in the present study. Serum concentrations of procollagen type-1 (PINP) were measured at Labor Augsburg MVZ GmbH (Augsburg, Germany). Methylmalonic acid and vitamins A, B2, B6, D3, and K1, as well as amino acids alanine, arginine, glutamine, leucine, lysine, and proline were measured in plasma at Bevital AS (Bergen, Norway). Plasma concentrations of fibroblast growth factor 23 (FGF23), α-klotho, and PTH were measured at the Institute of Agricultural and Nutritional Sciences, Martin Luther University Halle-Wittenberg (Halle, Germany). Serum concentrations of holotranscobalamin, vitamin B12, and folate were determined at the Department of Clinical Chemistry and Laboratory Medicine, University of Saarland (Homburg, Germany). Serum concentrations of selenium and selenoprotein P (SePP) were measured at the Institute of Nutritional Science, University of Potsdam (Potsdam, Germany). Fatty acids in plasma phospholipids were determined at the Department of Molecular Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke (Germany) [19]. The total n-3 fatty acids included the sum of linolenic acid (C18:3n3), eicosapentaenoic acid (C20:5n3), docosapentaenoic acid (C22:5n3), and docosahexaenoic acid (C22:6n3). We calculated the combined vitamin B12 indicator (4cB12) from concentrations of holotranscobalamin, vitamin B12, total homocysteine, and methylmalonic acid according to the published equation (adjusted for age) [20]. The participants collected their urine over 24 h before their second visit to the study center. Concentrations of calcium and magnesium in 24 h urine were

measured at Labor 28 GmbH (Berlin, Germany) and concentrations of urinary iodine at the Laboratory of Human Nutrition, Institute of Food, Nutrition and Health, ETH Zurich (Switzerland) [21].

2.5. Sample Size Estimation

The sample size was calculated by assuming a clinically relevant difference of 5% in BUA between vegans and omnivores. Along with a level of significance of 5% and a power of 80%, a total of 72 participants were required (36 vegans, 36 omnivores) (G*power, (*t*-test for independent samples), version 3.1., Heinrich Heine University, Dusseldorf, Germany).

2.6. Statistical Analysis

Normally distributed variables are reported as mean ± standard derivation. Skewed variables are reported as median (interquartile range). Categorical variables are reported as *n* (percentages). A Student's *t*-test or Mann–Whitney *U* test was used to compare the continuous variables between vegans and omnivores, and a chi-square test was used for categorical variables. The RRR was described in detail by Hoffmann et al., including the SAS software code and its application in nutritional epidemiology [22]. RRR appears to be a promising tool for characterizing the relationships between bone health and a comprehensive profile of biomarkers. The RRR determines the linear combinations of predictor variables (biomarkers) that explain a maximum variation in the response variables (BUA and SOS). In this analysis, we used 28 bone-relevant biomarkers as predictor variables. In detail, we included the nutritional biomarkers, i.e., vitamins (combined vitamin B12 indicator, A, B6, B2, K1, folate), amino acids (alanine, arginine, glutamine, leucine, lysine, proline), total n-3 fatty acids, zinc, SePP, urinary magnesium, urinary iodine, TSH, along with biomarkers of calcium homeostasis (PTH, vitamin D3, urinary calcium), biomarkers of bone turnover (CTX, PINP, osteocalcin, alkaline phosphatase), biomarkers of the FGF23–α-klotho axis (α-klotho, FGF23), and the inflammatory biomarker hsCRP. Due to missing values of BUA (n = 1) and CTX (n = 1), the RRR analysis comprised 70 participants (36 vegans, 34 omnivores). All skewed variables were log-transformed for the analyses. As the number of response variables determines the number of extracted patterns, the current RRR created two patterns. To ensure that the observed variation of bone-relevant biomarkers reflected the different profiles of vegans and omnivores, the RRR patterns were derived using the pooled data of vegans and omnivores. Only the first pattern was retained for the analyses, as this pattern contributed the largest proportion of explained variance (first pattern: 34.4%, second pattern: 5.3%). The description of the bone-health-related pattern focused on those predictors with factor loadings ≥0.20, which were considered the main contributors of a score. Each participant received a factor score for the identified pattern; this score ranked the participants according to the degree to which they conformed to the pattern. Distributions of the main contributors were compared across tertiles of the pattern scores, and analysis of variance (ANOVA) was used to assess the linear trends. Investigating the main contributors across tertiles of the pattern scores, additional analyses were carried out using a multivariable-adjusted analysis of covariance (ANCOVA), including the additional adjustment of the month of assessment (January–July, model 1), a sex- and age-adjusted model 2, as well as a lifestyle model 3 (BMI, smoking status, physical activity, alcohol consumption). Moreover, sensitivity analyses were performed after the exclusion of postmenopausal women (n = 6) and one woman with surgical menopause. Linear regression models were used to estimate the associations between diet groups (vegan/omnivores) with BUA (unadjusted, model 1) and adjusted for lifestyle factors (model 2), including age, sex, smoking status, educational level, BMI, physical activity, and alcohol consumption. Models 3 and 4 were adjusted for the biomarker pattern score, while model 4 was additionally for lifestyle factors. All statistical analyses were performed using SAS software, version 9.4 (SAS Institute, Cary, NC, USA). Any p-values < 0.05 were considered statistically significant.

3. Results

Table 1 shows the basic characteristics of the 72 participants according to a vegan or omnivorous diet (n = 36 each). The median duration of veganism was 4.8 years (IQR: 3.1–8.7). Due to sex- and age-matched inclusion of the participants, we observed no differences in sex and age (Table 1). Moreover, no differences in anthropometric measurements, physical activity, smoking, education, or alcohol consumption were observed between the groups (all $p > 0.05$). However, compared to omnivores (33.3%), vegans (97.2%) were more likely to take supplements, especially supplements of vitamin B12 (91.7%).

Table 1. Characteristics of the study population according to a vegan or omnivorous diet.

Characteristics	Vegans (n = 36)	Omnivores (n = 36)	p-Value
Duration vegan diet (years)	4.8 (3.1–8.7)		
Men	50.0% (18)	50.0% (18)	1.00
Age (years)	37.5 (32.5–44.0)	38.5 (32.0–46.0)	0.75
Anthropometry			
BMI (kg/m^2)	22.9 ± 3.2	24.0 ± 2.1	0.08
Fat mass (%)	24.1 ± 7.8	26.2 ± 7.7	0.27
Muscle mass (%)	33.9 ± 5.2	33.5 ± 5.2	0.72
Waist circumference (cm)			
Women	73.1 ± 6.9	77.2 ± 6.2	0.07
Men	84.5 ± 8.9	86.0 ± 6.1	0.56
Education (%)			0.60
Low	0.0% (0)	2.8% (1)	
Intermediate	30.6% (11)	30.6% (11)	
High	69.5% (25)	66.7% (24)	
Lifestyle			
Physical activity (h/week)	2.8 (0.88–3.75)	2.3 (1.2–4.1)	0.69
Walking (h/week)	7.0 (5.0–12.0)	5.5 (3.5–11.8)	0.15
Smoking status			0.30
Non-smoker	66.7% (24)	58.3% (21)	
Ex-smoker	22.2% (8)	16.7% (6)	
Smoker	11.1% (4)	25.0% (9)	
Alcohol consumption (g/d)			
Women	0.10 (0.00–4.69)	0.21 (0.02–4.88)	0.22
Men	0.04 (0.00–2.00)	3.85 (0.36–16.2)	0.09
Taking supplements	97.2% (35)	33.3% (12)	<0.0001
Vitamin B12	91.7% (33)	8.3% (3)	<0.0001
Vitamin D3	50.0% (18)	11.1% (4)	0.0003

Variables expressed as percentage (n), mean ± SD, or median (IQR). BMI: body mass index.

Compared to omnivores, vegans showed lower mean values of all QUS parameters (Table 2). However, only the difference in BUA levels reached statistical significance (vegans: 111.8 ± 10.7 dB/MHz, omnivores: 118.0 ± 10.8 dB/MHz, p = 0.02). In addition, a regression revealed that omnivores had 6.2 dB/MHz higher BUA levels compared to vegans (p = 0.02, model 1, Table S1), and the association was even stronger after adjusting for lifestyle factors (model 2, Table S1). The bone resorption marker CTX was higher in vegans (0.45 ± 0.19 ng/mL) compared to omnivorous participants (0.36 ± 0.16 ng/mL, p = 0.03). Concerning the calcium homeostasis, vegans had lower urinary calcium levels (p = 0.004) and were more likely to have higher PTH concentrations compared to omnivores (p = 0.09). Moreover, vegans had higher α-klotho concentrations than omnivores (p = 0.01). Omnivores had higher concentrations of vitamin A and B2, whereas vegans showed higher concentrations of vitamin K1 and folate. The concentrations of vitamin B12 and B6 did not differ between the dietary groups. Vegans had higher concentrations of glutamine and lower concentrations of lysine compared to omnivores ($p < 0.0001$, Table 2), whereas there were no differences in the other amino acids (i.e., alanine, arginine, leucine, and proline).

Moreover, vegans had a lower level of urinary iodine compared to omnivores ($p < 0.0001$), while the TSH concentration ($p = 0.34$) did not differ. Furthermore, vegans had lower concentrations of zinc ($p = 0.03$), SePP ($p < 0.0001$), and total n-3 fatty acids ($p < 0.0001$).

Table 2. Characteristics of bone parameters and biomarkers according to a vegan or omnivorous diet.

Characteristics	Vegans (n = 36)	Omnivores (n = 36)	p-Value
Quantitative ultrasound			
BUA (dB/MHz) [a]	111.8 ± 10.7	118.0 ± 10.8	0.02
SOS (m/s) [a]	1581.7 ± 28.3	1592.3 ± 9.27	0.20
SI [a]	97.3 ± 13.3	104.3 ± 16.9	0.05
Bone turnover			
CTX (ng/mL) [a]	0.45 ± 0.19	0.36 ± 0.16	0.03
Osteocalcin (ng/mL)	20.8 ± 5.49	18.2 ± 6.83	0.08
PINP (µg/L)	60.7 ± 17.0	52.7 ± 18.2	0.06
Alkaline phosphatase (U/L)	64.5 (57.0–80.0)	59.5 (50.5–79.5)	0.13
Calcium homeostasis			
PTH (pg/mL)	52.3 ± 21.0	44.1 ± 19.0	0.09
Vitamin D3 (nmol/L)	63.2 (21.5–88.1)	45.4 (34.6–68.6)	0.49
Urinary calcium (mg/L)	55.5 (36.5–73.0)	86.0 (49.0–165.5)	0.004
FGF23–α-klotho axis			
α-Klotho (pg/mL)	780.3 (621.1–976.2)	640.1 (520.8–770.2)	0.01
FGF23 (RU/mL)	64.5 (54.4–83.2)	63.6 (57.7–72.5)	0.75
Vitamin B12 status			
Vitamin B12 (pmol/L)	337.9 (218.0–559.1)	267.6 (227.2–364.5)	0.12
Holotranscobalamin (pmol/L)	89.4 (58.9–205.0)	84.3 (67.6–100.4)	0.35
Total homocysteine (µmol/L)	8.60 (6.70–11.3)	8.75 (7.25–10.5)	0.90
Methylmalonic acid (µmol/L)	0.17 (0.15–0.22)	0.18 (0.16–0.21)	0.62
4cB12	0.54 (0.07–1.24)	0.42 (0.19–0.70)	0.47
Vitamins			
Vitamin A (µmol/L)	1.80 (1.56–1.92)	2.07 (1.80–2.33)	0.004
Vitamin B2 (nmol/L)	6.00 (4.39–10.70)	9.05 (6.82–11.8)	0.03
Vitamin B6 (nmol/L)	67.2 (49.1–89.4)	78.8 (47.1–99.7)	0.62
Vitamin K1 (nmol/L)	1.55 (1.30–2.23)	0.78 (0.54–1.13)	<0.0001
Folate (ng/mL)	10.9 (7.71–12.8)	7.82 (6.36–11.2)	0.03
Amino acids			
Alanine (µmol/L)	373.2 ± 98.1	348.7 ± 66.2	0.22
Arginine (µmol/L)	64.1 (52.7–74.4)	69.1 (59.0–76.0)	0.35
Glutamine (µmol/L)	629.4 ± 83.2	546.9 ± 64.3	<0.0001
Leucine (µmol/L)	117.5 (103.6–137.0)	120.0 (114.4–143.8)	0.07
Lysine (µmol/L)	128.5 (119.0–147.7)	171.4 (152.3–189.3)	<0.0001
Proline (µmol/L)	174.7 (146.5–244.4)	174.6 (139.2–195.7)	0.24
Iodine and thyroid			
Urinary iodine (µg/L)	28.1 (17.1–41.6)	74.1 (41.5–101.7)	<0.0001
TSH (µg/L)	2.13 ± 0.92	2.35 ± 1.05	0.34
Other bone-related biomarkers			
Zinc (µg/dL)	79.3 ± 11.6	87.3 ± 13.3	0.008
Selenium (µg/L)	67.7 (59.8–82.1)	76.2 (68.4–83.5)	0.11
SePP (mg/L)	3.26 (2.61–4.47)	4.97 (4.22–5.46)	<0.0001
hsCRP (mg/L)	0.39 (0.21–0.88)	0.63 (0.24–1.74)	0.25
Total n-3 fatty acids (%)	3.07 (2.66–3.53)	5.11 (4.22–5.77)	<0.0001
Urinary magnesium (mg/L)	57.0 (44.8–66.9)	56.4 (43.5–81.9)	0.88

Variables expressed as percentage or mean ± SD or median (IQR); [a] n = 71 (vegan n = 36, omnivores n = 35). BUA (ultrasound attenuation), SOS (speed of sound), SI (stiffness index), CTX (b-CrossLaps), PINP (procollagen type-1), PTH (parathyroid hormone), FGF23 (fibroblast growth factor 23), 4cB12 (four markers combined vitamin B12 indicator), TSH (thyroid-stimulating hormone), SePP (selenoprotein P), hsCRP (high-sensitivity C-reactive protein).

Exploratory RRR

An exploratory RRR was applied to investigate the relationship between bone health (BUA and SOS) and the profile of 28 nutrition- and bone-related biomarkers. The first derived biomarker pattern score explained 34.4% of the total variance in BUA and SOS (35.9% for BUA, 32.9% for SOS). Twelve out of the 28 biomarkers were identified to contribute most to bone health. This pattern consisted of the following main contributors (factor loading of $\geq$0.20) with positive factor loadings for lysine (0.35), urinary iodine (0.31), TSH (0.30), SePP (0.30), vitamin A (0.28), leucine (0.24), α-klotho (0.20), total n-3 fatty acids (0.20), urinary calcium (0.20), urinary magnesium (0.20), and vitamin B6 (0.20), and negative factor loading for FGF23 (−0.23) (Figure 1).

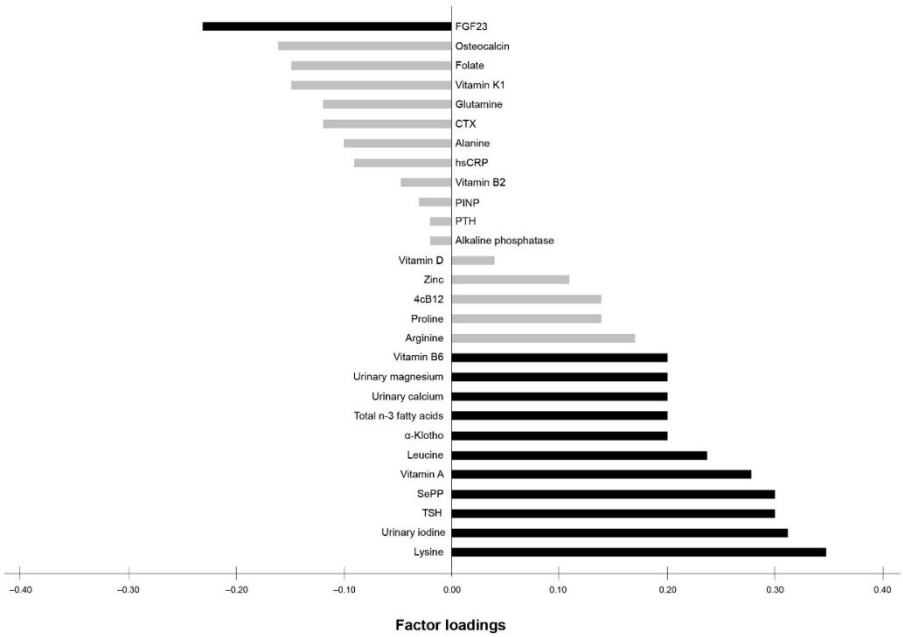

Figure 1. Factor loadings of all 28 biomarkers according to the biomarker pattern score explaining the maximum variation in BUA and SOS. Factor loadings are correlations between biomarkers and the biomarker pattern score. Black bars indicate biomarkers with factor loadings $\geq$ 0.20, which are considered as major contributors to the score. Grey bars indicate biomarkers with factor loadings < 0.20. FGF23 (fibroblast growth factor 23), CTX (b-CrossLaps), hsCRP (high-sensitivity C-reactive protein), PINP (procollagen type-1), PTH (parathyroid hormone), 4cB12 (four markers combined vitamin B12 indicator), SePP (selenoprotein P), TSH (thyroid-stimulating hormone).

An ANOVA across tertiles of the biomarker pattern score showed that the levels of all QUS parameters were significantly higher across the tertiles (Table 3). Accordingly, participants in the highest tertile (T3) had, on average, 11.1% higher BUA levels compared to the first tertile (T1) (p for trend < 0.0001). Furthermore, we observed an increase in SOS (T1 to T3: 2.6%, p for trend < 0.0001), as well as SI (T1 to T3: 18.5%, p for trend < 0.0001) across the tertiles of the biomarker pattern score, while the percentage of vegans decreased. In detail, the first tertile comprised 70% vegans, the second tertile had 61% vegans, and the third tertile included 26% vegans (p for trend = 0.009). Moreover, across the tertiles, we observed a positive association with physical activity (p for trend = 0.01). We observed no association between other lifestyle factors across tertiles (Table 3). Interestingly, a regression model revealed the high impact of the biomarker pattern score on bone health independent of the

diet group, as the model detected no difference in BUA between vegans and omnivores after adjustment of the biomarker pattern score (model 3, Table S1).

Table 3. Characteristics of the bone parameters and biomarkers with factor loadings ≥ 0.20 according to tertiles of the first biomarker pattern score obtained using reduced rank regression.

Characteristics	T1 (*n* = 23)	T2 (*n* = 24)	T3 (*n* = 23)	*p* for Trend
Vegans/omnivores	16/7	14/10	6/17	0.009
Duration vegan diet (years)	3.5 (3.1–6.0)	4.9 (2.3–6.3)	8.2 (4.2–12.2)	0.27
Men	39.1% (9)	50.0% (12)	56.5% (13)	0.49
Age (years)	40.0 (35.0–47.0)	36.0 (31.0–44.5)	35.0 (31.0–44.0)	0.09
BMI (kg/m^2)	22.4 ± 2.5	24.0 ± 3.1	23.7 ± 2.5	0.13
Physical activity (h/week)	1.50 (0.67–3.54)	2.42 (1.07–3.44)	2.67 (1.75–4.33)	0.01
Smoker	26.1% (6)	16.7% (4)	13.0% (3)	0.17
Alcohol consumption (g/d)				
Women	0.27 (0.01–9.90)	0.10 (0.01–2.50)	0.13 (0.02–1.51)	0.16
Men	2.00 (0.21–19.8)	0.03 (0.00–1.99)	1.16 (0.00–4.40)	0.42
Quantitative ultrasound				
BUA (dB/MHz)	108.8 ± 10.8	113.2 ± 9.06	122.4 ± 9.37	<0.0001
SOS (m/s)	1569.1 ± 27.4	1581.5 ± 28.2	1611.7 ± 33.4	<0.0001
SI	91.8 ± 12.9	98.1 ± 12.1	112.7 ± 14.3	<0.0001
Calcium homeostasis				
Urinary calcium (mg/L)	60.0 (39.0–82.0)	55.5 (40.0–103.5)	82.0 (50.0–167.0)	0.20
FGF23–α-klotho axis				
α-Klotho (pg/mL)	666.4 (515.8–865.9)	652.5 (557.8–807.4)	763.0 (689.6–860.4)	0.21
FGF23 (RU/mL)	73.7 (58.9–91.3)	62.6 (57.7–70.9)	63.9 (50.3–78.0)	0.04
Vitamins				
Vitamin A (µmol/L)	1.77 (1.53–1.95)	1.91 (1.61–2.21)	2.04 (1.79–2.31)	0.003
Vitamin B6 (nmol/L)	60.0 (44.1–84.1)	72.3 (46.4–95.0)	84.4 (53.3–126.0)	0.01
Amino acids				
Leucine (µmol/L)	117.7 (106.5–136.8)	118.2 (106.7–137.6)	118.9 (111.7–152.8)	0.14
Lysine (µmol/L)	129.7 (113.9–155.8)	146.3 (128.4–165.8)	166.1 (146.3–187.5)	0.0002
Iodine and thyroid				
Urinary iodine (µg/L)	26.7 (14.8–53.3)	44.6 (29.7–63.2)	70.7 (34.1–103.6)	0.002
TSH (µg/L)	1.75 ± 0.81	2.38 ± 1.12	2.64 ± 0.83	0.002
Other bone-related biomarkers				
SePP (mg/L)	3.37 (2.32–4.77)	3.82 (3.07–5.25)	5.08 (4.15–5.32)	0.0004
Total n-3 fatty acids (%)	3.45 (2.79–4.32)	3.98 (3.02–4.93)	4.36 (3.68–5.65)	0.03
Urinary magnesium (mg/L)	50.2 (44.0–59.0)	59.1 (43.3–93.0)	59.1 (46.6–74.3)	0.19

Variables expressed as a percentage or mean ± SD or median (IQR). BMI (body mass index), BUA (ultrasound attenuation), SOS (speed of sound), SI (stiffness index), FGF23 (fibroblast growth factor 23), TSH (thyroid-stimulating hormone), SePP (selenoprotein P).

Regarding the main contributors of the pattern, an ANOVA across tertiles of the biomarker pattern score showed significant positive associations with vitamin A (p for trend = 0.003), vitamin B6 (p for trend = 0.01), the amino acid lysine (p for trend = 0.0002), SePP (p for trend = 0.0004), and n-3 fatty acids (p for trend = 0.03). Furthermore, participants had higher concentrations of urinary iodine and TSH (both p for trend = 0.002) across the tertiles. As depicted in Table 3, according to the FGF23–α-klotho axis, FGF23 concentrations showed inverse associations (p for trend = 0.04), whereas α-klotho levels were higher in participants in T3 compared to T1; however, these were not statistically significant across the tertiles (p for trend = 0.21). Furthermore, the urinary calcium levels (T1: median 60.0 mg/L vs. T3: 82.0 mg/L), and levels of urinary magnesium (T1: 50.2 mg/L vs. T3: 59.1 mg/L) were higher in participants in T3, although not statistically significant across the tertiles (both p for trend > 0.19). Regarding leucine, no association across the tertiles was

observed (p for trend = 0.14). In addition to the main contributors of the pattern, zinc was positively associated across tertiles (p for trend = 0.02, Table S2).

In the sensitivity analyses, after the additional adjustment according to the month of assessment, sex, age, and lifestyle variables, i.e., BMI, smoking status, physical activity, and alcohol consumption, effectively no changes in the results were observed (data not shown). In addition, the exclusion of postmenopausal women and women with surgical menopause did not change the results (data not shown).

4. Discussion

The present study observed differences in bone health between vegans and omnivores, showing lower mean values of all QUS parameters in vegans compared to omnivores; however, only differences in the BUA levels reached statistical significance. We also detected differences in biomarkers related to bone health between vegans and omnivores, and an exploratory biomarker pattern was further derived, revealing a combination of biomarkers contributing to bone health. This pattern provides a possible explanation of the lower bone health in vegans compared to omnivores.

Up till now, few studies [7,23–28] have investigated the association between a vegan diet and bone health, showing lower BMD in vegans compared to omnivores. In 2019, Iguacel et al. [6] concluded in a systemic review and meta-analysis that a vegan diet was associated with decreased BMD at different sites (lumbar spine, femoral neck, whole body) compared to an omnivorous diet [6]. Moreover, the authors suggested that the lower BMD values found in vegans could be clinically relevant because the fracture risk was also found to be higher in vegans than in omnivores [6]. None of the included studies used QUS data for the assessment of bone health. However, the results of our RBVD study are in agreement, also showing reduced bone health in vegans compared to omnivores.

Scientific evidence suggests that some specific nutrients derived mainly from animal food sources are found in lower quantities in vegans, which could adversely affect bone health. It is well known that vitamin B12 is the most critical nutrient when following a vegan diet [10,12]. Regarding bone health, it has been proposed that a deficiency in vitamin B12 can negatively affect bone development and maintenance [6]. However, we observed no differences in any of the blood parameters assessing vitamin B12 status [21]. Next to vitamin B12, vitamin D also plays a central role in bone metabolism and mineralization. Vitamin D deficiency leads to increased bone turnover, resulting in decreased bone mineral density [29]. Furthermore, Busse et al. assumed that vitamin D deficiency decreases bone turnover and, in turn, leads to premature bone aging [30]. The impaired turnover of vitamin-D-deficient bone leads to hypo- and hypermineralized bone areas and increased fracture risk [30]. Due to the omission of food from animal origins, vegans are at higher risk of inadequate vitamin D supply [10,12,29], which may have adverse bone health effects. Furthermore, the endogenous vitamin D production might be limited in our study population living in Berlin (Germany) due to low sun exposure for several months of the year [29]. However, a sensitivity analysis revealed no change in the results after an adjustment for the month of blood collection. In agreement with the current evidence, the dietary intake of vitamin D3 is lower in vegans [21], but we observed no difference in the vitamin D3 blood concentrations between vegans and omnivores, most likely because 50.0% of our vegans took vitamin D3 supplements.

We detected further differences in nutritional biomarkers between vegans and omnivores, which may contribute to the decreased bone health in vegans. A review of Dai and Koh [13] investigated the possible role of B vitamins in bone health, including evidence from in vitro and in vivo experimental studies, as well as observational and intervention studies. Next to vitamin B12, the results of this review suggest a protective role of vitamins B2 and B6 in bone health [13]. Interestingly, in agreement with the reduced bone health of vegans in the RBVD study, we also observed lower plasma concentrations of vitamin B2 in vegans, which is explained by the lower dietary intake compared to omnivores [21]. Indeed, a few studies have shown that the status of vitamin B2 is considered deficient in ≈30% of

vegans [31,32]. Regarding vitamin A, Davey et al. noticed a lower mean intake of retinol in vegans compared to omnivores, fish-eaters, and ovo-lacto-vegetarians in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Oxford study [12]. Although no significant difference in the intake of vitamin A equivalents was observed in the RBVD study [21], the plasma concentrations of vitamin A were lower in vegans compared to omnivores. However, the role of vitamin A regarding bone health may be ambiguous. On the one hand, it has been found that vitamin A promotes skeletal health [33]. On the other hand, an epidemiological study demonstrated that an excessive intake of vitamin A or high serum vitamin A are also related to adverse skeletal health, including accelerating bone loss, decreasing bone mineral density, and increasing the incidence of fractures [33].

As oily fish and, to a lesser extent, dairy foods and meat are the primary sources of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [34,35], the intake of n-3 fatty acids while following a vegan diet may be lower than in omnivores [10]. Indeed, lower plasma levels of n-3 fatty acids in vegans compared to omnivores were observed in the present study. The n-3 fatty acids EPA and DHA are suggested to stimulate osteoblast survival, promote osteoblastogenesis, and prevent bone resorption by altering membrane function, regulating calcium balance, and enhancing osteoblast activity [36]. Furthermore, the involvement of EPA and DHA in preosteoblast differentiation and maturation was associated with their anti-inflammatory effects, i.e., reducing the synthesis of inflammatory PGE2 and modulating peroxisome proliferators-activated receptor gamma (PPARgamma) and lower levels of inflammatory cytokines, e.g., interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) [36]. Regarding bone health, a recent meta-analysis on observational studies noticed that a higher dietary intake of n-3 fatty acids was significantly associated with a lower risk of hip fracture [37]. In addition, two systematic reviews/meta-analyses based on randomized controlled trials indicated associations between n-3 fatty acids and improved BMD [38,39].

Different minerals have an impact on bone metabolism. It has been observed that selenium and the selenium-transport protein SePP (constituting the majority of selenium in blood) were positively correlated with BMD [40,41], even if SePP might be more relevant because of its proposed function as the essential selenium transporter to the bones [42]. Vegans had a lower intake of selenium [10], as well as lower concentrations of total serum selenium [41]. In fact, this was also seen in the present study; however, statistical significance was observed only for SePP. Next, zinc has also been found to be important in the regulation of bone homeostasis, as many zinc-related proteins are involved in the regulation of cellular function in osteoblasts and osteoclasts [43]. Zinc stimulates cell differentiation, cell proliferation, and mineralization in osteoblasts [43]. Indeed, a study showed lower BMD for the hip, spine, and distal wrist of men in the lowest plasma zinc quartile compared to men with higher plasma zinc concentrations [44]. Accordingly, the present study demonstrated lower serum zinc concentrations in vegans, as well as lower BUA levels, compared to omnivores. Furthermore, the macro minerals calcium and magnesium are known as important contributors to bone health [43]. In fact, 99% of the body's calcium resides in the skeleton and about 60% of all magnesium in the body is found in bone [43]. As concentrations in the blood are carefully regulated within narrow limits, the present study used 24 h urine samples to better reflect the calcium and magnesium statuses. A switch from an omnivorous to a vegetarian diet demonstrated a rise in the urinary excretion of magnesium [45]. Kidneys are able to retain magnesium during deprivation by reducing its excretion or excrete magnesium in cases of excess intake [46]. Therefore, the renal excretion of the filtered load has been found to vary from 0.5 to 70% [46]. Nevertheless, the homeostasis also depends on the absorption in the intestine. In fact, it is noteworthy that the intestinal absorption of magnesium is not directly proportional to dietary magnesium intake but is rather dependent on the individual magnesium status [46]. It has been found that the lower the magnesium level, the more this element is absorbed in the gut; thus, relative magnesium absorption is high when intake is low and vice versa [46]. The individual adaption of magnesium might provide a possible explanation for why the present study

observed no differences in urinary magnesium concentrations between vegans and omnivores, despite the observed higher intake of magnesium in vegans [12], which is supported by our dietary data. Regarding calcium, a switch from an omnivorous diet to a vegetarian diet is associated with a decrease in the excretion of calcium [45]. In detail, Knurick et al. found that the daily calcium excretion was significantly higher (≈34%) in the omnivores as compared to individuals adhering to vegetarian diets [7]. The present study also showed a lower excretion of calcium in vegans compared to omnivores (≈36%). This was likely caused by the lower intake of calcium in vegans as urinary calcium concentrations reflect dietary intake [47].

A vegan diet may also include healthy constituents that counterbalance the negative effects on bone health. In fact, plant-based diets are high in vitamin K [7,8] and folate [7,12]. Accordingly, our RBVD study demonstrated higher dietary intake [21] and higher concentrations of folate and vitamin K in the blood of vegans compared to omnivores. Vitamin K is known as a cofactor for the optimal mineralization of bone and is positively associated with BMD [11]. In addition, several epidemiologic studies found a significant relationship between high folate intake/concentrations and increased BMD or reduced fracture risk [7,13,14].

Lifestyle factors may influence or cover potential associations between dietary habits and BMD [6]. Scientific evidence suggests that vegans tend to show a healthier lifestyle compared to omnivores, which might have an important impact on BMD [9], i.e., higher levels of physical activity [12], lower smoking rates [12], lower consumption of alcohol [12], and lower BMI. However, as the present study detected no relevant differences in these lifestyle factors between vegans and omnivores, no impact on the levels of QUS measurements was expected.

Exploratory RRR

As discussed above, several nutrients require particular attention for bone health in vegans. However, ascribing the lower BUA levels (in some degree SOS and SI) of vegans to a single nutrient or biomarker is likely oversimplistic, given the complexity of the homeostatic regulatory mechanisms of bones. In fact, complex interconnections between nutrients, foods, and dietary patterns imply that no single element of a diet can provide the complete picture of dietary effects on health [48]. Based on this, an exploratory systematic approach was adapted to detect a biomarker pattern that revealed a combination of biomarkers that contributes to bone health, i.e., the RRR identified a pattern based on twelve biomarkers as main contributors (factor loading $\geq$ 0.20) explaining a maximum variation in BUA and SOS in our population. Highly important, the ANOVA demonstrated positive associations between all QUS parameters across the tertiles of the biomarker pattern score. This might be of clinical relevance, as it has been reported that even relatively small changes in bone health, e.g., a 10% increase in bone mass, reduced fracture risk by as much as 50% [9].

The identified biomarker pattern was characterized by biomarkers with positive factor loadings for lysine, urinary iodine, TSH, Sepp, vitamin A, leucine, α-klotho, total n-3 fatty acids, urinary calcium, urinary magnesium, and vitamin B6, and a negative factor loading for FGF23. Regarding the main contributors, the ANOVA supported positive associations of vitamin A and B6, SePP, and n-3 fatty acids across the tertiles of the biomarker pattern score. This is in agreement with the aforementioned recent evidence showing that these biomarkers are suggested to be components with beneficial properties according to bone health [38–41].

Interestingly, urinary iodine and TSH also seem to have an important role in bone health, identifying them as strong contributors to the biomarker pattern. In fact, a recent epidemiological study reported that urinary iodine levels were significantly lower in women with postmenopausal osteoporosis and were associated with the total T-score [49]. Regarding TSH, a population-based register cohort study that included healthy participants without a known thyroid disease (n = 222,138) observed associations between low TSH

concentrations with an increased long-term risk of hip fracture (45% increase in hip fracture risk for each SD reduction in TSH level) [50]. Similarly, Murphy et al. also noticed a 43% increase in nonvertebral fracture risk for each SD reduction in TSH levels in 2374 euthyroid postmenopausal women [51].

Furthermore, the RRR also identified the plasma amino acids leucine and lysine as the main contributors to the biomarker pattern. Mechanistic evidence indicated that leucine and lysine (in addition to arginine, alanine, proline, and glutamine) stimulate insulin secretion in vitro [52], which has been proposed to promote osteoblast growth and differentiation [53,54]. Additionally, it has been shown that leucine is the most potent of the branched-chain amino acids for the stimulation of muscle protein synthesis [55], which is critical for the maintenance of adequate bone strength and density [54]. Similarly, Jennings et al. demonstrated that the dietary intake of lysine, leucine (in addition to arginine, alanine, proline, and glutamic acid) was associated with higher BMD [54].

The FGF23–α-klotho axis was also identified as a main contributor to the biomarker pattern. FGF23 was inversely associated. FGF23 plays a key role in balancing mineral ion homeostasis and bone mineralization [56], where it reduces the renal phosphate uptake and the secretion of parathyroid hormone, respectively [57,58]. Moreover, it has been noticed that FGF23 decreases 1,25-dihydroxyvitamin D concentrations by downregulating the expression of vitamin-D-metabolizing enzymes [57,58]. The critical role of FGF23 in mineral ion homeostasis was first identified in human genetic and acquired rachitic disease [56], showing that an excess of FGF23 levels cause several types of hypophosphatemic rickets/osteomalacia, which are characterized by impaired mineralization of the bone matrix [56,57]. This is in agreement with the present study, which found that FGF23 was the biomarker with the strongest negative factor loading in our exploratory RRR. Nevertheless, more research is needed because until now, only a few cross-sectional studies have investigated the association of FGF23 with BMD in apparently healthy participants, providing controversial results [59–62]. Furthermore, until now, only a few epidemiological studies [60,63,64] have investigated the associations between circulating α-klotho and bone health and showed conflicting results.

To conclude, the exploratory RRR revealed a combination of twelve biomarkers that might have contributed to bone health in our study population. As the present study revealed a decreased percentage of vegans across the tertiles of the biomarker pattern score corresponding with increasing QUS levels, it might be hypothesized that the detected combination of biomarker concentrations contributed to the impaired bone health in vegans. However, as the RBVD was a small study, replication in an independent study population is needed to confirm the results. To the best of our knowledge, the present study is the first to apply RRR to detect an exploratory biomarker pattern that may reveal a combination of biomarkers that are relevant to bone health. Usually, RRR has been efficiently used in nutritional epidemiology to identify dietary patterns [65]. The validation of the derived patterns is highly recommended [65]. Further limitations of our study deserve to be mentioned. In the present study, we used the QUS measurements as a proxy of BMD, commonly measured using the dual energy X-ray absorptiometry technique (DEXA). However, validation studies against DEXA suggested the usefulness of QUS in diagnosing osteoporosis and future fracture risk [66]. Therefore, QUS represents a valid, inexpensive, easy, and quick alternative measurement tool without radiation. Moreover, the cross-sectional design does not allow for causal inference. Moreover, the study included middle-aged men and women from a small area (Berlin, Germany); thus, the results may not be generalizable to other populations. However, the RBVD study provided comprehensive high-quality data as a result of the standardized procedures, including the collection of blood and urine, in combination with extensive information from computer-based questionnaires, a dietary assessment using a 3-day weighed food protocol, and anthropometric measurements.

In conclusion, the study observed differences in bone health between vegans and omnivores, along with differences in biomarkers related to bone health. In addition, an exploratory biomarker pattern was derived that revealed a combination of biomarkers, pro-

viding a possible explanation of a reduced bone health in vegans compared to omnivores. Additional studies are required to confirm these findings.

Supplementary Materials: The following are available online at https://www.mdpi.com/2072-6643/13/2/685/s1, Table S1. Regression models of diet (vegans/omnivores) on broadband ultrasound attenuation (BUA), Table S2. Characteristics of all predictor variables included in the reduced rank regression (RRR) (including those in Table 3) according to the tertiles of the first biomarker pattern score.

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Institutional Review Board Statement: The study was approved by the Ethics Committee of Charité University Medical Center Berlin (No. EA4/121/16) and was 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 datasets generated during and/or analyzed during the current RBVD study are not publicly available due to provisions of the written informed consent.

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Conflicts of Interest: The authors state that they have no conflict of interest.

References

1. Janssen, M.; Busch, C.; Rödiger, M.; Hamm, U. Motives of consumers following a vegan diet and their attitudes towards animal agriculture. *Appetite* **2016**, *105*, 643–651. [CrossRef]
2. Allès, B.; Baudry, J.; Méjean, C.; Touvier, M.; Péneau, S.; Hercberg, S.; Kesse-Guyot, E. Comparison of Sociodemographic and Nutritional Characteristics between Self-Reported Vegetarians, Vegans, and Meat-Eaters from the NutriNet-Santé Study. *Nutrients* **2017**, *9*, 1023. [CrossRef]
3. Lee, Y.; Park, K. Adherence to a Vegetarian Diet and Diabetes Risk: A Systematic Review and Meta-Analysis of Observational Studies. *Nutrients* **2017**, *9*, 603. [CrossRef]
4. Kahleova, H.; Levin, S.; Barnard, N.D. Vegetarian Dietary Patterns and Cardiovascular Disease. *Prog. Cardiovasc. Dis.* **2018**, *61*, 54–61. [CrossRef]
5. Dinu, M.; Abbate, R.; Gensini, G.F.; Casini, A.; Sofi, F. Vegetarian, vegan diets and multiple health outcomes: A systematic review with meta-analysis of observational studies. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 3640–3649. [CrossRef] [PubMed]
6. Iguacel, I.; Miguel-Berges, M.L.; Gómez-Bruton, A.; A Moreno, L.; Julián, C. Veganism, vegetarianism, bone mineral density, and fracture risk: A systematic review and meta-analysis. *Nutr. Rev.* **2019**, *77*, 1–18. [CrossRef] [PubMed]
7. Knurick, J.R.; Johnston, C.S.; Wherry, S.J.; Aguayo, I. Comparison of Correlates of Bone Mineral Density in Individuals Adhering to Lacto-Ovo, Vegan, or Omnivore Diets: A Cross-Sectional Investigation. *Nutrients* **2015**, *7*, 3416–3426. [CrossRef]
8. Tucker, K.L. Vegetarian diets and bone status. *Am. J. Clin. Nutr.* **2014**, *100*, 329S–335S. [CrossRef]
9. Office of the Surgeon General (US). *Determinants of Bone Health: Bone Health and Osteoporosis: A Report of the Surgeon General*; Office of the Surgeon General (US): Rockville, MD, USA, 2004; Volume 6. Available online: https://www.ncbi.nlm.nih.gov/books/NBK45503/ (accessed on 19 February 2021).
10. Richter, M.; Boeing, H.; Grünewald-Funk, D.; Heseker, H.; Kroke, A.; Leschik-Bonnet, E.; Oberritter, H.; Strohm, D.; Watzl, B. Vegan Diet Position of the German Nutrition Society (DGE). *Ernaehrungs Umschau Int.* **2016**, *63*, 92–104. [CrossRef]
11. Palermo, A.; Tuccinardi, D.; D'Onofrio, L.; Watanabe, M.; Maggi, D.; Maurizi, A.R.; Greto, V.; Buzzetti, R.; Napoli, N.; Pozzilli, P.; et al. Vitamin K and osteoporosis: Myth or reality? *Metababolism* **2017**, *70*, 57–71. [CrossRef]
12. Davey, G.K.; Spencer, E.A.; Appleby, P.N.; Allen, N.E.; Knox, K.H.; Key, T.J. EPIC–Oxford:lifestyle characteristics and nutrient intakes in a cohort of 33 883 meat-eaters and 31 546 non meat-eaters in the UK. *Public Health Nutr.* **2003**, *6*, 259–268. [CrossRef]
13. Dai, Z.; Koh, W.-P. B-Vitamins and Bone Health–A Review of the Current Evidence. *Nutrients* **2015**, *7*, 3322–3346. [CrossRef] [PubMed]

14. Kalimeri, M.; Leek, F.; Wang, N.X.; Koh, H.R.; Roy, N.C.; Cameron-Smith, D.; Kruger, M.C.; Henry, C.J.; Totman, J.J. Folate and Vitamin B-12 Status Is Associated with Bone Mineral Density and Hip Strength of Postmenopausal Chinese-Singaporean Women. *JBMR Plus* **2020**, *4*. [CrossRef] [PubMed]
15. Goltzman, D.; Mannstadt, M.; Marcocci, C. Physiology of the Calcium-Parathyroid Hormone-Vitamin D Axis. *Front. Horm. Res.* **2018**, *50*, 1–13. [CrossRef] [PubMed]
16. Menzel, J.; Biemann, R.; Longree, A.; Isermann, B.; Mai, K.; Schulze, M.B.; Abraham, K.; Weikert, C. Associations of a vegan diet with inflammatory biomarkers. *Sci. Rep.* **2020**, *10*, 1933–1938. [CrossRef]
17. General Electric Company. Achilles EXPII—Affordable and Convenient Fracture Risk Assessment Using Quantitative Ultrasound. Available online: https://www3.gehealthcare.com/en/nonav-marquee/~{}/media/57f9c197945d4eadb9c29221fc815624.ashx2017 (accessed on 19 February 2021).
18. The InterAct Consortium Validity of a short questionnaire to assess physical activity in 10 European countries. *Eur. J. Epidemiol.* **2012**, *27*, 15–25. [CrossRef]
19. Weitkunat, K.; Schumann, S.; Nickel, D.; Hornemann, S.; Petzke, K.J.; Schulze, M.B.; Pfeiffer, A.F.H.; Klaus, S. Odd-chain fatty acids as a biomarker for dietary fiber intake: A novel pathway for endogenous production from propionate. *Am. J. Clin. Nutr.* **2017**, *105*, 1544–1551. [CrossRef]
20. Fedosov, S.N.; Brito, A.; Miller, J.W.; Green, R.; Allen, L.H. Combined indicator of vitamin B12 status: Modification for missing biomarkers and folate status and recommendations for revised cut-points. *Clin. Chem. Lab. Med.* **2015**, *53*, 1215–1225. [CrossRef]
21. Weikert, C.; Trefflich, I.; Menzel, J.; Obeid, R.; Longree, A.; Dierkes, J.; Meyer, K.; Herter-Aeberli, I.; Mai, K.; Stangl, G.I.; et al. Vitamin and Mineral Status in a Vegan Diet. *Dtsch. Arztebl. Int.* **2020**, *117*, 575–582.
22. Hoffmann, K.; Schulze, M.B.; Schienkiewitz, A.; Nöthlings, U.; Boeing, H. Application of a New Statistical Method to Derive Dietary Patterns in Nutritional Epidemiology. *Am. J. Epidemiol.* **2004**, *159*, 935–944. [CrossRef]
23. Chiu, J.-F.; Lan, S.-J.; Yang, C.-Y.; Wang, P.-W.; Yao, W.-J.; Su, I.-H.; Hsieh, C.-C. Long-Term Vegetarian Diet and Bone Mineral Density in Postmenopausal Taiwanese Women. *Calcif. Tissue Int.* **1997**, *60*, 245–249. [CrossRef]
24. Barr, S.I.; Prior, J.C.; Janelle, K.; Lentle, B.C. Spinal Bone Mineral Density in Premenopausal Vegetarian and Nonvegetarian Women: Cross-Sectional and Prospective Comparisons. *J. Am. Diet. Assoc.* **1998**, *98*, 760–765. [CrossRef]
25. Lau, E.; Kwok, T.; Woo, J.; Ho, S.C. Bone mineral density in Chinese elderly female vegetarians, vegans, lacto-vegetarians and omnivores. *Eur. J. Clin. Nutr.* **1998**, *52*, 60–64. [CrossRef]
26. Outila, T.A.; Kärkkäinen, M.U.M.; Seppänen, R.H.; Lamberg-Allardt, C.J.E. Dietary Intake of Vitamin D in Premenopausal, Healthy Vegans was Insufficient to Maintain Concentrations of Serum 25-hydroxyvitamin D and Intact Parathyroid Hormone Within Normal Ranges During the Winter in Finland. *J. Am. Diet. Assoc.* **2000**, *100*, 434–441. [CrossRef]
27. Fontana, L.; Shew, J.L.; Holloszy, J.O.; Villareal, D.T. Low Bone Mass in Subjects on a Long-term Raw Vegetarian Diet. *Arch. Intern. Med.* **2005**, *165*, 684–689. [CrossRef] [PubMed]
28. Ho-Pham, L.T.; Nguyen, P.L.T.; Le, T.T.T.; Doan, T.A.T.; Tran, N.T.; Nguyen, T.V.; Le, T.A. Veganism, bone mineral density, and body composition: A study in Buddhist nuns. *Osteoporos. Int.* **2009**, *20*, 2087–2093. [CrossRef]
29. Ambroszkiewicz, J.; Klemarczyk, W.; Gajewska, J.; Chełchowska, M.; Franek, E.; Laskowska-Klita, T. The influence of vegan diet on bone mineral density and biochemical bone turnover markers. *Pediatr. Endocrinol. Diabetes Metab.* **2010**, *16*, 201–204.
30. Busse, B.; Bale, H.A.; Zimmermann, E.A.; Panganiban, B.; Barth, H.D.; Carriero, A.; Vettorazzi, E.; Zustin, J.; Hahn, M.; Ager, J.W., 3rd; et al. Vitamin D Deficiency Induces Early Signs of Aging in Human Bone, Increasing the Risk of Fracture. *Sci. Transl. Med.* **2013**, *5*, 193ra188. [CrossRef] [PubMed]
31. Majchrzak, D.; Singer, I.; Männer, M.; Rust, P.; Genser, D.; Wagner, K.-H.; Elmadfa, I. B-Vitamin Status and Concentrations of Homocysteine in Austrian Omnivores, Vegetarians and Vegans. *Ann. Nutr. Metab.* **2006**, *50*, 485–491. [CrossRef]
32. Schüpbach, R.; Wegmuller, R.; Berguerand, C.; Bui, M.; Herteraeberli, I. Micronutrient status and intake in omnivores, vegetarians and vegans in Switzerland. *Eur. J. Nutr.* **2017**, *56*, 283–293. [CrossRef] [PubMed]
33. Navarro-Valverde, C.; Caballero-Villarraso, J.; Mata-Granados, J.M.; Casado-Diaz, A.; Sosa-Henríquez, M.; Malouf-Sierra, J.; Nogues-Solan, X.; Rodriguez-Mañas, L.; Cortés-Gil, X.; Delgadillo-Duarte, J.; et al. High Serum Retinol as a Relevant Contributor to Low Bone Mineral Density in Postmenopausal Osteoporotic Women. *Calcif. Tissue Int.* **2018**, *102*, 651–656. [CrossRef] [PubMed]
34. Burdge, G.C.; Tan, S.-Y.; Henry, C.J. Long-chain n-3 PUFA in vegetarian women: A metabolic perspective. *J. Nutr. Sci.* **2017**, *6*, e58. [CrossRef] [PubMed]
35. Tur, J.A.; Bibiloni, M.M.; Sureda, A.; Pons, A. Dietary sources of omega 3 fatty acids: Public health risks and benefits. *Br. J. Nutr.* **2012**, *107*, S23–S52. [CrossRef]
36. Bao, M.; Zhang, K.; Wei, Y.; Hua, W.; Gao, Y.; Li, X.; Ye, L. Therapeutic potentials and modulatory mechanisms of fatty acids in bone. *Cell Prolif.* **2019**, *53*, e12735. [CrossRef]
37. Sadeghi, O.; Djafarian, K.; Ghorabi, S.; Khodadost, M.; Nasiri, M.; Shab-Bidar, S. Dietary intake of fish, n-3 polyunsaturated fatty acids and risk of hip fracture: A systematic review and meta-analysis on observational studies. *Crit. Rev. Food Sci. Nutr.* **2017**, *59*, 1320–1333. [CrossRef]
38. Orchard, T.S.; Pan, X.; Cheek, F.; Ing, S.W.; Jackson, R.D. A systematic review of omega-3 fatty acids and osteoporosis. *Br. J. Nutr.* **2012**, *107*, S253–S260. [CrossRef]

39. Abdelhamid, A.; Hooper, L.; Sivakaran, R.; Hayhoe, R.P.G.; Welch, A.; the PUFAH Group. The Relationship Between Omega-3, Omega-6 and Total Polyunsaturated Fat and Musculoskeletal Health and Functional Status in Adults: A Systematic Review and Meta-analysis of RCTs. *Calcif. Tissue Int.* **2019**, *105*, 353–372. [CrossRef]
40. Hoeg, A.; Gogakos, A.; Murphy, E.; Mueller, S.; Köhrle, J.; Reid, D.M.; Glüer, C.C.; Felsenberg, D.; Roux, C.; Eastell, R.; et al. Bone Turnover and Bone Mineral Density Are Independently Related to Selenium Status in Healthy Euthyroid Postmenopausal Women. *J. Clin. Endocrinol. Metab.* **2012**, *97*, 4061–4070. [CrossRef]
41. Hoeflich, J.; Hollenbach, B.; Behrends, T.; Hoeg, A.; Stosnach, H.; Schomburg, L. The choice of biomarkers determines the selenium status in young German vegans and vegetarians. *Br. J. Nutr.* **2010**, *104*, 1601–1604. [CrossRef] [PubMed]
42. Pietschmann, N.; Rijntjes, E.; Hoeg, A.; Stoedter, M.; Schweizer, U.; Seemann, P.; Schomburg, L. Selenoprotein P is the essential selenium transporter for bones. *Metallomics* **2014**, *6*, 1043–1049. [CrossRef]
43. Della Pepa, G.; Brandi, M.L. Microelements for bone boost: The last but not the least. *Clin. Cases Miner. Bone Metab.* **2016**, *13*, 181–185. [CrossRef]
44. Hyun, T.H.; Barrett-Connor, E.; Milne, D.B. Zinc intakes and plasma concentrations in men with osteoporosis: The Rancho Bernardo Study. *Am. J. Clin. Nutr.* **2004**, *80*, 715–721. [CrossRef]
45. Nouvenne, A.; Ticinesi, A.; Morelli, I.; Guida, L.; Borghi, L.; Meschi, T. Fad diets and their effect on urinary stone formation. *Transl. Androl. Urol.* **2014**, *3*, 303–312.
46. Jahnen-Dechent, W.; Ketteler, M. Magnesium basics. *Clin. Kidney J.* **2012**, *5*, i3–i14. [CrossRef]
47. Foley, K.F.; Boccuzzi, L. Urine Calcium: Laboratory Measurement and Clinical Utility. *Lab. Med.* **2010**, *41*, 683–686. [CrossRef]
48. Tapsell, L.C.; Neale, E.P.; Satija, A.; Hu, F.B. Foods, Nutrients, and Dietary Patterns: Interconnections and Implications for Dietary Guidelines. *Adv. Nutr.* **2016**, *7*, 445–454. [CrossRef] [PubMed]
49. Arslanca, T.; Korkmaz, V.; Arslanca, S.B.; Karadag, B.; Ergün, Y. Body iodine status in women with postmenopausal osteoporosis. *Menopause* **2018**, *25*, 320–323. [CrossRef]
50. Abrahamsen, B.; Jørgensen, H.L.; Laulund, A.S.; Nybo, M.; Brix, T.H.; Hegedüs, L. Low Serum Thyrotropin Level and Duration of Suppression as a Predictor of Major Osteoporotic Fractures—The Openthyro Register Cohort. *J. Bone Miner. Res.* **2014**, *29*, 2040–2050. [CrossRef]
51. Murphy, E.; Glüer, C.C.; Reid, D.M.; Felsenberg, D.; Roux, C.; Eastell, R.; Williams, G.R. Thyroid Function within the Upper Normal Range Is Associated with Reduced Bone Mineral Density and an Increased Risk of Nonvertebral Fractures in Healthy Euthyroid Postmenopausal Women. *J. Clin. Endocrinol. Metab.* **2010**, *95*, 3173–3181. [CrossRef] [PubMed]
52. Liu, Z.; Jeppesen, P.B.; Gregersen, S.; Chen, X.; Hermansen, K. Dose- and Glucose-Dependent Effects of Amino Acids on Insulin Secretion from Isolated Mouse Islets and Clonal INS-1E Beta-Cells. *Rev. Diabet. Stud.* **2008**, *5*, 232–244. [CrossRef] [PubMed]
53. Yang, J.; Zhang, X.; Wang, W.; Liu, J. Insulin stimulates osteoblast proliferation and differentiation through ERK and PI3K in MG-63 cells. *Cell Biochem. Funct.* **2010**, *28*, 334–341. [CrossRef]
54. Jennings, A.; MacGregor, A.; Spector, T.; Cassidy, A. Amino Acid Intakes Are Associated with Bone Mineral Density and Prevalence of Low Bone Mass in Women: Evidence from Discordant Monozygotic Twins. *J. Bone Miner. Res.* **2015**, *31*, 326–335. [CrossRef]
55. Fujita, S.; Volpi, E. Amino Acids and Muscle Loss with Aging. *J. Nutr.* **2006**, *136*, 277S–280S. [CrossRef] [PubMed]
56. Guo, Y.-C.; Yuan, Q. Fibroblast growth factor 23 and bone mineralisation. *Int. J. Oral Sci.* **2015**, *7*, 8–13. [CrossRef] [PubMed]
57. Fukumoto, S. FGF23 and Bone and Mineral Metabolism. In *Bone Regulators and Osteoporosis Therapy. Handbook of Experimental Pharmacology*; Springer: Cham, Switzerland, 2019; Volume 262, pp. 281–308. [CrossRef]
58. Richter, B.; Faul, C. FGF23 Actions on Target Tissues—With and Without Klotho. *Front. Endocrinol.* **2018**, *9*, 189. [CrossRef] [PubMed]
59. Isakova, T.; Cai, X.; Lee, J.; Katz, R.; Cauley, J.A.; Fried, L.F.; Hoofnagle, A.N.; Satterfield, S.; Harris, T.B.; Shlipak, M.G.; et al. Associations of FGF23 With Change in Bone Mineral Density and Fracture Risk in Older Individuals. *J. Bone Miner. Res.* **2016**, *31*, 742–748. [CrossRef] [PubMed]
60. Han, W.; Bai, X.-J.; Han, L.-L.; Sun, X.-F.; Chen, X.-M. The relationship between serum fibroblast growth factor 23, Klotho, and lumbar spine bone mineral density in northern Chinese postmenopausal women. *Menopause* **2019**, *26*, 546–553. [CrossRef] [PubMed]
61. Shen, J.; Fu, S.; Song, Y. Relationship of Fibroblast Growth Factor 23 (FGF-23) Serum Levels with Low Bone Mass in Postmenopausal Women. *J. Cell. Biochem.* **2017**, *118*, 4454–4459. [CrossRef]
62. Jovanovich, A.; Bůžková, P.; Chonchol, M.; Robbins, J.; Fink, H.A.; De Boer, I.H.; Kestenbaum, B.; Katz, R.; Carbone, L.; Lee, J.; et al. Fibroblast Growth Factor 23, Bone Mineral Density, and Risk of Hip Fracture Among Older Adults: The Cardiovascular Health Study. *J. Clin. Endocrinol. Metab.* **2013**, *98*, 3323–3331. [CrossRef]
63. Zheng, S.; Chen, Y.; Zheng, Y.; Zhou, Z.; Li, Z. Correlation of serum levels of fibroblast growth factor 23 and Klotho protein levels with bone mineral density in maintenance hemodialysis patients. *Eur. J. Med Res.* **2018**, *23*, 18. [CrossRef]
64. Chalhoub, D.; Marques, E.; Meirelles, O.; Semba, R.D.; Ferrucci, L.; Satterfield, S.; Nevitt, M.; Cauley, J.A.; Harris, T.; Health ABC Study. Association of Serum Klotho with Loss of Bone Mineral Density and Fracture Risk in Older Adults. *J. Am. Geriatr. Soc.* **2016**, *64*, e304–e308. [CrossRef] [PubMed]

65. Weikert, C.; Schulze, M.B. Evaluating dietary patterns: The role of reduced rank regression. *Curr. Opin. Clin. Nutr. Metab. Care* **2016**, *19*, 341–346. [CrossRef] [PubMed]
66. Myint, P.K.; Clark, A.B.; Kwok, C.S.; Loke, Y.K.; Yeong, J.K.-Y.; Luben, R.N.; Wareham, N.J.; Khaw, K.-T. Bone Mineral Density and Incidence of Stroke: European prospective investigation into cancer-norfolk population-based study, systematic review, and meta-analysis. *Stroke* **2014**, *45*, 373–382. [CrossRef] [PubMed]

2.2.2. Einfluss der urinären potenziellen renalen Säurelast auf die Knochengesundheit

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Sowohl Veganismus als auch eine hohe Säurelast in der Nahrung, werden als mögliche Einflussfaktoren für eine schlechtere Knochengesundheit diskutiert [74]. Allerdings ist die Rolle der Basen- oder Säurelast in der Ernährung für die Knochengesundheit von Veganer:innen bisher unzureichend untersucht worden [74].

In dieser Originalarbeit wurden in 24-Stunden-Urinproben die uPRAL von 34 Veganer:innen und 35 Mischköstler:innen der RBVD-Studie bestimmt [74]. Es zeigte sich, dass Veganer:innen im Vergleich zu Mischköstler:innen signifikant niedrigere uPRAL-Wert hatten (mittlerer Unterschied: -34.5 mEq/24 h, $p<0.0001$) [74]. Zudem hatten Veganer:innen eine geringere 24-Stunden-Phosphatausscheidung im Urin ($p=0.0004$), sowie eine geringere 24-Stunden-Sulfatausscheidung ($p=0.01$) und einen höheren pH-Wert im Urin ($p<0.0001$) [74].

Auch bei der etwas geringeren Studienpopulation im Vergleich zur Originalarbeit 3, hatten die Veganer:innen niedrigere BUA-Werte im Vergleich zur Mischkostgruppe [74]. Es zeigte sich, dass innerhalb des Spektrums von alkalischer bis niedriger Säurelast (uPRAL) kein Zusammenhang mit der Knochengesundheit festgestellt werden konnte [74].

Insgesamt bestätigten die Ergebnisse dieser Arbeit die erwarteten unterschiedlichen Säure-Basen-Profile von Veganer:innen und Mischköstler:innen mit einem ausgeprägten Basenüberschuss bei veganer Ernährungsweise, jedoch ohne Einfluss auf die Knochengesundheit [74].

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Urinary Potential Renal Acid Load (uPRAL) among Vegans Versus Omnivores and Its Association with Bone Health in the Cross-Sectional Risks and Benefits of a Vegan Diet Study

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Abstract: Both veganism and high dietary acid load are linked to unfavorable bone health. However, the specific role of dietary alkali or acid load for the bone health of vegans is so far unknown. Thus, the renal biomarker for dietary acid or alkali load, i.e., urinary potential renal acid load (uPRAL), was measured in 24 h urine samples of 34 vegans and 35 omnivores (50.7% males). Bone health was assessed via calcaneal quantitative ultrasound. Associations between uPRAL and bone health indices were examined using multivariable general linear models. Compared to omnivores, vegans had a significantly lower uPRAL (mean difference = −34.5 mEq/24 h, $p < 0.0001$), a lower 24 h urinary phosphate excretion ($p = 0.0004$), a lower 24 h urinary sulfate excretion ($p = 0.01$), and a higher urine pH value ($p < 0.0001$). Broadband ultrasound attenuation (BUA) was lower among vegans versus omnivores ($p = 0.037$), yet it was not associated with uPRAL irrespective of adjustments. This study confirms different acid-base profiles of vegans and omnivores, with a pronounced alkaline excess among vegans and a rather low acid load among a group of omnivores with moderate protein intake. Within this spectrum of alkaline to low acid load, no association with bone health was found.

Keywords: 24 h urine; BUA; cross-sectional study; QUS; urinary phosphate; urinary PRAL; urinary sulfate; vegan

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1. Introduction

Vegan diets, which exclude any food of animal sources, have increased in popularity, but they may entail diverse health implications. Amongst the most prominent health concerns associated with veganism is lower bone health [1], as indicated by lower bone mineral density (BMD) and higher fracture rates among vegans compared to omnivores in two meta-analyses of observational studies [2,3].

Compared to typical Western omnivorous diets, vegan diets are usually characterized by lower protein and higher fruit and vegetable intakes—diet components with established importance for the acid-base status [4]. Accordingly, studies estimating acid load from dietary intake data report a lower acid load of vegan diets [5–8].

Yet, multiple approaches exist for the assessment of acid load, which can be based on either dietary intake or urinary excretion. A key advantage of urinary excretion-based approaches—such as urinary potential renal acid load (uPRAL)—over dietary-based approaches is their ability to reflect actual intakes and the individual bioavailability of acid-base relevant anions and cations [9]. Hence, uPRAL is considered a direct biomarker of diet-dependent acid load [9]. To our knowledge, this valuable approach has not been used so far to investigate the presumed difference in diet-dependent acid load between vegans and omnivores.

Dietary-induced shifts in acid-base balance with mild systemic pH and bicarbonate adaptations still within the physiological range are thought to induce small endocrine-metabolic changes with diverse long-term health consequences, including bone health [9,10]. Although it remains a topic of debate kindled by conflicting results [11], notable evidence links high dietary acid load to unfavorable bone parameters or fractures [12–15]. Interestingly, it was argued that the low acid load of vegan diets would protect vegans' bones [4], at least in a state of sufficient nutrient supply [16]. Yet on the contrary, it has also been reasoned that benefits of alkalization for bone status may be confined to people consuming diets with pronounced acid load and/or people with relevant predispositions, e.g., kidney dysfunction, metabolic syndrome, frailty or osteopenia [11,17,18]. The latter is an interesting argumentative approach, since it would imply that vegans, with their presumably already low dietary acid load, may not additionally profit from further dietary alkalization with regard to bone health. Hence, the complex association of vegan diet, dietary acid or alkali load and bone health needs to be further investigated.

Against this background, the present study aimed to:

(i) Characterize uPRAL, 24 h urinary excretion profiles of acid-base relevant ions and urinary pH of vegans in comparison to omnivores.
(ii) Investigate if the expected very low acid, i.e., alkaline, loads of vegan diets may contribute to alleviating the unfavorable association of veganism with poor bone health.

2. Materials and Methods

2.1. Design and Study Population

The "Risks and Benefits of a Vegan Diet" (RBVD) study is a cross-sectional study conducted at the study center of the German Federal Institute of Risk Assessment (BfR) in Berlin, Germany, from January to July 2017. The study was approved by the Ethics Committee of the Charité-Universitätsmedizin Berlin (No. EA4/121/16) and written informed consent was obtained from all participants.

Details on the RBVD study were published previously [19]. Briefly, recruitment of participants was based on announcements in selected grocery stores and restaurants in Berlin (Germany), followed by a screening for eligibility criteria, and a sex- and age-matched selection of 36 vegans and 36 omnivores (for sample size calculations see [20]). Eligibility criteria included: age of 30–60 years, strictly vegan (excluding any animal-based foods) or omnivorous diet (minimal weekly consumption of three servings of meat or two servings of meat plus two servings of processed meat products), minimal diet adherence of 1 year, BMI < 30 kg/m^2, absence of current infection or serious diseases (such as cancer, myocardial infarction, stroke, diabetes), absence of medication with glucocorticoids or proton pump inhibitors, absence of pregnancy or breastfeeding. The primary outcome of the RBVD study was the difference in the bone health parameter broadband ultrasound attenuation (BUA) between vegans and omnivores. The samples size was calculated to achieve a power of 80% at a significance level of $\alpha = 0.05$ to discern a clinically relevant difference in BUA of at least 5% [19,20]. Acid-base parameters represent secondary outcomes.

Due to missing data in 24 h urinary excretions of sodium and/or potassium, three participants were excluded, resulting in a data set including 69 participants for uPRAL analyses. The data set for bone health analyses included $n = 68$ due to the exclusion of one further participant, as explained below.

2.2. Bone Strength and Microstructure Parameters

Quantitative ultrasound (QUS) parameters of bone strength and microstructure (BUA [dB/MHz], speed of sound (SOS [m/s]) and stiffness index (SI)) were estimated twice bilaterally at the *os calcaneus* by trained study staff using Achilles EXPII (General Electric Healthcare, Little Chalfont, UK). Anatomical or medical conditions (ankle edema, trauma, or fracture) hampering a valid QUS measurement resulted in unilateral measures in the case of $n = 4$ participants and complete exclusion of $n = 1$ participant. For statistical analysis, arithmetic means were calculated of all available measurements.

2.3. Urine Sampling and Analysis

Participants were instructed on the standard procedures of 24 h urine collection. The 24 h urine sample had to be collected in provided preservative-free plastic containers starting on the day preceding the study visit (excluding the first morning micturition) and ending on the morning of the study visit (including the first morning micturition). At the study center, collected samples were mixed, aliquoted, and analyzed on the same day or stored at −80 °C for subsequent analyses. Same-day analysis included measurement of urine pH at the study center using a pH meter (Knick Portamess, Berlin, Germany) in addition to external measurements of urinary concentrations of calcium and magnesium by flame atomic absorption spectrometry (AAS NovAA ®350), as well as sodium and potassium by ion-selective electrodes at the Labor28 GmbH (Berlin, Germany). Subsequent analysis in 2021 included measurement of urine concentrations of sulfate, phosphate and chloride by Dionex 2000i/SP ion chromatography with an ion Pac AS4A column (Dionex GmbH, Idstein, Germany) at the laboratory of the DONALD Study (Dortmund, Germany).

uPRAL was calculated from 24 h urinary ion excretions according to the following equation of Remer and Manz [21]:

$$\begin{aligned} \text{uPRAL (mEq/24 h)} &= \text{Cl (mmol/24 h)} + 2 * \text{SO}_4\text{(mmol/24 h)} + 1.8 * \text{PO}_4\text{(mmol/24 h)} \\ &\quad -\text{Na (mmol/24 h)} - \text{K (mmol/24 h)} - 2 * \text{Mg (mmol/24 h)} - 2 \\ &\quad *\text{Ca (mmol/24 h)} \end{aligned} \quad (1)$$

2.4. Dietary Intake Assessment

Dietary intake was assessed by three-day weighed dietary records, capturing two weekdays and one weekend day. Participants were instructed to weigh every consumed item as well as leftovers with a provided electronic food scale and to record detailed information on the item and its preparation. Daily nutrient intakes, i.e., total energy, protein, and alcohol, were calculated using the EAT-Software (University of Paderborn, 3.5.5), which is based on data from the German Nutrient Database (Bundeslebensmittelschlüssel (BLS), version 3.02) and represents averages over the three recorded days.

Identification of potential misreporting was based on the relation of recorded total energy intake to estimated basal metabolic rate [22], applying established cut-offs for underreporting [23] and overreporting [24].

2.5. Assessment of Covariates

Anthropometric measurements were taken in duplicate by trained staff according to standard procedures, with participants being barefoot and in underwear. Standing height (cm) was determined using a flexible stadiometer (SECA, Hamburg, Germany) and body weight (kg) was measured using an electronic digital scale (Omron BF511, Omron Healthcare Ltd., Kyoto, Japan). Waist circumference was measured at the midpoint between lower ribs and iliac crest. Duplicate measurements were averaged and used for calculation of body mass index (BMI, kg/m^2).

Questionnaires were used for inquiry of educational attainment, smoking habits, current and previous diseases, family disease history, intake of medication or supplements, and physical activity. Physical activity was created and categorized according to the "recreational index" of the InterAct Consortium [25], which is based on sum duration of walking, cycling and sports (averaged for summer and winter, in h/week) multiplied by standard metabolic equivalent of task (MET) estimates (3.0 for walking and 6.0 for cycling and sports). Categories were then dichotomized to represent moderate to high physical activity (yes/no) corresponding with >33.75 MET h/week.

2.6. Statistical Analyses

Statistical analyses were conducted with the SAS statistical software package version 9.4 (SAS Institute Inc., Cary, NC, USA).

Characteristics of the study population are presented as mean ± SD or median (25th, 75th percentile) for normal or non-normal continuous variables, respectively, and as absolute frequencies (percentages) for categorical variables. Tests for differences between vegans and omnivores included independent two-sample Student's *t* test or Satterthwaite *t* test (in case of heteroscedasticity) for normal continuous variables, and Mann–Whitney U test for non-normal continuous variables. Associations of uPRAL with urine pH and of dietary protein intake with 24 h urinary excretions of phosphate and sulfate were analyzed by Spearman correlations.

Multivariable linear associations between uPRAL and bone health outcomes (primary outcome: BUA) were analyzed in general linear models (PROC GLM) and compared to robust regressions (PROC ROBUSTREG).

Basic models (model A) represent unadjusted models including the predictor uPRAL only. Adjusted models (models B to D) were constructed by inclusion of a priori fixed covariates along with hierarchical inclusion of covariates, significantly predicting the outcome and substantially modifying the association (change of $\beta_{uPRAL} \geq 10\%$). A priori covariates included sex, age, smoking status (current, ex, non-smoker), BMI (kg/m^2), moderate to high physical activity (yes/no), alcohol intake (g/d), protein intake (g/d), and 24 h urinary calcium excretion (mmol/24 h). Covariates considered in the hierarchical approach comprised veganism (yes/no), high educational attainment (yes/no), season of study visit (January to March/April to July), total energy intake (MJ/d), duration of veganism (months), urine volume (L/24 h), use of oral contraceptives, antirheumatic or antihypertensive medication (yes/no), and supplementation with calcium, magnesium or vitamin D (yes/no). For comparability, identical adjusted models were used for all outcomes. Results of multivariable linear associations are presented as adjusted least-square means (95%-CI) of bone health outcomes in sex-specific tertiles of uPRAL along with parameters of linear trends (p_{trend}, β_{trend}, SE_{trend}) from models with uPRAL as a continuous variable.

Sensitivity analyses separately investigated the relevance of menopausal status, under- and overreporting (yes/no), and prevalence of rheumatic or thyroid diseases (yes/no) by additional inclusion of the respective variable to the fully adjusted models.

3. Results

Characteristics of the study sample (n = 69) are presented in Table 1. Among the 34 vegans included in this analysis, veganism was followed for 4.9 years in median. No significant differences in sex, age, or socioeconomic and lifestyle factors were detected between both diet groups, except for lower BMI among vegans as compared to omnivores. Most importantly, vegans were characterized by significantly lower uPRAL and higher urine pH (both $p < 0.0001$).

Table 1. Characteristics of vegans and omnivores (n = 69).

Characteristics	*n*	Vegans	Omnivores	*p*
n	69	34	35	
Sex (*n*, % females)	69	17 (50%)	17 (48.6%)	0.9
Age (years)	69	36.5 (32.0, 41.0)	38.0 (32.0, 46.0)	0.7
Anthropometric data				
BMI (kg/m^2)	69	22.2 (20.3, 24.9)	23.7 (22.3, 25.2)	0.034
Socioeconomic status and lifestyle data				
High educational attainment (*n*, %)	69	23 (67.6%)	24 (68.6%)	>0.9
Duration of veganism (years)	69	4.9 (3.1, 11.1)	0	
Physical activity (*n*, %) [a]	69			0.7
Inactive		5 (14.7%)	4 (11.4%)	
Active		29 (85.3%)	31 (88.6%)	

Table 1. *Cont.*

Characteristics	*n*	Vegans	Omnivores	*p*
Smoking status (*n*, %)	69			0.2
Non-smoker		23 (67.6%)	21 (60.0%)	
Ex-smoker		8 (23.5%)	5 (14.3%)	
Current smoker		3 (8.8%)	9 (25.7%)	
Menopausal status (*n*, %) [b]	33			0.5
Pre/peri		13 (76.5%)	14 (82.4%)	
Post		3 (17.6%)	3 (17.6%)	
Dietary intake data				
Total energy (MJ/d)	69	9.6 (7.7, 11.7)	10.0 (8.9, 11.5)	0.4
Protein (%en)	69	12.2 (10.9, 15.7)	14.6 (12.3, 16.6)	0.019
Protein (g/kg body weight/d)	69	1.0 (0.9, 1.4)	1.2 (1.1, 1.5)	0.06
Alcohol (g/d)	69	0.05 (0.00, 2.00)	1.16 (0.02, 13.7)	0.026
24 h urinary excretion data				
uPRAL (mEq/24 h)	69	−37.3 ± 31.5	−2.8 ± 22.5	<0.0001
Urine volume (L/24 h)	69	2.2 (1.6, 2.9)	1.9 (1.5, 2.6)	0.3
Chloride (mmol/24 h)	69	132 (94, 190)	147 (102, 203)	0.6
Sulfate (mmol/24 h)	69	14.7 (10.1, 18.9)	20.8 (13.6, 24.2)	0.012
Phosphate (mmol/24 h)	69	18.9 (14.1, 24.0)	26.5 (21.1, 39.0)	0.0004
Sodium (mmol/24 h)	69	143 (102, 184)	138 (100, 198)	0.9
Potassium (mmol/24 h)	69	82 (59, 106)	69 (61, 107)	0.7
Calcium (mmol/24 h)	69	2.9 (1.8, 4.5)	4.1 (3.1, 5.8)	0.046
Magnesium (mmol/24 h)	69	5.4 (4.0, 7.1)	4.2 (3.3, 6.8)	0.1
Urinary pH	69	6.7 (6.4, 7.1)	6.2 (5.9, 6.4)	<0.0001
Bone health parameters [c]				
BUA (dB/MHz)	68	112 ± 11	118 ± 11	0.037
SOS (m/s)	68	1584 ± 27	1593 ± 40	0.3
SI	68	98 ± 13	104 ± 17	0.1

Results presented as mean ± SD, median (25th, 75th percentile) or absolute (relative) frequencies. BUA—broadband ultrasound attenuation; SOS—speed of sound; SI—stiffness index. [a] Moderate to high physical activity equals > 33.75 MET h/week of walking, cycling and sports. [b] $n = 33$ due to 1 missing menopausal status among the total of 34 female participants; % calculated based on the total *n* of females among vegans and omnivores, respectively; postmenopausal status includes operative menopause. [c] Reduced sample sizes ($n = 68$) result from exclusion of 1 participant due to a missing QUS measurement.

As is noticeable in the urine ionogram (Figure 1), the lower uPRAL of vegans was mainly driven by lower phosphate and sulfate excretions ($p = 0.0004$ and $p = 0.01$, respectively), which corresponded with lower protein intake among vegans vs. omnivores ($p = 0.02$; Table 1; correlation with protein intake: $r_{Spearman} = 0.41$, $p = 0.0005$ for phosphate and $r_{Spearman} = 0.63$, $p < 0.0001$ for sulfate).

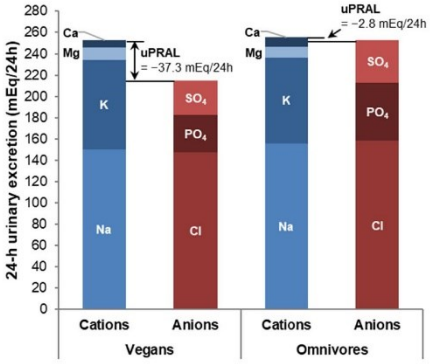

Figure 1. Urine ionogram of mean 24 h urinary excretions of cations and anions related to uPRAL (in mEq/24 h) among 34 vegans and 35 omnivores.

Further, a strong inverse correlation between uPRAL and urine pH was evident ($r_{Spearman} = -0.85$, $p < 0.0001$; Figure 2).

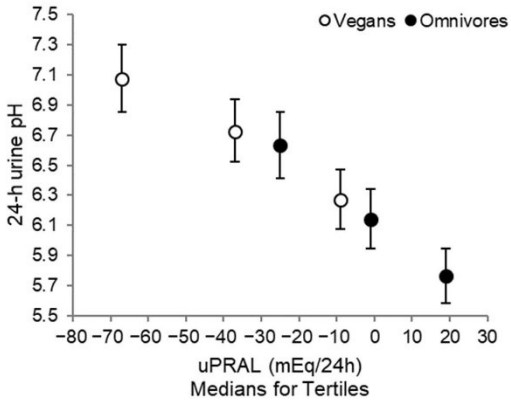

Figure 2. Geometric means and 95%-CI of 24 h urine pH in tertiles of uPRAL for vegans ($n = 34$) and omnivores ($n = 35$).

Among the parameters of bone health, BUA was significantly lower among vegans in comparison to omnivores ($p = 0.037$; $n = 68$; Table 1), which was independent of sex, age, and BMI ($p = 0.045$).

No significant association was observed between uPRAL and any QUS parameter (BUA, SOS or SI) irrespective of adjustment for veganism, sex, age, BMI, smoking status, physical activity, urinary calcium excretion, alcohol consumption, and protein intake (Table 2). Robust regression and sensitivity analyses (considering under- or overreporting, rheumatic or thyroid diseases, and menopausal status) produced grossly similar results.

Table 2. Association of uPRAL with quantitative ultrasound parameters of bone health ($n = 68$) presented as linear trends and least-squared means of bone health parameters in uPRAL tertiles.

	Predictor: uPRAL					
	T1 ($n = 22$) −53.6 (−67.0, −44.1) [a]	**T2 ($n = 24$) −15.2 (−21.5, −10.9) [a]**	**T3 ($n = 22$) 14.1 (4.0, 20.2) [a]**	β_{trend}	SE_{trend}	p_{trend}
Vegans/Omnivores [b]	19/3	10/14	5/17			
BUA (dB/MHz)						
Model A	114 (109–119)	113 (109–117)	118 (113–123)	+0.024	0.042	0.6
Model B	116 (111–121)	112 (108–117)	116 (111–121)	−0.031	0.048	0.5
Model C	116 (111–121)	113 (109–117)	116 (111–121)	−0.041	0.046	0.4
Model D	116 (111–121)	114 (109–118)	115 (111–120)	−0.030	0.048	0.5
SOS (m/s)						
Model A	1584 (1570–1599)	1584 (1570–1598)	1597 (1583–1611)	+0.166	0.128	0.2
Model B	1586 (1570–1603)	1584 (1570–1598)	1595 (1580–1611)	+0.129	0.152	0.4
Model C	1587 (1571–1602)	1585 (1572–1599)	1593 (1578–1608)	+0.100	0.147	0.5
Model D	1587 (1571–1604)	1586 (1572–1600)	1592 (1576–1607)	+0.127	0.159	0.4
SI						
Model A	100 (93–106)	99 (92–105)	106 (99–112)	+0.062	0.058	0.3
Model B	102 (94–109)	98 (92–104)	104 (97–111)	+0.015	0.068	0.8
Model C	102 (95–109)	99 (93–105)	103 (96–110)	+0.001	0.065	>0.9
Model D	102 (95–109)	100 (94–106)	102 (96–109)	+0.015	0.069	0.8

Presented are least-squared means (95%-CI) of bone health outcomes in sex-specific tertiles of the predictor uPRAL unless otherwise indicated. Linear trends (β_{trend}, SE_{trend}, p_{trend}) were obtained in general linear models with uPRAL as a continuous variable. BUA—broadband ultrasound attenuation; SOS—speed of sound; SI—stiffness index; T—tertile. Model A: unadjusted; Model B: adjusted for veganism (yes/no); Model C: additionally adjusted for sex, age (y); Model D: additionally adjusted for BMI (kg/m^2), smoking status (current vs. ex vs. non-smoker), moderate to high physical activity (yes/no), alcohol intake (g/d), protein intake (g/d), 24 h urinary calcium excretion (mmol/24 h). [a] Medians (25th, 75th percentiles) for predictor uPRAL in tertiles. [b] Numbers of vegans and omnivores in tertiles of predictor uPRAL.

Of note, as shown in Table 2, five vegans fall into the third uPRAL tertile, equivalent to moderate acid load (median uPRAL = 14.1 mEq/24 h), and three omnivores fall into the first uPRAL tertile, equivalent to pronounced alkali load (median uPRAL = −53.6 mEq/24 h).

4. Discussion

The present study has characterized uPRAL, 24 h urinary ion excretions and urine pH among vegans and omnivores and investigated the specific role of dietary acid or alkali load for bone health parameters in the healthy participants of the RBVD study. Our findings demonstrate and confirm that pronounced alkali load and higher urine pH result from vegan versus omnivorous diets. Further, the present biomarker-based results indicate that higher alkali loads among vegans can be attributable, to a relevant degree, to a lower phosphate and sulfur intake corresponding with lower protein intakes. Despite the clear differences in uPRAL and urinary pH between both diet groups, the omnivores of our study were also characterized by a rather low dietary acid load. Thus, as an important finding, no association between dietary alkalinity and bone health parameters could be detected for this particular range from low acid to high alkaline load.

4.1. Acid Load of Vegan Diets

In comparison to vegetarian or omnivorous diets, vegan diets are reported to comprise the lowest acid loads, as estimated by algorithms based on dietary intake data [5–8,26]. Yet, this is the first study to corroborate a pronounced difference in uPRAL among vegans versus omnivores based on urinary excretion data.

Previous studies have reported a higher mean urine pH among vegans (6.2–6.7) as compared to omnivores (5.7–6.2), corresponding to the lower acid load of their diets [5,8], which is also in line with our results. Yet, to our knowledge, no other study investigated urinary excretion profiles of ions relevant to acid load among vegans. The observed lower 24 h urinary excretion of phosphate and sulfate corresponds well with the lower protein intake among vegans of our study.

The few vegans in the third uPRAL tertile and few omnivores in the first uPRAL tertile demonstrate that vegan diets are not inevitably alkalizing and omnivorous diets are not inevitably acidifying, highlighting the importance of food choices with regard to a balanced and varied diet.

4.2. Bone Health

Although a favorable low acid load of vegetarian and vegan diets has been presumed to counteract their bone-detrimental effect [4], this hypothesis, especially among vegans, has not been tested to date.

In general agreement with our results, lower bone health (lower BMD levels and higher fracture rates) among vegans over omnivores was reported in a recent review [1], two meta-analyses of observational studies [2,3] and a recent prospective study [27]. A biomarker pattern of multiple bone-relevant nutrients and hormones appeared important to bone health in our previous analysis of the RBVD data [20]. Among the major contributors to the pattern were urinary calcium and magnesium, two principal constituents of the acid-base system.

Bone loss in metabolic acidosis is a long-known phenomenon [28,29] attributable to a direct effect of extracellular protons on osteoclasts, osteoblasts and matrix proteins and an indirect effect via deregulation of the growth hormone/IGF-axis and elevated glucocorticoids [9,10,30]. Similar, yet more subtle, cellular and endocrine-metabolic effects on bones have been observed with dietary-induced low-grade acidotic states well within the physiological range [9,10]. These rather small effects are expected to culminate in impaired bone health if net acid-producing diets are maintained habitually for an extended time [9–11].

Despite the biological plausibility for the bone-detrimental effect of dietary acid load and several findings of links to fracture risk or prediction scores [13–15], evidence appears

inconsistent [11]. Upon closer consideration of our results, however, it is highly relevant that our study population was not only healthy but also captured a rather low spectrum of dietary acid load, reaching from highly negative (i.e., alkaline) to only moderately positive (i.e., acidic) uPRAL values. Our null findings in this low spectrum of uPRAL might be interpreted as indirect support for the findings indicating that bones benefit from alkalization in predisposed individuals, e.g., with renal functional losses or subclinical acidosis (mild shifts of blood pH and bicarbonate towards the lower range), or in subjects habitually consuming high-PRAL diets [11,17,18]. Further, the rather low protein intake of our study population merits attention. Especially the combination of low protein intake and low dietary acid load, as in our study, appeared to decouple potential acid load effects from bone health [18,31]. Notwithstanding, it needs to be stressed that our study was not designed to test any of those hypotheses and therefore these possible explanations remain speculative.

Among other reasons discussed for conflicting findings [9,11,32], methodological differences appear most convincing. The detection of the rather small effects of low-grade metabolic acidosis on bone health demands detailed high-quality data and a thorough understanding of all relevant factors influencing the association between acid load and bone health. To begin with, only sufficiently detailed assessment of dietary acid load—ideally based upon repeated 24 h urinary excretion of acid-base biomarkers—will ensure an appropriate reflection of habitual low-grade acidosis [9]. Direct measurement of net acid excretion (NAE, via 24 h urinary ammonium, titratable acids and bicarbonate excretions) is generally considered as the best available measure of net endogenous acid production [9]. Yet, use of 24 h urinary ion excretions for the calculation of uPRAL in our study represents a good and well-established biomarker, which specifically captures the dietary impact on the acid-base status [9]. Using uPRAL instead of NAE, however, omits dietary noncombustible organic acids (e.g., phenolic, quinic, or benzoic acids), which will conceivably differ between diet groups consuming different amounts of fruit and vegetables. Since fruit and vegetables are not only sources of organic acids but also main contributors to alkalization, the net contribution of organic acids is not of primary relevance [31]. A specific limitation considering our acid-base assessment is the use of a single 24 h urine sample, which can only capture a short snapshot of exposure.

Moreover, in order to discern the specific impact of dietary acid load on bone health, it is of the utmost importance to consider the complex interrelations of all influential factors. Adjustment for either protein intake or its biomarker, urinary nitrogen excretion, appears particularly mandatory. Protein is known to elicit counter-directed effects on bone: a bone-detrimental effect through contribution to acid load on the one side and a strong direct bone-anabolic effect on the other side. A lack of consideration of this complex bi-directional role of proteins for bone metabolism may lead to partial or complete masking of associations [9,33,34].

Finally, different methods for assessment of bone health (peripheral quantitative computed tomography (pQCT), dual-energy X-ray absorptiometry (DEXA), and QUS) all associate—although to different degrees—with osteoporosis and fracture risk [35–39]. Assessment of bone health by QUS in our study instead of the elaborate pQCT or the widely applied DEXA may be seen as a limitation of our study, yet these methods partly reflect different properties of bone (areal vs. volumetric BMD, bone mass, size, structure, elasticity and compartment-specific properties) [35,38,39]. Up to now, it is not fully elucidated if and to what extent the effects of low-grade metabolic acidosis are specific to bone site, compartment (trabecular or cortical), mass and structural properties of bone or differ in specific age spans and across sexes. Generally, endocrinological and nutritional influences are assumed to act site-unspecifically [38]. However, differential changes in cortical and trabecular compartments were observed in ammonium chloride-induced chronic metabolic acidosis among rats [40]. Compartment-specific bone loss was further reported in the growing and mature skeleton (following immobilization) [38] and throughout aging [41]. Whereas the growing skeleton adapts to disuse at the periosteal surface, the mature skeleton

reacts on endocortical and trabecular surfaces [38]. Throughout aging, trabecular bone is continuously lost, starting in young adulthood, possibly following a declining course of the IGF-system, while cortical bone is lost mainly upon menopause in women and in elderly men, resulting from the evolving sex-steroids deficiency [41]. Congruent with this compartment-specificity across age, prospective studies in youths found associations of dietary acid load with cortical area (apart from BMC) [13,31,33], whereas two [12,15] out of three compartment-specific studies among adults [12,15,42] indicated predominant effects on trabecular bone. Three studies performing calcaneal QUS-measurements reported inverse associations between dietary PRAL and BUA among women (partly restricted to those with a fracture history) [14,43,44]. Given that 95% of the calcaneus is composed of trabecular bone [35,37], calcaneal BUA also mainly reflects trabecular properties. Of note, the effect sizes in these studies were relatively low (1.5–3% lower BUA for highest versus lowest quantiles of acid load) [14,43,44]. Thus, our non-significant inverse association might also be explained, at least in part, by insufficient power to detect such a small difference (a sample size of 72 was calculated as necessary to discern a 5% difference in BUA between vegans and omnivores [20]). Apart from the specific limitations extensively discussed above, the relatively small and non-representative convenience sample and the cross-sectional study design also call for consideration.

The major strengths of our study include the successful matching of recruited vegans and omnivores, accurate and standardized data assessment, especially biomarker measurement of dietary acid or alkali load based on 24 h urinary anion and cation excretions, and the investigation of its association with bone health parameters.

5. Conclusions

Our study provides an elaborate characterization of uPRAL, 24 h urinary ion excretions and urinary pH among healthy vegans and omnivores. It confirms different acid-base profiles of both diet groups, with a pronounced alkaline excess among vegans and a balanced to low acid load among the omnivores of our study. Further, it demonstrates that within this spectrum of alkaline to low acid load, combined with low to moderate protein intakes, no benefits for bone health could be seen.

It is important to note that a vegan diet is not per se alkalizing. With unfavorable food choices, the benefits of the alkali load of usual vegan diets can be lost.

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Institutional Review Board Statement: The study was approved by the Ethics Committee of Charité University Medical Center Berlin (No. EA4/121/16) and was 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 datasets generated and/or analyzed during the current Risks and Benefits of a Vegan Diet study are not publicly available due to provisions of the data protection regulations.

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References

1. Ogilvie, A.R.; McGuire, B.D.; Meng, L.; Shapses, S.A. Fracture Risk in Vegetarians and Vegans: The Role of Diet and Metabolic Factors. *Curr. Osteoporos. Rep.* **2022**, 1–11. [CrossRef] [PubMed]
2. Ho-Pham, L.T.; Nguyen, N.D.; Nguyen, T.V. Effect of vegetarian diets on bone mineral density: A Bayesian meta-analysis. *Am. J. Clin. Nutr.* **2009**, *90*, 943–950. [CrossRef] [PubMed]
3. Iguacel, I.; Miguel-Berges, M.L.; Gomez-Bruton, A.; Moreno, L.A.; Julian, C. Veganism, vegetarianism, bone mineral density, and fracture risk: A systematic review and meta-analysis. *Nutr. Rev.* **2019**, *77*, 1–18. [CrossRef] [PubMed]
4. Burckhardt, P. The role of low acid load in vegetarian diet on bone health: A narrative review. *Swiss Med. Wkly.* **2016**, *146*, w14277. [CrossRef]
5. Ausman, L.M.; Oliver, L.M.; Goldin, B.R.; Woods, M.N.; Gorbach, S.L.; Dwyer, J.T. Estimated net acid excretion inversely correlates with urine pH in vegans, lacto-ovo vegetarians, and omnivores. *J. Ren. Nutr.* **2008**, *18*, 456–465. [CrossRef]
6. Cupisti, A.; D'Alessandro, C.; Gesualdo, L.; Cosola, C.; Gallieni, M.; Egidi, M.F.; Fusaro, M. Non-Traditional Aspects of Renal Diets: Focus on Fiber, Alkali and Vitamin K1 Intake. *Nutrients* **2017**, *9*, 444. [CrossRef]
7. Johnston, C.S.; Bliss, C.; Knurick, J.R.; Scholtz, C. Rapid Eating Assessment for Participants [shortened version] scores are associated with Healthy Eating Index-2010 scores and other indices of diet quality in healthy adult omnivores and vegetarians. *Nutr. J.* **2018**, *17*, 89. [CrossRef]
8. Knurick, J.R.; Johnston, C.S.; Wherry, S.J.; Aguayo, I. Comparison of correlates of bone mineral density in individuals adhering to lacto-ovo, vegan, or omnivore diets: A cross-sectional investigation. *Nutrients* **2015**, *7*, 3416–3426. [CrossRef]
9. Remer, T.; Krupp, D.; Shi, L. Dietary protein's and dietary acid load's influence on bone health. *Crit. Rev. Food. Sci. Nutr.* **2014**, *54*, 1140–1150. [CrossRef]
10. Carnauba, R.A.; Baptistella, A.B.; Paschoal, V.; Hubscher, G.H. Diet-Induced Low-Grade Metabolic Acidosis and Clinical Outcomes: A Review. *Nutrients* **2017**, *9*, 538. [CrossRef]
11. Frassetto, L.; Banerjee, T.; Powe, N.; Sebastian, A. Acid Balance, Dietary Acid Load, and Bone Effects—A Controversial Subject. *Nutrients* **2018**, *10*, 517. [CrossRef] [PubMed]
12. de Jonge, E.A.L.; Koromani, F.; Hofman, A.; Uitterlinden, A.G.; Franco, O.H.; Rivadeneira, F.; Kiefte-de Jong, J.C. Dietary acid load, trabecular bone integrity, and mineral density in an ageing population: The Rotterdam study. *Osteoporos Int.* **2017**, *28*, 2357–2365. [CrossRef] [PubMed]
13. Esche, J.; Johner, S.; Shi, L.; Schonau, E.; Remer, T. Urinary Citrate, an Index of Acid-Base Status, Predicts Bone Strength in Youths and Fracture Risk in Adult Females. *J. Clin. Endocrinol. Metab.* **2016**, *101*, 4914–4921. [CrossRef] [PubMed]
14. Hayhoe, R.P.G.; Abdelhamid, A.; Luben, R.N.; Khaw, K.T.; Welch, A.A. Dietary acid-base load and its association with risk of osteoporotic fractures and low estimated skeletal muscle mass. *Eur. J. Clin. Nutr.* **2020**, *74*, 33–42. [CrossRef] [PubMed]
15. Jehle, S.; Hulter, H.N.; Krapf, R. Effect of potassium citrate on bone density, microarchitecture, and fracture risk in healthy older adults without osteoporosis: A randomized controlled trial. *J. Clin. Endocrinol. Metab.* **2013**, *98*, 207–217. [CrossRef]
16. Galchenko, A.; Gapparova, K.; Sidorova, E. The influence of vegetarian and vegan diets on the state of bone mineral density in humans. *Crit. Rev. Food Sci. Nutr.* **2021**, 1–17. [CrossRef]
17. Frassetto, L.A.; Hardcastle, A.C.; Sebastian, A.; Aucott, L.; Fraser, W.D.; Reid, D.M.; Macdonald, H.M. No evidence that the skeletal non-response to potassium alkali supplements in healthy postmenopausal women depends on blood pressure or sodium chloride intake. *Eur. J. Clin. Nutr.* **2012**, *66*, 1315–1322. [CrossRef]
18. Macdonald, H.M.; Black, A.J.; Aucott, L.; Duthie, G.; Duthie, S.; Sandison, R.; Hardcastle, A.C.; Lanham New, S.A.; Fraser, W.D.; Reid, D.M. Effect of potassium citrate supplementation or increased fruit and vegetable intake on bone metabolism in healthy postmenopausal women: A randomized controlled trial. *Am. J. Clin. Nutr.* **2008**, *88*, 465–474. [CrossRef]
19. Weikert, C.; Trefflich, I.; Menzel, J.; Obeid, R.; Longree, A.; Dierkes, J.; Meyer, K.; Herter-Aeberli, I.; Mai, K.; Stangl, G.I.; et al. Vitamin and Mineral Status in a Vegan Diet. *Dtsch. Arztebl. Int.* **2020**, *117*, 575–582. [CrossRef]
20. Menzel, J.; Abraham, K.; Stangl, G.I.; Ueland, P.M.; Obeid, R.; Schulze, M.B.; Herter-Aeberli, I.; Schwerdtle, T.; Weikert, C. Vegan Diet and Bone Health—Results from the Cross-Sectional RBVD Study. *Nutrients* **2021**, *13*, 685. [CrossRef]
21. Remer, T.; Manz, F. Estimation of the renal net acid excretion by adults consuming diets containing variable amounts of protein. *Am. J. Clin. Nutr.* **1994**, *59*, 1356–1361. [CrossRef]
22. Schofield, W.N. Predicting basal metabolic rate, new standards and review of previous work. *Hum. Nutr. Clin. Nutr.* **1985**, *39* (Suppl. 1), 5–41. [PubMed]
23. Black, A.E. Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. *Int. J. Obes. Relat. Metab. Disord.* **2000**, *24*, 1119–1130. [CrossRef] [PubMed]
24. Johansson, L.; Solvoll, K.; Bjorneboe, G.E.; Drevon, C.A. Under- and overreporting of energy intake related to weight status and lifestyle in a nationwide sample. *Am. J. Clin. Nutr.* **1998**, *68*, 266–274. [CrossRef] [PubMed]
25. InterAct, C.; Peters, T.; Brage, S.; Westgate, K.; Franks, P.W.; Gradmark, A.; Tormo Diaz, M.J.; Huerta, J.M.; Bendinelli, B.; Vigl, M.; et al. Validity of a short questionnaire to assess physical activity in 10 European countries. *Eur. J. Epidemiol.* **2012**, *27*, 15–25. [CrossRef]
26. Ströhle, A.; Waldmann, A.; Koschizke, J.; Leitzmann, C.; Hahn, A. Diet-dependent net endogenous acid load of vegan diets in relation to food groups and bone health-related nutrients: Results from the German Vegan Study. *Ann. Nutr. Metab.* **2011**, *59*, 117–126. [CrossRef]

27. Tong, T.Y.N.; Appleby, P.N.; Armstrong, M.E.G.; Fensom, G.K.; Knuppel, A.; Papier, K.; Perez-Cornago, A.; Travis, R.C.; Key, T.J. Vegetarian and vegan diets and risks of total and site-specific fractures: Results from the prospective EPIC-Oxford study. *BMC Med.* **2020**, *18*, 353. [CrossRef]
28. Mitch, W.E. Metabolic and clinical consequences of metabolic acidosis. *J. Nephrol.* **2006**, *19* (Suppl. 9), S70–S75.
29. Raphael, K.L. Metabolic Acidosis in CKD: Core Curriculum 2019. *Am. J. Kidney Dis.* **2019**, *74*, 263–275. [CrossRef]
30. Esche, J.; Shi, L.; Sanchez-Guijo, A.; Hartmann, M.F.; Wudy, S.A.; Remer, T. Higher diet-dependent renal acid load associates with higher glucocorticoid secretion and potentially bioactive free glucocorticoids in healthy children. *Kidney Int.* **2016**, *90*, 325–333. [CrossRef]
31. Remer, T.; Manz, F.; Alexy, U.; Schoenau, E.; Wudy, S.A.; Shi, L. Long-term high urinary potential renal acid load and low nitrogen excretion predict reduced diaphyseal bone mass and bone size in children. *J. Clin. Endocrinol. Metab.* **2011**, *96*, 2861–2868. [CrossRef] [PubMed]
32. Dawson-Hughes, B. Acid-base balance of the diet-implications for bone and muscle. *Eur. J. Clin. Nutr.* **2020**, *74*, 7–13. [CrossRef] [PubMed]
33. Alexy, U.; Remer, T.; Manz, F.; Neu, C.M.; Schoenau, E. Long-term protein intake and dietary potential renal acid load are associated with bone modeling and remodeling at the proximal radius in healthy children. *Am. J. Clin. Nutr.* **2005**, *82*, 1107–1114. [CrossRef] [PubMed]
34. Thorpe, M.; Mojtahedi, M.C.; Chapman-Novakofski, K.; McAuley, E.; Evans, E.M. A positive association of lumbar spine bone mineral density with dietary protein is suppressed by a negative association with protein sulfur. *J. Nutr.* **2008**, *138*, 80–85. [CrossRef]
35. Chin, K.Y.; Ima-Nirwana, S. Calcaneal quantitative ultrasound as a determinant of bone health status: What properties of bone does it reflect? *Int. J. Med. Sci.* **2013**, *10*, 1778–1783. [CrossRef]
36. Gonnelli, S.; Cepollaro, C. The use of ultrasound in the assessment of bone status. *J. Endocrinol. Invest.* **2002**, *25*, 389–397. [CrossRef]
37. Guglielmi, G.; Adams, J.; Link, T.M. Quantitative ultrasound in the assessment of skeletal status. *Eur. Radiol.* **2009**, *19*, 1837–1848. [CrossRef] [PubMed]
38. Hart, N.H.; Newton, R.U.; Tan, J.; Rantalainen, T.; Chivers, P.; Siafarikas, A.; Nimphius, S. Biological basis of bone strength: Anatomy, physiology and measurement. *J. Musculoskelet Neuronal. Interact.* **2020**, *20*, 347–371.
39. Link, T.M.; Kazakia, G. Update on Imaging-Based Measurement of Bone Mineral Density and Quality. *Curr. Rheumatol. Rep.* **2020**, *22*, 13. [CrossRef]
40. Gasser, J.A.; Hulter, H.N.; Imboden, P.; Krapf, R. Effect of chronic metabolic acidosis on bone density and bone architecture in vivo in rats. *Am. J. Physiol. Renal. Physiol.* **2014**, *306*, F517–F524. [CrossRef]
41. Riggs, B.L.; Melton, L.J.; Robb, R.A.; Camp, J.J.; Atkinson, E.J.; McDaniel, L.; Amin, S.; Rouleau, P.A.; Khosla, S. A population-based assessment of rates of bone loss at multiple skeletal sites: Evidence for substantial trabecular bone loss in young adult women and men. *J. Bone Miner Res.* **2008**, *23*, 205–214. [CrossRef] [PubMed]
42. Pedone, C.; Napoli, N.; Pozzilli, P.; Lauretani, F.; Bandinelli, S.; Ferrucci, L.; Antonelli-Incalzi, R. Quality of diet and potential renal acid load as risk factors for reduced bone density in elderly women. *Bone* **2010**, *46*, 1063–1067. [CrossRef] [PubMed]
43. Welch, A.A.; Bingham, S.A.; Reeve, J.; Khaw, K.T. More acidic dietary acid-base load is associated with reduced calcaneal broadband ultrasound attenuation in women but not in men: Results from the EPIC-Norfolk cohort study. *Am. J. Clin. Nutr.* **2007**, *85*, 1134–1141. [CrossRef] [PubMed]
44. Wynn, E.; Lanham-New, S.A.; Krieg, M.A.; Whittamore, D.R.; Burckhardt, P. Low estimates of dietary acid load are positively associated with bone ultrasound in women older than 75 years of age with a lifetime fracture. *J. Nutr.* **2008**, *138*, 1349–1354. [CrossRef] [PubMed]

2.3. PFAS in der veganen Ernährung

2.3.1. Unterschiede in den Blutkonzentrationen von PFAS zwischen Veganer:innen und Mischköstler:innen

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Die Ergebnisse der Originalpublikation werden im Folgenden zusammengefasst und können dem Abstract der Publikation ähneln. Die deutsche Übersetzung erfolgte durch die Autorin.

Neben der externen Exposition durch Luft und Staub ist der Verzehr von Lebensmitteln und Trinkwasser für den Menschen ein Hauptweg der Exposition von PFAS, wobei tierische Lebensmittelhauptgruppen, wie Fisch oder andere Meeresfrüchte, Eier sowie Fleisch und Fleischerzeugnisse, maßgeblich zur PFAS Exposition beitragen [75]. Aufgrund der langen HWZ im menschlichen Blut können sich langkettige PFAS nach der Aufnahme mit Trinkwasser, Lebensmitteln oder über andere Quellen anreichern [75]. Durch den vollständigen Verzicht tierischer Produkte im Zuge einer veganen Ernährungsweise kann vermutet werden, dass Veganer:innen niedrigere PFAS-Konzentrationen im Blut, im Vergleich zu Mischköstler:innen, haben könnten [75]. Darüber hinaus sind niedrigere Konzentrationen von Gesamtcholesterin und LDL-Cholesterin einer der gut dokumentierten Ernährungseffekte in Folge einer veganen Ernährungsweise [75]. Zudem wurde auch in epidemiologischen Studien beobachtet, dass erhöhte Konzentrationen von Gesamtcholesterin und LDL-Cholesterin mit höheren PFAS-Werten assoziiert sind [75].

Im Rahmen dieser Originalarbeit wurden die vier wichtigsten PFAS (PFOS, PFOA, PFNA, PFHxS) in der RBVD-Studie umfassend untersucht, sowie die Summe dieser vier Verbindungen [75]. Veganer:innen hatten im Vergleich zur Mischkostgruppe im Median niedrigere Plasmakonzentrationen für PFOS (Vegan: 2.31 ng/ml (IQR: 1.37-3.59); Mischkost: 3.57 ng/ml (IQR: 1.94-5.14), p=0.02) und für PFNA (Vegan: <0.25 ng/ml (IQR:<0.25-0.30); Mischkost: 0.41 ng/ml (IQR:0.33-0.58), p<0.0001) [75]. Keine signifikanten Median-Unterschiede wurden für PFOA (Vegan: 1.69 ng/ml (IQR.1.35-2.75); Mischkost: 1.44 ng/ml (IQR: 0.98-2.61), p=0.26) und PFHxS (Vegan: 1.96 ng/ml (IQR: 0.88-3.75); Mischkost: 1.79 ng/ml (IQR: 0.92-2.74), p=0.70) festgestellt [75].

Die stärksten Korrelationen mit verschiedenen Lebensmittelgruppen wurden zwischen den PFOA-Konzentrationen und dem Wasserkonsum beobachtet (bei der gesamten Studienpopulation, n=72) und zwischen PFOS- und PFNA-Konzentrationen und dem Verzehr von Fleisch und Fleischprodukten (Mischkostgruppe n=36) [75].

Die Arbeit bestätigte die niedrigen mediane Konzentrationen von LDL-Cholesterin bei Veganer:innenn, im Vergleich zu Mischköstler:innen (Vegan: 86.5 mg/dl (IQR:68.5-97.0); Mischkost: 115.5 mg/dl (IQR:93.5-136.0), p=0.001) [75]. Jedoch hatte keine der untersuchten PFAS einen Einfluss auf den Unterschied im LDL-Cholesterin zwischen Veganer:innenn und Mischköstler:innen [75].

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Internal exposure to perfluoroalkyl substances (PFAS) in vegans and omnivores

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ABSTRACT

Perfluoroalkyl substances (PFAS) are a complex group of anthropogenic compounds with exceptional properties. Due to their high persistence and mobility, they have caused ubiquitous environmental contamination and in part accumulate in the food chain. In the general population, diet is the main source of PFAS exposure, with the important sources fish and meat. As a vegan diet implies the complete exclusion of any animal products, it might be expected that vegans have lower blood levels of PFAS compared to omnivores. Furthermore, lower levels of cholesterol is one of the well-documented nutritional effects in vegans, but cholesterol levels were also found to be associated with higher PFAS levels in epidemiological studies.

To examine the relations of internal PFAS levels and the levels of cholesterol in vegans and omnivores, the cross-sectional "Risks and Benefits of a Vegan Diet" (RBVD) study was used involving 36 vegans and 36 omnivores from Berlin/Germany. Nine perfluoroalkyl substances were quantified in plasma using a triple-stage quadrupole mass spectrometer.

Lower median plasma concentrations were found in vegans compared to omnivores for perfluorooctane sulfonic acid (PFOS) (2.31 vs. 3.57 ng/ml, respectively; $p = 0.02$) and for perfluorononanoic acid (PFNA) (<0.25 vs. 0.41 ng/ml, respectively; $p < 0.0001$). No significant differences of the median concentrations were observed for perfluorooctanoic acid (PFOA) (1.69 vs. 1.44 ng/ml, respectively, $p = 0.26$) and perfluorohexane sulfonic acid (PFHxS) (1.96 vs. 1.79 ng/ml, respectively; $p = 0.70$). The strongest correlations with food groups, derived from a food frequency questionnaire, were observed between levels of PFOA and water consumption (in case of the total study population, $n = 72$), and between levels of PFOS as well as PFNA and the consumption of 'meat and meat products' (in case of the omnivores, $n = 36$). Levels of Low Density Lipoprotein (LDL) cholesterol were confirmed to be considerably lower in vegans compared to omnivores (86.5 vs. 115.5 mg/dl, respectively; $p = 0.001$), but no associations between the four main PFAS and LDL cholesterol were observed (all $p > 0.05$) at the low exposure level of this study.

According to the results of our study, a vegan diet may be related to lower PFAS levels in plasma. We highlight the importance of the adjustment of dietary factors like a vegan diet in case of epidemiological studies dealing with the impact of PFAS on the levels of blood lipids.

1. Introduction

Perfluoroalkyl substances (PFAS) are a complex group of man-made chemicals composed of a fluorinated carbon backbone of varying length, primarily terminated by a carboxylate (perfluoroalkyl carboxylic acids, PFCAs) or a sulfonate (perfluorooctane sulfonic acids, PFSAs) as

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functional group. The combination of the polar and non-polar structure makes PFAS 'amphiphilic' providing water and oil repellency, and the strength of their carbon-fluorine bonds results in extremely high chemical and thermal stability. Since decades, the compounds have been used for the production of many consumer products like nonstick cookware, breathable textiles or protective coatings for paper, food packing materials, and carpets. From these everyday objects, PFAS are released and have been found – due to their high persistence and mobility – to cause ubiquitous environmental contamination and in part to accumulate in the food chain (Sunderland et al., 2019).

Consumption of food and drinking water is the main route of background exposure in humans. Internal exposure to PFAS in individuals can easily be determined by an analysis of serum or plasma. Four compounds, namely the PFCAs perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA), and the PFSAs perfluorooctane sulfonic acid (PFOS) and perfluorohexane sulfonic acid (PFHxS), typically represent more than 90% of detectable PFAS in serum/plasma of adults in industrialized countries. In the European adult population, median concentrations of PFOS, PFOA, PFHxS, and PFNA were found to be 7.7, 1.9, 0.67 and 0.61 ng/ml, respectively, based on studies from 2007/2008 and onwards (EFSA Panel on Contaminants in the Food Chain, 2020). This pattern results from the occurrence in food and drinking water on the one hand, and from accumulation due to half-lives up to several years in humans on the other hand. The latter is due to missing metabolic degradation and low urinary excretion (EFSA Panel on Contaminants in the Food Chain, 2020).

According to the recent evaluation of the European Food Safety Authority (EFSA), 'Fish and other seafood' was the most important contributor to the mean Lower Bound (LB) exposure in case of PFOS and PFOA, followed by 'Eggs and egg products', 'Meat and meat products', and 'Fruit and fruit products' (EFSA Panel on Contaminants in the Food Chain, 2020). Therefore, internal background exposure to these two substances in humans can be expected to be influenced by their dietary habits. Over the last years, plant-based diets have become increasingly popular in Germany and many other western countries, not merely due to increasing awareness of suffering animals or environmental problems, but also because of expected health benefits (Janssen et al., 2016). As a vegan diet implies the complete exclusion of any animal products, it might be expected that vegans have lower blood levels of PFAS compared to omnivores. Studies on this issue are yet missing. Therefore, the first aim of this investigation was to compare the internal PFAS exposure of German vegans and omnivores. For this purpose, PFAS was analyzed in samples of the 'Risks and Benefits of a Vegan Diet' (RBVD) study in 36 vegans and 36 omnivores aged 30–60 years (Menzel et al., 2020, 2021; Weikert et al., 2020).

While a broad spectrum of toxic effects of different PFAS was observed in experimental animals primarily at higher doses, epidemiological studies conducted in recent years revealed associations of certain biological parameters and levels of PFAS in serum/plasma even in the higher background range (EFSA Panel on Contaminants in the Food Chain, 2020). Using data of reduced formation of vaccine antibodies in one-year old children (Abraham et al., 2020), EFSA derived a tolerable weekly intake (TWI) of 4.4 ng/kg body weight for the sum of PFOS, PFOA, PFHxS and PFNA. According to the modelling of EFSA, such an intake corresponds to an internal level of 6.9 ng/ml for the sum of these four PFAS in women at the age of 35 years (EFSA Panel on Contaminants in the Food Chain, 2020).

Regarding possible changes of lipid metabolism, positive associations have been observed especially between high background levels of PFOS/PFOA and levels of Low Density Lipoprotein (LDL) cholesterol (Frisbee et al., 2010; Steenland et al., 2009). In this context, a vegan diet may be an undervalued confounding factor: An on average lower level of LDL cholesterol is one of the well-documented nutritional effects in vegans compared to omnivores (Yokoyama et al., 2017), resulting from the missing intake of animals fats. As outlined above, vegans may concurrently have lower external and internal PFAS exposure, resulting from missing intake of foods of animal origin with relatively high PFAS content. Therefore, the second aim of this investigation was to compare the impact of internal PFAS exposure and of a vegan diet on blood lipid levels, especially with regard on levels of LDL cholesterol in the RBVD study.

2. Methods

2.1. Study population

Participants of the present RBVD study were recruited by announcement (flyer) in (organic/vegan) supermarkets and investigated between January 2017 and July 2017 at the German Federal Institute for Risk Assessment (BfR) in Berlin (Weikert et al., 2020). A phone screening was performed including a brief explanation of the study and checking inclusion criteria (age 30–60 years, following the diet at least one year) and exclusion criteria (BMI $\geq$30, cardiovascular disease, type 2 diabetes, cancer, pregnancy, breastfeeding, current infection). Hypercholesterolemia and taking lipid-lowering medications were no study exclusion criteria. The final study population comprises 36 vegans and 36 omnivores, who were matched by sex and age. In the present study, an omnivorous diet was defined as the consumption of at least three portions of meat per week or two portions of meat and two portions of processed meat (e.g. cold cuts, sausages) per week, whereas a vegan diet was defined as no consumption of any animal food products. Each participant visited the study center twice - on their first visit, participants gave their written informed consent, received instructions to document their diet, and got material to collect urine. At the second visit, a fasting blood sample was collected, anthropometric measurements were performed and lifestyle characteristics as well as a food frequency questionnaire were assessed. The time span was on average 2 weeks between the two visits in the study center (minimum 1 week to maximum 4 weeks). The study was approved by the Ethics Committee of Charité – Universitätsmedizin Berlin (No. EA4/121/16) and was conducted in accordance with the Declaration of Helsinki.

2.2. Assessment of lifestyle characteristics

Anthropometric measurements, i.e. weight, height, and waist circumference, were taken by trained and quality-monitored personnel on participants wearing only light underwear. Body weight was assessed by an electronic digital scale (Omron BF511, Omron Healthcare Ltd., Kyoto, Japan) and the height was measured using a flexible anthropometer (SECA 213, Hamburg, Germany). Waist circumference was defined as in the horizontal plane midway between the lowest ribs and the iliac crest. Information on physical activity, educational level and smoking status was assessed by computer-based questionnaires. In the RBVD study the physical activity has been determined by a physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC)-study, provided by the Human Study Center of the German Institute of Human Nutrition Potsdam-Rehbruecke (InterAct et al., 2012). Physical activity contains the sum of average hours in summer and winter per week spent on cycling, sports and gardening. Walking comprises the sum of average hours per week during summer and winter. Further, occupational activity was assessed in the RBVD study. The validated EPIC-Potsdam Study food frequency questionnaire (FFQ) collects semi-quantitatively for each food item information on the usual portion size and the average frequency of intake of 102 food items during the past 12 months (Nothlings et al., 2007). Portion size for each item was estimated via image of different portion sizes or with standard portion sizes e.g. a cup (150 ml). Food groups were derived from the FFQs and available as g/d. Individual food groups were summed up to derive food groups, as a basis serves the classification of EFSA: 'Fruits', 'Vegetables (including fungi)', 'Starchy roots and tubers', 'Waters', 'Grains and grain based products', 'Meat and meat products', 'Fish and other seafood' and 'Eggs' (supplemental Table 1).

2.3. Blood collection and laboratory analysis

About 60 ml of venous blood was collected from fasting participants at the BfR study center.

The accredited medical laboratory (Labor 28 GmbH, Berlin, Germany) measured routine biomarkers including plasma concentrations of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides on the same day. Plasma samples used for PFAS assessment were stored at −80 °C in freezers until time of analysis. PFAS were measured at the Bavarian Health and Food Safety Authority. The following compounds were analyzed using 100 µl plasma: perfluorodecanoate (PFDA), PFNA, PFOA, perfluorohexanoic acid (PFHxA), PFOS, PFHxS, perfluorobutane sulfonate (PFBS), perfluorododecanoate (PFDoDA), and 3H-perfluoro-3-[(3-methoxy-propoxy) propanoate] (ADONA). Sample preparation, analysis and quality criteria have been previously described in detail by Mosch et al. (2010). In brief, an online extraction LC-MS/MS system was used, and the compounds were quantified with a triple-stage quadrupole mass spectrometer (API 5500 QTRAP™ Applied Biosystems, Darmstadt, Germany) equipped with a TurboIonSpray® interface. The perfluorinated substances and the corresponding isotope-labeled internal standards were purchased from Wellington Laboratories (Ontario, Canada). Limit of quantification (LOQ) in plasma was 0.25 ng/ml, based on a tenfold peak-to-noise ratio. Values below the LOQ were assigned the half value. The sum of PFAS was defined including PFOS, PFOA, PFHxS and PFNA ('PFAS sum'). Accuracy of the analysis was ensured by External Quality Assurance Schemes (EQUAS) for PFOS and PFOA (http://www.g-equas.de/).

2.4. Statistics

Normally distributed variables were reported as mean and standard derivation (SD). Skewed variables were reported as median and interquartile range (IQR). Categorical variables were reported as percentage. A Student's *t*-test or Mann-Whitney *U* test was used to compare continuous variables between vegans and omnivores, and a chi square test was used for categorical variables.

To investigate the association of veganism with biomarkers of the lipid metabolism, compared to omnivores, an analysis of variance (ANOVA) was performed for model 1 (unadjusted). Additionally, a multivariable adjusted analysis of covariance (ANCOVA) was conducted to detected differences between vegans and omnivores in model 2 (adjusted for several PFAS and the PFAS sum) and model 3 (additionally adjusted for age, sex, smoking status, education, waist circumference, and physical activity). The model was not adjusted for recent weight changes as the study did not assess data on weight changes. Blood lipid concentrations were skewed, thus variables were log-transformed for ANOVA or ANOVA, afterwards back-transformed and expressed as geometric means and 95%-confidence intervals (95%-CI).

To investigate a potential relationship between PFAS plasma levels and blood lipids, we used linear regression models and also a restricted cubic spline (RCS) regression analyses to investigate nonlinear associations. Three knots were used, located at the 5th, 50th and 95th percentiles. The RCS regression models fitted with generalized estimating equations were constructed using the SAS macro %RCS_Reg (v1.50) developed by Desquilbet and Mariotti (2010). Not only levels of blood lipids, but also those of PFAS were skewed distributed. Therefore, all variables were log-transformed for the analyses. The analyses were performed in unadjusted models (model 1), adjusted for type of diet (model 2) as well as additionally adjusted for age, sex, smoking status, education, waist circumference, and physical activity (model 3).

To investigate potential correlations between PFAS plasma levels and food groups, we calculated Spearman (partial) correlations for the total and the omnivorous sample. Correlation analyses between individual PFAS and individual food groups were performed in an unadjusted model (model 1) for omnivores, and for the total sample, model 1 was adjusted for type of diet. Model 2 was additionally adjusted for age, sex, smoking status, education, waist circumference, and physical activity.

The statistical analyses were performed using SAS software, version 9.4 (SAS institute, Cary, N.C., USA), IBM SPSS Statistics for Windows, Version 26.0 (Armonk, NY: IBM Corp) and R software (version 3.6.3). Test findings with p values of <0.05 were considered statistically significant.

3. Results

The general characteristics of the 72 sex- and age matched participants are shown in Table 1, according to vegan or omnivorous diet (n = 18 men and 18 women each). The median duration of veganism was 4.8 years (IQR: 3.1–8.7). Median age was 37.5 years (range 30–57) in vegans and 38.5 years (range 30–57) in omnivores, respectively. No relevant differences in anthropometric measurements, physical activity, smoking, education, were observed between the groups. Regarding occupational activity, 16.7% (n = 6) of vegans and 8.4% (n = 3) of omnivores reported a high level of intensive occupational activity. 61.1% (n = 22) of vegans and 77.8% (n = 28) of omnivores stated a high level of sedentary occupational activity. None of the participants took any lipid-lowering medications.

The following PFAS were analyzed: PFOS, PFOA, PFHxS, PFNA, PFDA, PFBS, PFHxA, PFDoDA and ADONA. Levels of the main four contaminants, PFOS, PFOA, PFHxS and PFNA are given in Table 2. PFOS and PFOA were quantifiable in all the 72 participants, whereas PFHxS and PFNA were below the LOQ in two samples and 22 samples, respectively. In case of PFDA, 58 samples were below the LOQ. Of the 14 samples quantifiable (range of 0.26–0.49 ng/ml), 13 were from omnivores. These values were not considered in the following evaluation. Levels of the other four compounds (PFBS, PFHxA, PFDoDA and ADONA) were not found above the LOQ.

Table 1
Characteristics of the study population according to vegan or omnivorous diet.

	Vegans (n = 36)	Omnivores (n = 36)	*p-value*
Duration vegan diet [years]	4.8 (3.1–8.7)		
Men	50.0% (18)	50.0% (18)	
Age [years]	37.5 (32.5–44.0)	38.5 (32.0–46.0)	*0.75*
Anthropometry			
BMI [kg/m^2]	22.9 ± 3.2	24.0 ± 2.1	*0.08*
Waist circumference [cm]			
Women	73.1 ± 6.9	77.2 ± 6.2	*0.07*
>80	8.3% (3)	13.9% (5)	
Men	84.5 ± 8.9	86.0 ± 6.1	*0.56*
>94	5.6% (2)	5.6% (2)	
Education [%]			*0.60*
Low	0.0% (0)	2.8% (1)	
Intermediate	30.6% (11)	30.6% (11)	
High	69.4% (25)	66.7% (24)	
Lifestyle			
Physical Activity [h/week]	2.8 (0.88–3.75)	2.3 (1.2–4.1)	*0.69*
Walking [h/week]	7.0 (5.0–12.0)	5.5 (3.5–11.8)	*0.15*
Smoking status			*0.30*
Non-smoker	66.7% (24)	58.3% (21)	
Ex-Smoker	22.2% (8)	16.7% (6)	
Smoker	11.1% (4)	25.0% (9)	
Blood lipids			
Total cholesterol [mg/dl]	157.0 (137.0–180.5)	203.5 (178.5–222.5)	<0.0001
HDL cholesterol [mg/dl]	56.5 (50.5–71.5)	61.5 (51.5–80.5)	0.21
LDL cholesterol [mg/dl]	86.5 (68.5–97.0)	115.5 (93.5–136.0)	0.001
Triglyceride [mg/dl]	71.0 (53.0–90.5)	85.0 (52.0–120.5)	0.26

Variables expressed as percentage (n), mean ± SD or median (IQR).

Table 2
PFAS according to a vegan or omnivorous diet (n = 72).

	Vegans (n = 36)		Omnivores (n = 36)		
	Median (IQR)	Min - Max	Median (IQR)	Min - Max	*p-value*
PFOS [ng/ml]	*n > LOQ: 36*		*n > LOQ: 36*		
All (n = 36)	2.31 (1.37–3.59)	0.34–6.70	3.57 (1.94–5.14)	0.84–11.1	*0.02*
Men (n = 18)	2.31 (1.37–4.47)	0.34–6.70	4.65 (3.28–5.86)	0.84–10.8	*0.04*
Women (n = 18)	2.31 (1.38–2.75)	0.59–6.36	2.62 (1.87–3.96)	1.38–11.1	*0.21*
PFOA [ng/ml]	*n > LOQ: 36*		*n > LOQ: 36*		
All (n = 36)	1.69 (1.35–2.75)	0.26–4.24	1.44 (0.98–2.61)	0.62–4.65	*0.26*
Men (n = 18)	1.66 (1.46–2.79)	0.26–4.24	1.68 (1.18–2.92)	0.62–4.65	*0.73*
Women (n = 18)	1.75 (1.28.2.10)	0.72–3.80	1.18 (0.92–2.04)	0.64–3.35	*0.23*
PFHxS [ng/ml]	*n > LOQ: 35*		*n > LOQ: 35*		
All (n = 36)	1.96 (0.88–3.75)	<LOQ –11.2	1.79 (0.92–2.74)	<LOQ – 6.09	*0.70*
Men (n = 18)	2.14 (1.06–3.76)	<LOQ – 8.97	1.93 (1.33–2.70)	0.38–5.08	*0.76*
Women (n = 18)	1.74 (0.69–3.74)	0.26–11.2	1.79 (0.85–3.11)	<LOQ– 6.09	*0.83*
PFNA [ng/ml]	*n > LOQ: 16*		*n > LOQ: 34*		
All (n = 36)	<LOQ (<LOQ -0.30)	<LOQ – 0.49	0.41 (0.33–0.58)	<LOQ - 1.05	*<0.0001*
Men (n = 18)	<LOQ (<LOQ -0.30)	<LOQ – 0.42	0.49 (0.39–0.65)	<LOQ– 1.05	*<0.0001*
Women (n = 18)	<LOQ (<LOQ -0.30)	<LOQ – 0.49	0.35 (0.29–0.48)	<LOQ– 0.92	*0.003*
PFAS sum [ng/ml]					
All (n = 36)	6.41 (4.08–9.38)	0.84–21.4	7.65 (5.02–11.1)	2.02–21.6	*0.33*
Men (n = 18)	6.93 (3.98–9.96)	0.84–18.5	8.85 (7.28–12.0)	2.02–21.6	*0.18*
Women (n = 18)	5.96 (4.18–8.25)	1.78–21.4	6.18 (4.70–8.55)	2.44–20.6	*0.80*

LOQ: limit of quantification. PFAS sum: PFOS + PFOA + PFHxS + PFNA.

In the total study population (n = 72), median concentrations (IQR) of PFOS: 2.71 ng/ml (1.64–4.67), PFOA: 1.62 ng/ml (1.14–2.71), PFHxS: 1.84 ng/ml (0.91–3.19) and PFNA: 0.32 ng/ml (<0.25–0.44) were observed. The median concentration (IQR) of the PFAS sum was 7.05 ng/ml (4.72–9.85). The distributions of plasma levels of PFAS were skewed, as depicted in Fig. 1 for the lead compounds PFOS and PFOA in vegans and omnivores. According to the dietary group, data on plasma levels of the all four PFAS evaluated are compiled in Table 2 for the whole study group and separately for men and women. Regarding PFOA, no significant differences were observed between vegans and omnivores (p = 0.26), however, vegans were with tendency more likely to have higher PFOA concentrations compared to omnivores. In case of PFOS, the levels were significantly higher in omnivores (median 3.57 ng/ml, IQR: 1.94–5.14) compared to vegans (median 2.31 ng/ml, IQR: 1.37–3.59) (p = 0.02). The strongest difference was seen for PFNA with median values below the LOQ of 0.25 ng/ml (IQR: <0.25–0.30) for vegans compared to 0.41 ng/ml (IQR: 0.33–0.58) for omnivores (p < 0.0001). Of the 22 PFNA measurements below the LOQ, two were from omnivores, and 20 from vegans. No significant differences between vegans and omnivores were seen for PFHxS (p = 0.70) and the PFAS sum (p = 0.33). Considering the duration of the vegan diet, long-time vegans were with tendency more likely of lower level of the PFAS sum (PFOS + PFOA + PFHxS + PFNA, Fig. 2).

Regarding the level of the PFAS sum in women of childbearing age (≤45 years of age), a median value of 5.82 ng/ml (IQR: 4.44–7.76, range 1.78–21.4) was observed (n = 28, 15 vegans and 13 omnivores, median PFAS sum 5.74 vs. 5.91 ng/ml, respectively; p = 0.53). Ten of these 28 women (36%) exceeded the EFSA derived plasma level of 6.9 ng/ml corresponding to the TWI in women at the age of 35 years.

Correlations between PFAS and eight selected food groups are visually summarized in Fig. 3, and specific correlation coefficients are presented in supplemental Table 2. In the total population (n = 72), the strongest correlations were observed between PFOA and water consumption (model 2 correlation coefficient 0.34, p = 0.01, supplemental Table 2). Regarding the consumption of 'meat and meat products' in omnivores (n = 36), the strongest correlations were observed with the concentrations of PFOS (model 2 correlation coefficient 0.38, p = 0.04) and PFNA (model 2 correlation coefficient 0.50, p = 0.01, supplemental Table 2). Supplemental Table 3 shows the intake of the food groups based on FFQ data in vegans and omnivores.

Levels of LDL and total cholesterol were considerably lower in vegans compared to omnivores (Tables 1 and 3). As shown in Table 3, after adjustment of several PFAS (model 2), none of the investigated PFAS, neither the PFAS sum, alter the differences of LDL cholesterol between vegans and omnivores (Table 3) indicating no relevant impact of PFAS on the association between vegans/omnivores and LDL cholesterol. After further adjustment for lifestyle factors (model 3), the difference between both diet groups got smaller, especially omnivores had lower concentrations of LDL cholesterol. Nevertheless, further adjustment of PFAS sum (model 3, Table 3) as well as for PFOS, PFOA, PFHxS or PFNA did not alter the difference of blood lipid concentrations. We observed no relevant linear or non-linear associations between LDL cholesterol and different PFAS (on the log-scale), as depicted by splines in the supplemental Figure 1. Concerning other blood lipids, concentrations of HDL and total cholesterol as well as of triglycerides in vegans and omnivores also did not change after adjustment for different PFAS (data not shown). In line, linear regression analyses detected no associations between PFAS and blood lipids (supplemental Table 4).

4. Discussion

The present cross-sectional study is the first study investigating differences in PFAS levels in vegans compared to omnivores. Significantly lower concentrations of PFOS and PFNA were observed in vegans compared to omnivores. Accordingly, we observed correlations with food groups expected to contribute most to the internal exposure with PFAS. At the present level of internal PFAS exposure, we did not observe relevant associations between PFAS and blood lipids in particular under consideration of the large differences in LDL and total cholesterol levels between vegans and omnivores.

Only very few data of recent years on internal exposure to PFAS are available for German adults. The internal plasma levels of PFAS measured in our total study group (residing in Berlin) were found to be – despite the high proportion of vegans – relatively high. The best

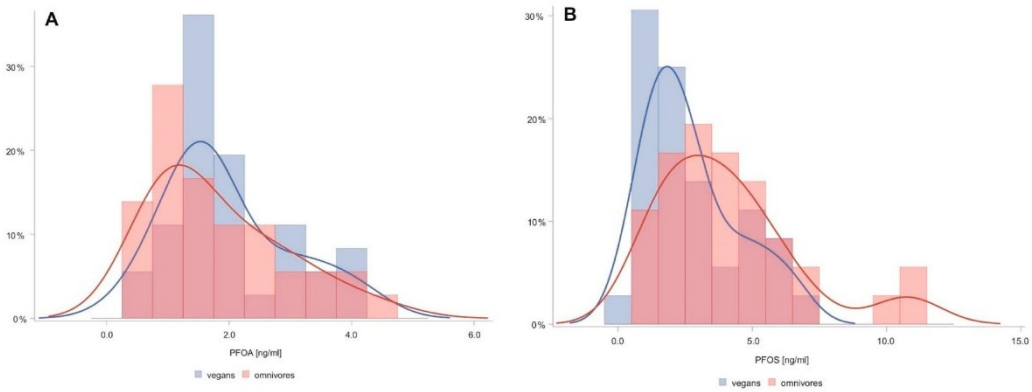

Fig. 1. Distributions of plasma PFOA and PFOS levels according to vegans and omnivores. Distribution of plasma PFOA levels [ng/ml] (A) and PFOS levels [ng/ml] (B) of the study population. Histograms depicted vegans in blue and omnivores in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

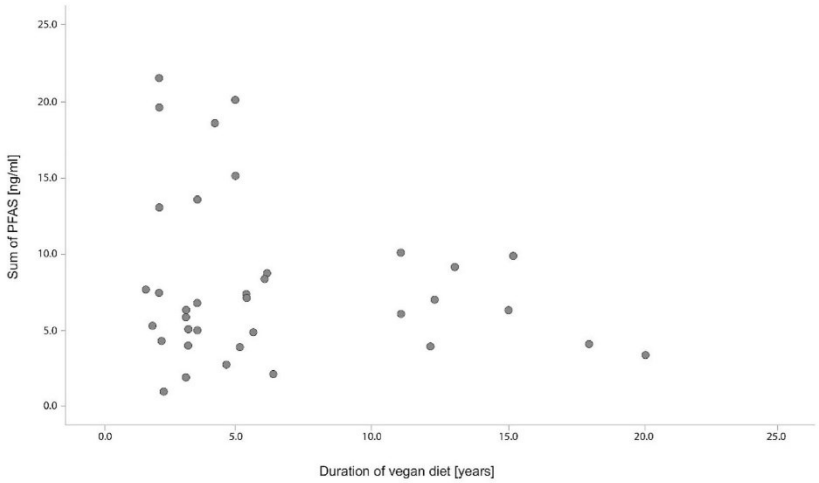

Fig. 2. Scatter plot of sum of PFAS (PFOS + PFOA + PFHxS + PFNA) according to the duration following a vegan diet. Scatter plot of sum of PFAS levels [ng/ml] to the duration of vegan diet [years] in vegans (n = 36).

comparison within Germany is possible with a group from Munich ("Side C", n = 158 blood donors, median age 39.5 years) investigated 2016 (Fromme et al., 2017), serving as a control for a contaminated region in Bavaria/Germany. Results revealed median levels of PFOS, PFOA, PFHxS, PFNA and the PFAS sum to be 2.1, 1.1, 0.5, 0.4 and 4.1 ng/ml, respectively (PFAS sum: personal communication of Prof. Fromme). Furthermore, data are available from the German Environmental Specimen Bank (n = 40 students aged 20–29 years from Münster), with median levels for the 2017/2019 sampling of PFOS, PFOA, PFHxS, PFNA and the PFAS sum of 2.6, 1.7, 0.5, 0.4 and 5.8 ng/ml, respectively (Gockener et al., 2020). The main difference between these investigations and our study are the surprising high levels of PFHxS, with a 3.7-fold higher median in Berlin (PFHxS median: 0.5 vs. 1.8 ng/ml). This may be due to regionally different nutritional habits or to higher concentrations in drinking water as well as due to differences in the socio-economic status. However, sample sizes of all studies were rather small and therefore also a chance finding cannot be excluded.

In the three investigations mentioned from Munich, Münster and Berlin, the sex ratios were exactly or roughly 1:1, and confirmed the higher levels of PFAS in men compared to women observed in many studies. As presented by EFSA, this is due to several factors. Besides a higher external exposure (e.g. due to a higher meat consumption), physiological differences including urinary elimination, menses, and use of oral contraceptives (2 vegans, 6 omnivores) have to be considered, as well as pregnancy and lactation (EFSA Panel on Contaminants in the Food Chain, 2020). In our study, the median levels of the PFAS sum were 8.2 ng/ml in men and 6.0 ng/ml in women. According to the recent risk assessment of EFSA, an internal level of the PFAS sum of up to 6.9 ng/ml corresponds to an external intake below the TWI in women of the childbearing age (EFSA Panel on Contaminants in the Food Chain,

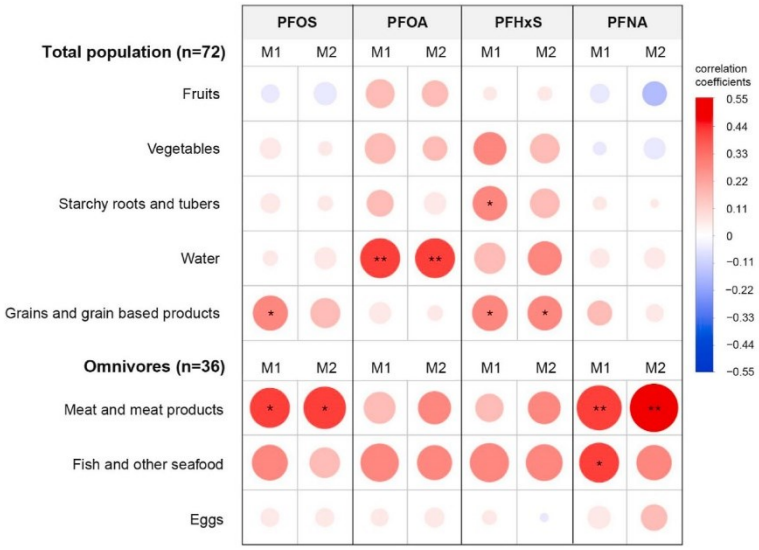

Fig. 3. Heatmap on correlations of 8 relevant food groups with PFAS plasma concentrations. Total population (n = 72) M1: adjusted for type of diet (vegan or omnivores), M2: additional adjustment for age, sex, smoking status, education, waist circumference, and physical activity; Omnivores (n = 36) M1: unadjusted model, M2: additional adjustment for age, sex, smoking status, education, waist circumference, and physical activity; **p < 0.01, *p < 0.05; Water includes drinking water, coffee, tea, and herbal tea.

Table 3
LDL cholesterol concentrations according to a vegan or omnivorous diet (n = 72).

	Vegans (n = 36)	Omnivores (n = 36)	*p-value*
LDL cholesterol [mg/dl]			
Model 1			
Unadjusted	86.3 (77.7–95.9)	110.3 (99.3–122.5)	*0.002*
Model 2			
PFOS	85.9 (77.1–95.8)	110.8 (99.4–123.5)	*0.002*
PFOA	86.2 (77.6–95.9)	110.4 (99.3–122.7)	*0.002*
PFHxS	86.3 (77.7–96.0)	110.3 (99.2–122.6)	*0.002*
PFNA	84.2 (74.6–95.1)	113.1 (100.1–127.7)	*0.003*
PFAS Sum	86.2 (77.6–95.9)	110.4 (99.3–122.8)	*0.002*
Model 3			
Lifestyle factors	86.1 (68.4–108.5)	103.6 (83.0–129.2)	*0.02*
Lifestyle factors + PFOS	85.7 (67.8–108.3)	104.0 (83.2–130.1)	*0.02*
Lifestyle factors + PFOA	86.3 (68.4–109.0)	103.5 (82.8–128.3)	*0.02*
Lifestyle factors + PFHxS	85.6 (67.8–108.0)	102.1 (81.5–128.0)	*0.02*
Lifestyle factors + PFNA	83.3 (65.5–106.0)	106.4 (84.7–133.6)	*0.01*
Lifestyle factors + PFAS Sum	85.7 (68.0–108.2)	103.4 (82.8–129.1)	*0.01*

expressed as geometric mean (95%-CI); Model 1: unadjusted, Model 2: adjusted for several PFAS, Model 3: additional adjusted for lifestyle factors i.e. age, sex, smoking status, education, waist circumference, physical activity; PFAS sum: PFOS + PFOA + PFHxS + PFNA.

2020). For this group (women between 18 and 45 years of age), 10 of 28 women (36%, maximum level 21.4 ng/ml) exceeded the level on our study, while 1 of 52 women exceeded it in Munich 2016 (2%, maximum level 7.2 ng/ml (Fromme et al., 2017)) and 6 of 20 women in Münster 2017/2019 (30%, maximum level 16.3 ng/ml (Gockener et al., 2020). These numbers demonstrate large regional differences in internal exposure to PFAS, and representative studies are necessary to get a more reliable picture of the proportion of women in the German population exceeding EFSA's internal level for the PFAS sum of 6.9 ng/ml corresponding the TWI.

The study detected significant differences in PFOS and PFNA concentrations between both diet groups, showing 54% and 240% higher median concentrations in omnivores compared to vegans, respectively. Obviously, this is due to the relatively high PFAS concentrations in food products of animals. According to the exposure assessment of EFSA (Annex A) for German Adults (EFSA Panel on Contaminants in the Food Chain, 2020), 'Fish and other seafood' was the most important contributor to the mean LB exposure, followed by 'Meat and meat products', 'Fruit and fruit products' and 'Eggs and egg products' in case of PFOS. In case of PFNA, 'Fruit and fruit products' and 'Fish and other seafood' were the most important contributors to the mean LB exposure. Therefore, the differences observed between vegans and omnivores are obviously better to explain by the diet in case of PFOS than in case of PFNA. Regarding the heat map generated from the FFQ data (Fig. 3), the pattern of PFOS and PFNA in omnivores seem comparable, with highest correlations for 'Meat and meat products' and 'Fish and other seafood'. However, due to the small number of participants of the study, the nutritional data from the FFQ with respect to the internal exposure to PFAS should be interpreted with caution. Nevertheless, our results are in line with another study. Lin et al. noticed that participants (n = 941 adults with pre-diabetes) with high consumption of meat, fried fish, and other fish/shellfish (but not omega-3 rich fish) had higher plasma concentrations of PFOS, PFHxS and PFNA (Lin et al., 2020).

Currently, PFAS levels in many food groups are found to be nearly completely below the presently available LOQs (EFSA Panel on Contaminants in the Food Chain, 2020). Therefore, more sensitive analytical methods are needed for the quantification of PFAS in foods. A higher proportion of quantified PFAS levels in food groups may lead to a better estimation of the contributions of different food groups to the exposure of different PFAS via food consumption and possibly to a change of the pattern of these contributions especially in case of PFNA.

Regarding PFOA and PFHxS in the two diet groups, no relevant differences were detected in vegans compared to omnivores. In case of PFOA, this is surprising, as EFSA identified 'Fish and other seafood', 'Eggs and egg products' as well as 'Meat and meat products' as most

important contributors to the mean LB exposure of PFOA (and PFOS) (EFSA Panel on Contaminants in the Food Chain, 2020). Interestingly, besides the above mentioned food categories, 'Alcoholic beverages' and 'Drinking water' were reported to be also important contributors to the mean LB exposure in case of PFOA in Germany (EFSA Panel on Contaminants in the Food Chain, 2020). Indeed, PFOA levels were found to have the highest correlations with the consumption of 'Water' in the total study group (see heat map, Fig. 3). In case of PFHxS, 'Fruit and fruit products', 'Alcoholic beverages' and 'Drinking water' were the only contributors to the mean LB exposure in Germany (EFSA Panel on Contaminants in the Food Chain, 2020). This is reflected in the heat map in case of 'Water' only, but as in case of PFNA, more sensitive analytical methods may change the pattern of the contributors to external exposure.

Epidemiological studies provided consistent findings of associations between serum levels of PFOS/PFOA and levels of cholesterol in populations with relatively high exposure (EFSA Panel on Contaminants in the Food Chain, 2020). In 2018, EFSA even used these associations with serum cholesterol levels to derive TWIs for both PFOS and PFOA (EFSA Panel on Contaminants in the Food Chain, 2018). However, a clear mode of action is missing. Since lower LDL cholesterol levels in vegans are one of the most highlighted health benefits of this diet (Benatar and Stewart, 2018), we thought to also analyze the relation between different PFAS and LDL cholesterol in our small study sample. Although a wide range of concentrations of LDL cholesterol was observed in our study population, we did not find any relevant relation between PFAS and LDL cholesterol. This may be due to the relatively low concentrations of PFAS in comparison to previous studies (EFSA Panel on Contaminants in the Food Chain, 2020). The distinctly lower levels of LDL cholesterol in vegans with concurrently lower levels of PFOS and PFNA strikingly raises the issue of confounding by diet in case of the above-mentioned epidemiological studies, and the extent of necessary adjustment to avoid false interpretations. Interestingly, in most studies investigating associations between PFAS and blood lipids, statistical models were adjusted – beside age, sex, smoking, alcohol intake and education – only for body mass index or waist circumference (EFSA Panel on Contaminants in the Food Chain, 2018). Only very few studies adjusted for further dietary variables such as saturated or animal fat intake (Eriksen et al., 2013; Nelson et al., 2010) or food groups such as meat or fish intake (Canova et al., 2020; Lin et al., 2020; Skuladottir et al., 2015) or healthy diet score (Donat-Vargas et al., 2019). Taking altogether, evidence for a causal relationship between PFAS and blood lipids is still not convincing and residual confounding still cannot be completely excluded to explain the observed associations between PFAS and blood lipids in many but not all studies. Further studies are necessary to clarify at one hand possible mechanisms and to investigate associations with improved statistical models including more potential confounders in particular dietary factors on the other hand.

Different methods exist for dietary assessments, and each has its advantages and disadvantages. Because of the long half-life time of PFAS, FFQ might be a good method assessing the past long-term diet. The EPIC-FFQ (Nothlings et al., 2007) captured the time span of the previous 12 months. We see the limitation of the present FFQ, which did not cover all variety of a vegan diet, as some food groups will not be fully assessed, thus the diet of vegans might be underestimated. However, in the present evaluation we analyzed only predetermined food groups, eaten by both omnivores and vegans, for example fruits, vegetables or water. Therefore, the use of the FFQ for our study purpose is acceptable. For the food groups of meat, fish or eggs, the analyses were only performed in omnivores to avoid bias due to non-consumption of the vegan population. Nevertheless, this study underlines the need for assessment tools in science for a past long-term diet also for participants following a plant-based diet (e.g. vegan, vegetarian) or the modification of already existing tools.

Limitations of our study deserve to be mentioned. The present RBVD study is relatively small (n = 72), including middle aged vegans and omnivores from a relatively small area (Berlin, Germany); therefore, the results may not be generalizable to other populations. Nevertheless, the RBVD study provides comprehensive high-quality data as a result of the standardized procedures in combination with extensive information from computer-based questionnaires and anthropometric measurements. Regarding FFQ, we see also some limitations concerning recall bias and under/over-reporting, attributed to reliance on participant's memory, inability to accurately estimate portion sizes and misinterpretation of the questions, or social desirability bias (Hooson Jzh et al., 2020). Other routes of exposure than diet such as ingestion of house dust, inhalation of indoor air, and dermal absorption may substantially contribute to the exposure to PFAS on an individual basis (Poothong et al., 2020), but these routes could not be considered in our study. Further, some packing materials and take-away food, as well as kitchen utensils might be potential sources of exposure to PFAS.

5. Conclusion

Lower levels of PFOS and PFNA, but not of PFOA and PFHxS were observed in vegans compared to omnivores. FFQ data allowed the identification of relevant food groups contributing to the levels of these four PFAS. The strong impact of a vegan diet on levels of blood lipids, especially on LDL cholesterol, was confirmed in our study. In contrast, the association of PFAS and LDL cholesterol was found to be negligible, possibly due to the relatively low levels of PFAS observed. However, we highlight the importance of the adjustment of dietary factors like a vegan diet in case of epidemiological studies dealing with the impact of PFAS on the levels of blood lipids.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2021.113808.

References

Abraham, K., Mielke, H., Fromme, H., Volkel, W., Menzel, J., Peiser, M., Zepp, F., Willich, S.N., Weikert, C., 2020. Internal exposure to perfluoroalkyl substances (PFASs) and biological markers in 101 healthy 1-year-old children: associations between levels of perfluorooctanoic acid (PFOA) and vaccine response. Arch. Toxicol. 94, 2131–2147.

Benatar, J.R., Stewart, R.A.H., 2018. Cardiometabolic risk factors in vegans; A meta-analysis of observational studies. PloS One 13, e0209086.

Canova, C., Barbieri, G., Zare Jeddi, M., Gion, M., Fabricio, A., Dapra, F., Russo, F., Fletcher, T., Pitter, G., 2020. Associations between perfluoroalkyl substances and lipid profile in a highly exposed young adult population in the Veneto Region. Environ. Int. 145, 106117.

Desquilbet, L., Mariotti, F., 2010. Dose-response analyses using restricted cubic spline functions in public health research. Stat. Med. 29, 1037–1057.

Donat-Vargas, C., Bergdahl, I.A., Tornevi, A., Wennberg, M., Sommar, J., Koponen, J., Kiviranta, H., Akesson, A., 2019. Associations between repeated measure of plasma perfluoroalkyl substances and cardiometabolic risk factors. Environ. Int. 124, 58–65.

EFSA Panel on Contaminants in the Food Chain, Knutsen, H.K., Alexander, J., Barregard, L., Bignami, M., Bruschweiler, B., Ceccatelli, S., Cottrill, B., Dinovi, M., Edler, L., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L.R., Nebbia, C.S., Oswald, I.P., Petersen, A., Rose, M., Roudot, A.C., Vleminckx, C., Vollmer, G., Wallace, H., Bodin, L., Cravedi, J.P., Halldorsson, T.I., Haug, L.S., Johansson, N., van

Loveren, H., Gergelova, P., Mackay, K., Levorato, S., van Manen, M., Schwerdtle, T., 2018. Risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food. EFSA J 16, e05194.
EFSA Panel on Contaminants in the Food Chain, Schrenk, D., Bignami, M., Bodin, L., Chipman, J.K., Del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L.R., Leblanc, J.C., Nebbia, C.S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Vleminckx, C., Wallace, H., Barregard, L., Ceccatelli, S., Cravedi, J.P., Halldorsson, T.I., Haug, L.S., Johansson, N., Knutsen, H.K., Rose, M., Roudot, A.C., Van Loveren, H., Vollmer, G., Mackay, K., Riolo, F., Schwerdtle, T., 2020. Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA J 18, e06223.
Eriksen, K.T., Raaschou-Nielsen, O., McLaughlin, J.K., Lipworth, L., Tjonneland, A., Overvad, K., Sorensen, M., 2013. Association between plasma PFOA and PFOS levels and total cholesterol in a middle-aged Danish population. PloS One 8, e56969.
Frisbee, S.J., Shankar, A., Knox, S.S., Steenland, K., Savitz, D.A., Fletcher, T., Ducatman, A.M., 2010. Perfluorooctanoic acid, perfluorooctanesulfonate, and serum lipids in children and adolescents: results from the C8 Health Project. Arch. Pediatr. Adolesc. Med. 164, 860–869.
Fromme, H., Wockner, M., Roscher, E., Volkel, W., 2017. ADONA and perfluoroalkylated substances in plasma samples of German blood donors living in South Germany. Int. J. Hyg Environ. Health 220, 455–460.
Gockener, B., Weber, T., Rudel, H., Bucking, M., Kolossa-Gehring, M., 2020. Human biomonitoring of per- and polyfluoroalkyl substances in German blood plasma samples from 1982 to 2019. Environ. Int. 145, 106123.
Hooson Jzh, J., Hutchinson Jyh, J., Warthon-Medina, M., Hancock, N., Greathead, K., Knowles, B., Vargas-Garcia, E., Gibson, L.E., Bush, L.A., Margetts, B., Robinson, S., Ness, A., Alwan, N.A., Wark, P.A., Roe, M., Finglas, P., Steer, T., Page, P., Johnson, L., Roberts, K., Amoutzopoulos, B., Burley, V.J., Greenwood, D.C., Cade, J. E., 2020. A systematic review of reviews identifying UK validated dietary assessment tools for inclusion on an interactive guided website for researchers. Crit. Rev. Food Sci. Nutr. 60, 1265–1289. www.nutritools.org.
InterAct, C., Peters, T., Brage, S., Westgate, K., Franks, P.W., Gradmark, A., Tormo Diaz, M.J., Huerta, J.M., Bendinelli, B., Vigl, M., Boeing, H., Wendel-Vos, W., Spijkerman, A., Benjaminsen-Borch, K., Valanou, E., de Lauzon Guillain, B., Clavel-Chapelon, F., Sharp, S., Kerrison, N., Langenberg, C., Arriola, L., Barricarte, A., Gonzales, C., Grioni, S., Kaaks, R., Key, T., Khaw, K.T., May, A., Nilsson, P., Norat, T., Overvad, K., Palli, D., Panico, S., Ramon Quiros, J., Ricceri, F., Sanchez, M.J., Slimani, N., Tjonneland, A., Tumino, R., Feskins, E., Riboli, E., Ekelund, U., Wareham, N., 2012. Validity of a short questionnaire to assess physical activity in 10 European countries. Eur. J. Epidemiol. 27, 15–25.
Janssen, M., Busch, C., Rodiger, M., Hamm, U., 2016. Motives of consumers following a vegan diet and their attitudes towards animal agriculture. Appetite 105, 643–651.
Lin, P.D., Cardenas, A., Hauser, R., Gold, D.R., Kleinman, K.P., Hivert, M.F., Fleisch, A.F., Calafat, A.M., Sanchez-Guerra, M., Osorio-Yanez, C., Webster, T.F., Horton, E.S., Oken, E., 2020. Dietary characteristics associated with plasma concentrations of per- and polyfluoroalkyl substances among adults with pre-diabetes: cross-sectional results from the Diabetes Prevention Program Trial. Environ. Int. 137, 105217.
Menzel, J., Abraham, K., Stangl, G.I., Ueland, P.M., Obeid, R., Schulze, M.B., Herter-Aeberli, I., Schwerdtle, T., Weikert, C., 2021. Vegan diet and bone health-results from the cross-sectional RBVD study. Nutrients 13.
Menzel, J., Biemann, R., Longree, A., Isermann, B., Mai, K., Schulze, M.B., Abraham, K., Weikert, C., 2020. Associations of a vegan diet with inflammatory biomarkers. Sci. Rep. 10, 1933.
Mosch, C., Kiranoglu, M., Fromme, H., Volkel, W., 2010. Simultaneous quantitation of perfluoroalkyl acids in human serum and breast milk using on-line sample preparation by HPLC column switching coupled to ESI-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci 878, 2652–2658.
Nelson, J.W., Hatch, E.E., Webster, T.F., 2010. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population. Environ. Health Perspect. 118, 197–202.
Nothlings, U., Hoffmann, K., Bergmann, M.M., Boeing, H., 2007. Fitting portion sizes in a self-administered food frequency questionnaire. J. Nutr. 137, 2781–2786.
Poothong, S., Papadopoulou, E., Padilla-Sanchez, J.A., Thomsen, C., Haug, L.S., 2020. Multiple pathways of human exposure to poly- and perfluoroalkyl substances (PFASs): from external exposure to human blood. Environ. Int. 134, 105244.
Skuladottir, M., Ramel, A., Rytter, D., Haug, L.S., Sabaredzovic, A., Bech, B.H., Henriksen, T.B., Olsen, S.F., Halldorsson, T.I., 2015. Examining confounding by diet in the association between perfluoroalkyl acids and serum cholesterol in pregnancy. Environ. Res. 143, 33–38.
Steenland, K., Tinker, S., Frisbee, S., Ducatman, A., Vaccarino, V., 2009. Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. Am. J. Epidemiol. 170, 1268–1278.
Sunderland, E.M., Hu, X.C., Dassuncao, C., Tokranov, A.K., Wagner, C.C., Allen, J.G., 2019. A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. J. Expo. Sci. Environ. Epidemiol. 29, 131–147.
Weikert, C., Trefflich, I., Menzel, J., Obeid, R., Longree, A., Dierkes, J., Meyer, K., Herter-Aeberli, I., Mai, K., Stangl, G.I., Muller, S.M., Schwerdtle, T., Lampen, A., Abraham, K., 2020. Vitamin and mineral status in a vegan diet. Dtsch Arztebl Int 117, 575–582.
Yokoyama, Y., Levin, S.M., Barnard, N.D., 2017. Association between plant-based diets and plasma lipids: a systematic review and meta-analysis. Nutr. Rev. 75, 683–698.

3. Diskussion

Die dargestellten Arbeiten untersuchten umfassend eine vegane Ernährungsweise, im Vergleich zu einer Mischkost, in Hinblick auf Unterschiede verschiedener inflammatorischer Biomarker, der Knochengesundheit und interner PFAS Exposition. Als ein weiterer Schwerpunkt wurden auch ernährungsbedingte und knochenrelevante Biomarker näher beleuchtet. Dabei wurden nicht nur Unterschiede in diesen Biomarkern zwischen beiden Ernährungsgruppen untersucht, sondern auch der Einfluss eines abgeleiteten explorativen Biomarkermusters auf die Knochengesundheit. Grundlage für die vorgestellten Arbeiten war die RBVD-Studie. Diese Studie konnte in den letzten Jahren trotz geringer Studiengröße zu verschiedensten Forschungsfragen erste Evidenz generieren.

3.1. Vegane und vegetarische Ernährungsformen und inflammatorische Biomarker

Es gilt als bekannt, dass low-grade Inflammationen mit einem erhöhten Risiko für verschiedene chronische Krankheiten assoziiert ist [17,19,20]. Zudem wird berichtet, dass Menschen, die einer veganen oder vegetarischen Ernährung folgen, ein verringertes Risiko für T2D, Herz-Kreislauferkrankungen oder für bestimmte Krebserkrankungen haben [11]. Daher wird diskutiert, dass ein vorteilhaftes inflammatorisches Biomarkerprofil von Menschen mit pflanzenbetonten Ernährungsformen als intermediärer Risikofaktor einen protektiven Einfluss auf das Erkrankungsrisiko haben könnte [72,73].

Mit dem Hintergrund, dass vegane Ernährungsweisen in Deutschland, als auch global immer beliebter werden, ist diese Ernährungsform in wissenschaftlichen Studien in Hinblick auf inflammatorische Biomarker nur wenig untersucht. Tatsächlich zeigte das systematische Review (Originalarbeit 2), das nur drei wissenschaftliche Publikationen [72,76,77] inflammatorische Biomarker mit veganer Ernährungsweise untersuchten [73]. Eine davon ist die RBVD-Studie, deren Ergebnisse ausführlich in Originalpublikation 1 beschrieben wurden und nachfolgend in das systematische Review eingeflossen sind [72]. Die RBVD-Studie stellt damit die umfangreichste Studie zu diesem Thema dar, denn hier wurden sieben inflammatorische Biomarker in Blutproben von Veganer:innen und Mischköstler:innen untersucht [72]. In den anderen beiden Publikationen wurde in kleinen veganen Untergruppen von Šebeková et al. (n=9) [77] nur CRP und von Franco-De-Moraes et al. (n=66) [76] drei inflammatorische Biomarker (CRP, E-Selektin, TNF-α) untersucht. Dabei zeigte Franco-de-Moraes et al. [76] höhere CRP-Werte bei Mischköstler:innen im Vergleich zu Veganer:innen, dagegen konnte Šebeková et al. [77] keine Unterschiede in den CRP-Werten feststellen. Auch wenn die RBVD-Studie tendenziell niedrigere CRP-Werte bei Veganer:innen feststellen konnte, erreichte dieser Unterschied im adjustierten Modell keine statistische Signifikanz [72]. Die gepoolten Ergebnisse dieser Einzelstudien zeigten jedoch in der Metaanalyse

(Originalpublikation 2), dass eine vegane Ernährungsweise mit niedrigeren CRP-Werten im Vergleich zu Mischköstler:innen assoziiert war [73].

Die vegetarische Ernährungsweise ist deutlich umfangreicher wissenschaftlich untersucht. So konnte das systematischen Review (Originalarbeit 2) insgesamt 14 Studien [72,76-88] einschließen, die inflammatorische Biomarker und Vegetarismus bei gesunden Personen untersucht haben [73]. Auch hier konnte eine Metaanalyse zeigen, dass eine vegetarische Ernährungsweise mit niedrigen CRP-Werten assoziiert war [73]. Weitere Metaanalysen, die pflanzenbetonte Ernährungsformen [26] bzw. vegetarisch-betonte Ernährungsmuster [29] untersuchten, zeigten ebenfalls niedrigere CRP-Werte [26,29]. Auch diese beiden Übersichtsarbeiten konnten keine umfassenden inflammatorische Profile untersuchen, weil andere inflammatorische Biomarker, neben CRP, wenig bis gar nicht in Einzelstudien untersucht wurden [26,29].

Ferner untersuchte Haghighatdoost et al. [28] in einem systematischen Review vegetarische Ernährungsformen und inflammatorische Biomarker. Die Autor:innen betonen hierbei, dass die Dauer der vegetarischen Ernährungsform einen Einfluss auf die Assoziation haben könnte [28]. So zeigten die Autor:innen, dass Vegetarier:innen, die sich seit mindestens 2 Jahren vegetarisch ernährten, signifikant niedrigere CRP-Konzentrationen im Vergleich zu Mischköstler:innen hatten [28]. Keine Assoziation wurde jedoch bei vegetarischen Studienteilnehmenden im Vergleich zu einer Mischkostgruppe beobachtet, die sich weniger als 2 Jahre, aber mindestens 6 Monate vegetarisch ernährten. [28]. Im Einklang mit diesen Beobachtungen zeigte auch die Sensitivitätsanalysen der Metaanalyse, dass Veganer:innen, die sich über 10 Jahre vegetarischen ernährten, im Vergleich zu Mischköstler:innen signifikant niedrigere CRP-Konzentrationen hatten [73]. Die mittlere Mittelwertdifferenz war bei Veganer:innen mit einer veganen Ernährung weniger als 10 Jahren nicht signifikant [73]. Auch die RBVD-Studie zeigte, dass Veganer:innen, die bereits mehr als 4.8 Jahren vegan lebten, tendenziell niedrigeren hsCRP-Wert hatten, im Vergleich zu Veganer:innen, die sich ≤ 4.8 Jahre vegan ernährten [72].

Zudem ist wichtig darauf hinzuweisen, dass Übergewicht und Adipositas mit erhöhten Konzentrationen inflammatorischer Biomarker assoziiert sind und die inflammatorischen Prozesse weiterführend auch in Verbindung mit dem erhöhten Risiko für chronische Erkrankungen dieser Bevölkerungsgruppe stehen [89]. In Übereinstimmung zeigte die RBVD-Studie, dass sowohl der BMI, als auch der Taillenumfang mit fast allen untersuchten inflammatorischen Biomarkern korrelierten, auch wenn adipöse Personen nicht bei der RBVD-Studie teilnehmen konnten (Ausschlusskriterium BMI ≥ 30 kg/m^2) [72]. In Originalarbeit 2 wurde eine mögliche Effektmodifikation durch den BMI auf die Assoziation zwischen einer veganen

Ernährungsweise und niedrigere CRP-Werten, im Vergleich zu Mischköstler:innen, durch eine Meta-Regression untersucht [73]. Es konnte jedoch keine Modifikation aufzeigt werden [73].

Insbesondere für vorerkrankte Populationen konnte in der Originalarbeit 2 (Metaanalyse) gezeigt werden, dass bei Patient:innen mit eingeschränkter Nierenfunktion eine vegetarische Ernährungsform stark mit niedrigen CRP-Konzentrationen assoziiert war [73]. Pflanzenbasierte Ernährungsweisen könnten Vorteile für diese vorerkrankte Personengruppen haben [73]. Tatsächlich werden pflanzenbasierte Ernährungsformen neben präventiven auch als therapeutische Maßnahmen bei zahlreichen chronischen Erkrankungen diskutiert. So wird vermutet, dass eine pflanzenbasierte Ernährung durch die verbundenen kardioprotektiven, antioxidativen und lipidsenkenden Eigenschaften die Entwicklung oder das Fortschreiten einiger Komplikationen bei einer chronischen Nierenerkrankung hemmt [90]. Dies könnte auch für andere kardiometabolische Erkrankungen gelten. So zeigten sich bei einer vegetarischen oder veganen Ernährung potenzielle Vorteile bei der Behandlung von T2D [91,92] sowie zusätzliche Vorteile in Hinblick auf Komorbiditäten, wie Herz-Kreislauf-Erkrankungen, Nierenerkrankungen und Neuropathie [91].

3.2. Vegane Ernährung und Knochengesundheit

Im Vergleich zu den inflammatorischen Biomarkern ist die Knochengesundheit in Hinblick auf eine pflanzenbasierte Ernährungsweise umfangreicher untersucht. Tatsächlich untersuchten bisher fünf (systematische) Übersichtsarbeiten [32-36] die Knochengesundheit (Knochendichte und/oder Frakturrate) in Hinblick auf Vegetarismus und Veganismus, davon auch drei mit Metaanalyse [34-36]. Bereits die erste Metaanalyse von Ho-Pham et al. aus dem Jahr 2009 berichtete, dass Veganer:innen und Vegetarier:innen eine niedrigere Knochendichte aufwiesen [35]. Die stärksten Assoziationen zeigten sich dabei für Veganer:innen im Vergleich zu Mischköstler:innen [35]. Drei weitere (systematische) Übersichtsarbeiten [33,34,36] berichteten ebenfalls über eine niedrigere Knochendichte bei Veganer:innen und Vegetarier:innen im Vergleich zur Mischkostgruppe, auch hier zeigten sich die stärksten Effektstärken bei den Veganer:innen [34,36]. Iquacel et al. zeigte zudem, dass Veganer:innen auch höhere Frakturraten hatten [36]. Diese Ergebnisse werden gestützt von einem aktuellen Review aus dem Jahr 2022 [32]. Die Ergebnisse der RBVD-Studie stehen im Einklang mit den Übersichtsarbeiten, dabei berücksichtigen diese Reviews fast ausschließlich Studien, die die Knochendichte mit der Dual-Energy-Röntgen-Absorptiometrie (DEXA) bestimmt haben [32-36]. Es wurden keine Studien eingeschlossen, die die Knochengesundheit auf Basis von Ultraschall basierenden Messmethoden untersuchten [32-36].

In Übereinstimmung mit diesen Übersichtsarbeiten zeigte jedoch auch unsere RBVD-Studie niedrige Werte in allen gemessenen Parametern des quantitativen Ultraschalls in der

Veganergruppe, auch wenn nur der wichtigste Parameter (BUA) statistische Signifikanz erreichte [46]. In unserer RBVD-Studie wurde die Knochengesundheit mittels QUS am Fersenbein erfasst [46]. Hierbei ist deutlich zu betonen, dass DEXA den Goldstandard für die Messung der Knochendichte sowie der Osteoporose-Diagnose darstellt [93]. Da die Parameter der QUS nicht nur ein Indikator für die Knochendichte sind, sondern auch für die Knochenarchitektur und die Elastizität [94,95], wird auf den Begriff der Knochendichte explizit im Rahmen der RBVD-Studie und der vorliegenden Arbeit verzichtet. Wissenschaftliche Studien zeigten jedoch, dass die auf Ultraschall basierenden Messmethoden eine ähnlich gute Risikoprädiktion in Hinblick auf das Frakturrisiko erlauben [96-98]. Gerade im Rahmen von epidemiologischen Studien, stellt QUS somit eine kostengünstige, einfache und schnelle Alternative zur Beurteilung der Knochengesundheit dar, die vor allem ohne Strahlung auskommt [46].

3.2.1. Ernährungsbedingte und knochenrelevante Biomarkern

Ein aktuelles systematisches Review aus dem Jahr 2021 untersuchte die Nährstoffzufuhr von Veganer:innen in Europa [10]. Das Review zeigte, dass Veganismus mit einer geringeren Aufnahme von Proteinen, verschiedener Vitamine (B2, Niacin (B3), B12, D) sowie Mineralstoffen und Spurenelementen Calcium, Kalium Jod, Zink und Selen assoziiert ist [10]. Zudem wurden auch Unterschiede in der Aufnahme von verschiedenen Fettsäuren gezeigt [10]. Diese Ergebnisse decken sich in weiten Teilen mit den potenziell kritischen Nährstoffen laut der DGE im Rahmen einer veganen Ernährung [8]. Auch die RBVD-Studie untersuchte den Versorgungsstatus bei veganer Ernährungsweise in Vergleich zu Mischköstler:innen [71]. Dabei wurde zum einen die Nährstoffzufuhr von einer Vielzahl von Makro- und Mikronutrienten aus der Nahrung (3-Tage-Wiegeprotokolle) und zum anderen die Nährstoffversorgung mittels Biomarker (Blut und Urin) untersucht [46,71]. In Hinblick auf die Nährstoffzufuhr aus der Nahrung entsprechen die Ergebnisse der RBVD-Studie in weiten Teilen den Ergebnissen des aktuellen systematischen Reviews [10]. Es zeigte sich, dass bei Veganer:innen höhere Aufnahmemengen von Ballaststoffen, aber auch von verschiedenen Vitaminen (Vitamin B6, E, K, Folat) sowie Eisen hatten [71]. Die Aufnahme von Vitamin B2, B3, B12, D sowie den Mineralstoffen Calcium und Jod war dagegen geringer im Vergleich zu der Mischkostgruppe [71]. Herauszuheben ist dabei, dass die Ergebnisse der Nährstoffzufuhr aus der Nahrung und die Nährstoffversorgung durch die Biomarker im Blut/Urin nicht in allen Nährstoffen deckungsgleich waren [71]. Tatsächlich zeigten sich u.a. im Vitamin B12- und Vitamin D-Status keine Unterschiede im Gruppenvergleich [71]. Das ist interessant, denn Vitamin B12 gilt als kritischster Nährstoff bei einer veganen Ernährungsweise [8,9]. Auf Basis der 3-Tage-Wiegeprotokolle wird das auch durch Daten der RBVD-Studie bestätigt, denn Vegan:innen hatten tatsächlich eine niedrige Zufuhr durch die Nahrung im Vergleich zur Mischkostgruppe

[71]. Dennoch ergibt sich in der RBVD-Studie kein erhöhtes Risiko für einen Vitamin-B12-Mangel bei den veganen Teilnehmenden, denn im Blut zeigten sich keine Gruppenunterschiede im Vitamin-B12-Indikator [46,71]. Daher wird vermutet, dass den Veganer:innen in der RBVD-Studie das Risiko eines Vitamin-B12-Mangels in Folge einer veganen Ernährungsweise bekannt ist, denn Vitamin B12 wird von fast allen Veganer:innen supplementiert (92%) [46,71]. Auch in Hinblick auf Vitamin D kann die Supplementierung eine plausible Erklärung sein (Supplementierung 50%) [46,71]. Denn auch Vitamin D gilt als potentiell kritischer Nährstoff in der Nährstoffzufuhr [8,10]. Tatsächlich sieht die RBVD-Studie aber keine Unterschiede in den Konzentrationen vom Vitamin D in Veganer:innen im Vergleich zu Mischköslter:innen, trotz der signifikant geringeren Nährstoffzufuhr über die Nahrung [46,71].

Die aktuelle wissenschaftliche Evidenz zeigte, dass es Unterschiede in der Nährstoffzufuhr und -versorgung wichtiger Nährstoffe zwischen Veganer:innen und Mischköstler:innen gibt. Viele dieser Nährstoffe haben auch einen wichtigen Einfluss auf Knochengesundheit. Originalpublikation 3 untersuchte daher ganz explizit 28 knochenrelevante und ernährungsbedingte Biomarker zwischen Veganer:innen und Mischköstler:innen [46]. Die niedrigere Knochengesundheit bei Veganer:innen einem einzigen Nährstoff oder Biomarker zuzuschreiben, ist angesichts der Komplexität der homöostatischen Regulationsmechanismen des Knochens zu einfach dargestellt [46]. In der Tat ist bekannt, dass es komplexe Zusammenhänge zwischen Nährstoffen, Lebensmitteln und Ernährungsmustern gibt, sodass kein einzelnes Element einer Ernährung ein vollständiges Bild der Auswirkungen der Ernährung auf die Gesundheit liefern kann [99]. Mit ähnlicher Sichtweise, betrachtet auch das aktuellste systematische Review von Oglivie et al. nicht nur die Knochendichte und Frakturrate, sondern thematisiert zusätzlich auch den Einfluss bestimmter Nährstoffe, inflammatorische Cytokine, sowie Darmmikrobiota auf die Knochengesundheit [32]. Die Originalpublikation 3 verfolgte weiterführend einen explorativen systemischen Ansatz, um eine Kombination von knochenrelevanten und ernährungsbedingten Biomarkern zu generieren, die die Varianz der Knochengesundheit maximal erklärt [46]. Das identifizierte Biomarkermuster beinhaltet 12 knochenrelevante und ernährungsbedingte Biomarker mit positiven Faktorladungen für Vitamin B6, Vitamin A, Omega-3-Fettsäuren, Calcium und Magnesium im Urin, Jod im Urin, TSH, Selenoprotein P, Lysin, Leucin, α-klotho und eine negative Faktorladung für FGF23 [46]. Auf diese knochenrelevanten und ernährungsbedingten Biomarker wird im Folgenden näher eingegangen

Ein systematisches Review zeigte, dass die Nährstoffaufnahme im Rahmen des Veganismus von Vitamin B6 hoch ist [10]. Interessanterweise wurde jedoch in der Deutschen Veganer Studie (n=93) auch aufgezeigt, dass obwohl die Vitamin B6-Zufuhr bei veganer Ernährung ausreichend war, die Konzentration von Vitamin B6 in den meisten Blutproben als

unzureichend galt [100]. Dies steht im Einklang mit den Ergebnissen der RBVD-Studie, auch hier war die Nährstoffaufnahme von Vitamin B6 bei Veganer:innen höher als bei Mischköstler:innen [71], die Blutkonzentrationen von Vitamin B6 unterschieden sich jedoch nicht [46,71]. Eine mögliche Erklärung berichtete Waldmann et al. [100], denn Lebensmittel tierischen Ursprungs enthalten hauptsächlich Pyridoxamin und Pyridoxal mit einer Bioverfügbarkeit von etwa 75% [100]. Vitamin B6 in Lebensmitteln pflanzlichen Ursprungs besteht hauptsächlich aus Pyridoxin und der phosphorylierten Derivate mit geringerer Bioverfügbarkeit [100]. Zudem ist ein großer Teil des Vitamin-B6-Gehaltes in pflanzlichen Lebensmitteln glucosyliert, dies verringert zusätzlich die Bioverfügbarkeit [100]. Eine Übersichtsarbeit [44] untersuchte die mögliche Rolle von B-Vitaminen auf die Knochengesundheit, dabei wurden Ergebnisse aus experimentellen In-vitro- und In-vivo-Studien sowie aus Beobachtungs- und Interventionsstudien berücksichtigt [44]. In Übereinstimmung mit den Ergebnissen der RBVD-Studie deuten die Ergebnisse dieser Arbeit auf eine protektive Rolle von Vitamin B6 auf die Knochengesundheit hin [44].

In Hinblick auf Vitamin A gibt es eine Vielzahl von Verbindungen mit Vitamin-A-Wirkung, dabei ist Retinol jedoch die zentrale Wirkungsform [101]. Das vorgeformte Vitamin A (aus Retinol und Retinylestern) ist ausschließlich in tierischen Lebensmitteln enthalten [101]. Verschiedene Provitamin-A-Carotinoiden sind meist in Pflanzen enthalten, die in unterschiedlichem Ausmaß in Vitamin A umgewandelt werden können [101]. Das wichtigste Provitamin A für die Vitamin-A-Versorgung des Menschen ist β-Carotin, da es eine hohe Umwandlungsrate in Retinol hat und auch in größeren Mengen verzehrt wird [101]. Für eine vegane Ernährungsweise sind Provitamin-A-Carotinoiden für die Aufrechterhaltung eines adäquaten Vitamin-A-Status von Bedeutung [101], daher gilt Vitamin A auch nicht als potenziell kritischer Nährstoff. Dennoch muss dabei die geringe Bioverfügbarkeit der Carotinoide, die variable Umwandlungseffizienz der Carotinoide, sowie Wechselwirkungen mit anderen Nahrungsinhaltsstoffen berücksichtigt werden [101]. Tatsächlich zeigte Davey et al. in der EPIC-Oxford-Studie (n=65.429), dass Veganer:innen die geringste durchschnittliche Aufnahme von Retinol über die Nahrung hatten, im Vergleich zu Mischköstler:innen, Fischesser:innen und Ovo-Lacto-Vegetarier:innen [43]. Die RBVD-Studie hingegen betrachtet die Aufnahme als Retinoläquivalente [71], das neben Retinol auch Provitamine (wie das β-Carotin) berücksichtigt. In Übereinstimmung mit einer aktuellen Studie von Dawczynski et al. [102] aus dem Jahr 2022 wurden keine Unterschiede zwischen Veganer:innen und Mischköstler:innen in beiden Studien festgestellt [71,102]. Wie oben beschrieben, zeigte auch Dawczynski et al., dass die Aufnahme von β-Carotin bei den Veganer:innen tatsächlich am höchsten war [102]. Die Konzentrationen von Vitamin A im Blut sind hingegen in beiden Studien in den Veganergruppen signifikant niedriger [46,102]. Ein aktuelles systematisches Review aus 2021 zeigte, dass Vitamin A sowohl positive als auch negative Auswirkungen auf die Knochengesundheit haben kann [103]. Zum einen erhält eine

adäquate Vitamin-A-Zufuhr über die Nahrung oder Nahrungsergänzungsmitteln die Knochengesundheit [103]. Ein anderes systematisches Review zeigte ebenfalls, dass eine höhere Zufuhr von Vitamin A mit einer höhere Knochendichte assoziiert war [104]. Zudem wirkt sich auch Provitamin-A positiv auf die Knochengesundheit aus, indem es die Osteoblastenaktivität und die Knochenbildung stimuliert sowie die osteoklastische Aktivität und die Knochenresorption hemmt [103]. Dennoch sollte dabei betrachtet werden, dass eine übermäßige Aufnahme von Vitamin A (Hypervitaminose A) mit einer niedrigen Knochendichte und auch mit einem erhöhten Frakturrisiko assoziiert ist, maßgeblich durch die Hemmung der Osteoblastendifferenzierung und Mineralisierung [103].

Langkettige Omega-3 Fettsäuren gelten im Rahmen einer veganen Ernährungsweise als potenziell kritische Nährstoffe [8]. Das gilt maßgeblich für EPA, da diese über pflanzliche Lebensmittel kaum zugeführt werden kann [8,9]. Leitzmann und Keller berichten, dass die Gesamtzufuhr von Omega-3-Fettsäuren bei Veganer:innen und Mischköstler:innen etwa gleich ist [9]. In Übereinstimmung zeigten auch die Daten der RBVD-Studie keine Unterschiede in der Gesamtzufuhr von Omega-3-Fettsäuren über die Nahrung zwischen Veganer:innen und Mischköstler:innen [105]. Es zeigte sich jedoch, dass Veganer:innen eine signifikant höhere Aufnahme von α-Linolensäure (ALA) im Vergleich zur Mischkostgruppe hatten [105]. Tatsächlich berichtete auch die Übersichtsarbeit von Burns-Whitmore et al. [106], dass die Mehrheit der eingeschlossenen Studien eine hohe Aufnahme von ALA bei Veganer: innen im Vergleich zu Mischköstler:innen hatten, dennoch gab es auch einzelne Studien die eine niedrigere Aufnahmen von ALA oder keine Unterschiede im Rahmen einer veganen Ernährungsweis feststellten [106]. Das Review zeigte in Übereinstimmung mit unserer RBVD-Studie auch, dass EPA und DHA von Veganer:innen über die Nahrung kaum aufgenommen werden [105,106]. Tatsächlich dienen nur maritime Mikroalgen als pflanzliche Quelle bei einer pflanzenbetonten Ernährungsweise [9]. Über diese Algen reichern sich EPA und DHA in der Nahrungskette aquatischer Organismen an, besondere in fettreichen Kaltwasserfischen [9]. Daher sind Veganer:innen möglicherweise auf die körpereigene Synthese angewiesen [9]. Der menschliche Organismus besitzt Enzymsysteme zur Kettenverlängerung und Desaturierung von ALA zu EPA und DHA [9,106]. Die Umwandlungsrate von ALA zu EPA (von 5%-12%) und DHA (<1%) sind beim Menschen jedoch limitiert [9,106]. Zudem wird berichtet, dass diese bereits limitierte Umwandlung durch eine hohe eine Zufuhr an Linolsäure (LA) weiter begrenzt wird, da beide Fettsäuren um die gleichen Enzymsysteme konkurrieren [9,107]. Tatsächlich zeigte Burns-Whitmore et al., ebenfalls in Übereinstimmung mit der RBVD-Studie, dass Veganer:innen eine höhere Aufnahme von LA hatten im Vergleich zu Mischköstler:innen [105,106]. Welch et al. zeigte ebenfalls in der EPIC-Norfolk-Studie deutliche Unterschiede in der Aufnahme von den langkettigen Omega-3-Fettsäuren zwischen Veganer:innen und Mischköstler:innen, dennoch hatten die Veganer:innen einen beachtlichen Plasmaanteil von

EPA und DHA [107]. Die Autor:innen vermuteten, dass die Umwandlungsrate auch variabler sein könnte [107]. Es ist denkbar, dass die Konversationsrate von ALA zu EPA und DHA doch höher sein konnte, wenn mit der Nahrung nur wenig EPA und DHA aufgenommen wird [107]. Diese Vermutung unterstützen auch die Ergebnisse der RBVD-Studie [105]. Denn trotz der sehr geringen Aufnahme von EPA und DHA von Veganer:innen, hatte die Gruppe der Veganer:innen einen beachtlichen Plasmaanteil von EPA und DHA, auch wenn der Anteil im Vergleich zur Mischkostgruppe signifikant niedriger war [105]. Darüber hinaus wurden in der RBVD-Studie keine ALA Unterschiede zwischen Veganer:innen und der Mischkostgruppe in den Plasmaphospholipiden gefunden, während die Aufnahme von ALA über die Nahrung bei Veganer:innen höher war [105]. Das Review von Burns-Whitmore et al. zeigte ebenso wie die RBVD-Studie, dass in den meisten Studien die Serum- als auch die Plasmaspiegel von EPA und DHA bei Veganer:innen im Vergleich zu Mischköstler:innen niedriger waren [106]. In Hinblick auf die Knochengesundheit wird vermutet, dass EPA und DHA einen positiven Einfluss haben. Es wird diskutiert, dass diese langkettigen Omega-3-Fettsäuren die Überlebensrate von Osteoblasten fördert, die Osteoblastogenese anregt und die Knochenresorption durch Veränderung der Membranfunktion, Regulierung des Calciumhaushalts und erhöhte Osteoblastenaktivität, verhindert [108]. Darüber hinaus ist die Beteiligung von EPA und DHA an der Differenzierung und Reifung von Präosteoblasten mit ihren anti-inflammatorischen Wirkungen assoziiert, z.B. reduzierte Synthese des entzündlichen PGE2 sowie niedrige Konzentrationen von Entzündungszytokinen, wie Interleukin-1 (IL-1), IL-6 und TNF-α [108]. In Bezug auf die Knochengesundheit stellte eine Metaanalyse mit Beobachtungsstudien (n=10 Studien) fest, dass ein hoher Verzehr von Omega-3-Fettsäuren signifikant mit einem geringeren Risiko für Hüftfrakturen assoziiert war [109]. Zwei systematische Übersichtsarbeiten (mit Metaanalyse [110]) auf der Grundlage von randomisierten kontrollierten Studien deuten auf eine Assoziation zwischen Omega-3-Fettsäuren und einer verbesserten Knochendichte hin [110,111].

Die Makromineralien Calcium und Magnesium sind bekannt als wichtige Faktoren für die Knochengesundheit [112]. Tatsächlich sind 99% des Calciums im Körper im Skelett gespeichert und etwa 60% des körpereigenen Magnesiums befinden sich in den Knochen [112]. Da die Blutkonzentrationen von Calcium und Magnesium innerhalb enger Grenzen reguliert werden, sind Konzentrationen im Blut kein geeigneter Indikator für die Überprüfung des Calciums– oder Magnesiumstatus [9]. Daher wurden in der RBVD-Studie 24-Stunden-Urinproben genutzt.

Physiologisch wird ein Großteil des Magnesiums im Blut glomerulär filtriert und zu 95% rückresorbiert [113]. Die Rückresorption von Magnesium in der Niere ist dabei maßgeblich abhängig von der Plasmakonzentration [113]. Tatsächlich kann die renale Magnesiumausscheidung über den Urin zwischen 0.5% und 70% der gefilterten Menge liegen

[113]. Die Nieren sind in der Lage, Magnesium während eines Mangels verstärkt rückzuresorbieren und so die Ausscheidung zu minimieren. Bei übermäßiger Aufnahme hingegen wird die Ausscheidung über den Urin gesteigert [113]. Dennoch hängt die Homöostase auch von der Resorption im Darm ab. In der Tat ist die intestinale Resorption von Magnesium nicht direkt proportional zur Magnesiumzufuhr mit der Nahrung, sondern vielmehr vom individuellen Magnesiumstatus abhängig [113]. Es wurde festgestellt, je niedriger der Magnesiumspiegel ist, desto mehr wird Magnesium im Darm resorbiert und umgekehrt [113]. Diese individuelle Anpassung von Magnesium könnte eine plausible Erklärung dafür sein, warum in der RBVD-Studie keine Unterschiede der Magnesiumausscheidung im Urin zwischen Veganer:innen und Mischköstler:innen beobachtet wurden [46], trotz der höchsten Magnesiumaufnahme über die Nahrung im Vergleich zu anderen Ernährungsformen bei Veganer:innen [10]. In Hinblick auf die Knochengesundheit zeigten verschiedene ältere und aktuelle Übersichtsarbeiten zum Teil mit Metaanalysen [112,114,115], dass eine höhere Magnesiumzufuhr mit einer besseren Knochendichte/niedriges Frakturrisiko assoziiert ist.

Laut der DGE gilt auch Calcium als ein potentiell kritischer Nährstoff [8]. Tatsächlich zeigte ein aktuelles Review, dass Veganer:innen eine niedrige Calciumzufuhr im Vergleich zu anderen Ernährungsformen hatten [10]. Dabei spielen niedrige Calciumgehalte pflanzlicher Lebensmittel, aber auch die Resorptionsbeeinträchtigungen durch verschiedene Hemmstoffe eine Rolle [9]. Milch- und Milchprodukte stellen die wichtigste Calciumquelle in Lebensmitteln dar, aber auch dunkle, grüne Gemüsesorten, verschiedene Nussarten und calciumreiche Mineralwasser sind ebenfalls gute Calciumlieferanten [9]. Die intestinale Calciumresorption wird jedoch von der Zusammensetzung der Nahrung beeinflusst [9]. Aus pflanzlichen Lebensmitteln wird aufgrund von enthaltenden Hemmstoffe teilweise weniger Calcium resorbiert [9]. Vitamin D dagegen gilt als calciumresorptionsfördernde Substanz [9]. Hemmstoffe sind z.B. Phytinsäure (z.B. in Getreide, Nüsse), Oxalsäure (z.B. in Spinat, Mangold) oder Zellulose, welche mit Calcium schwer lösliche Verbindungen bilden, die nicht oder nur eingeschränkt resorbierbar sind [9]. Diese Effekte spielen eine Rolle, wenn die Ernährung reich an Hemmstoffen ist und zudem wenig Calcium enthält, was bei einer veganen Ernährungsform der Fall sein kann [9]. Knurick et al. berichtete, dass die tägliche Calciumausscheidung bei Mischköstler:innen signifikant höher war (34%), als bei Vegetarier:innen [38]. Die Daten der RBVD-Studie zeigte ebenfalls eine höhere Calciumausscheidung bei der Mischkostgruppe im Vergleich zur den Veganer:innen (36%) [46]. Dies könnte auf die geringere Calciumzufuhr bei Veganer:innen zurückzuführen sein, da die Calciumkonzentration im Urin die Nahrungsaufnahme widerspiegelt [116]. Zudem könnte in der RBVD-Studie die geringere Calciumausscheidung, sowie die erhöhten PTH-Werte bei fast jedem dritten Veganer:innen auch als ein möglicher Hinweis auf eine physiologische Reaktion auf ein geringes Calciumangebot interpretiert werden [71].

Calcium ist mengenmäßig der wichtigste Mineralstoff und bildet mit anorganischem Phosphat in Form von Hydroxylapatit die Hartsubstanz von Knochen und Zähnen [9]. Bei einem Abfall des Calciumspiegels wird PTH ausgeschüttet, dieses setzt Calcium aus dem Knochen frei und verstärkt gleichzeitig die Rückresorption in der Niere [9]. Zudem wandelt es Vitamin D in die aktive Form um, welche die intestinale Resorption erhöht und synergetisch die Wirkung von PTH unterstützt [9]. Es gilt als unbestritten, dass eine adäquate Calciumzufuhr für eine normale Knochenentwicklung über verschiedene Lebensabschnitte wichtig ist [9]. Aber dennoch zeigen viele Studienergebnisse nicht immer Assoziationen zwischen der Calciumzufuhr über die Nahrung und der Knochengesundheit (Knochendichte und Frakturrisiko). So zeigte die Querschnittsstudie von Vannucci et al. aus Italien (n=1000) keine Assoziation der Calciumzufuhr aus der Nahrung und der Knochendichte [117]. Eine Metaanalyse (n=59 RCTs) von Tai et al. zeigte ebenso, dass eine Steigerung der Calciumzufuhr über die Nahrung nur einen geringen Einfluss auf die Knochendichte hatte (0.6%-1.8%) [118]. Auch eine Supplementierung hatte nur einen geringen Einfluss auf die Knochendichte [118]. Eine Metaanalyse von Bolland et al. berichtete, dass die Calciumzufuhr aus der Nahrung nicht mit dem Frakturrisiko assoziiert ist [119]. Andere Studienergebnisse lassen vermuten, dass nur eine niedrige Zufuhr von Calcium mit einem erhöhten Frakturrisiko assoziiert ist [117,120]. Die D-A-CH Gesellschaften empfehlen eine tägliche Calciumzufuhr für Erwachsene von 1000 mg/täglich [9]. Vannucci et al. berichtete, dass Personen mit einer niedrigen Calciumzufuhr (< 400 mg/Tag) im Vergleich zu Personen mit höheren Calciumzufuhr (≥ 400 mg/Tag) ein signifikant höheres Risiko für Frakturen hatten [117]. Appleby et al. zeigte mit den Daten der EPIC-Oxford Studie (n=34.696), dass vor allem Veganer:innen mit einer Zufuhr von weniger als 525 mg/Tag ein erhöhtes Frakturrisiko hatten [120]. Eine schwedische Studie mit den Daten einer Mammographie-Kohorte (n=50.22 Frauen), zeigten eine nicht-lineare Assoziation, so hatten Frauen im ersten Quintil mit einer niedrigen Calciumzufuhr (≤ 750 mg/Tag) ein erhöhtes Osteoporose- und Frakturrisiko [121]. Jede weitere Calciumerhöhung (2. bis 5. Quintil) führte hingegen zu keiner Verringerung des Frakturrisikos. Im 5. Quintil mit der hohen Calciumzufuhr stiegt das Risiko für Frakturen sogar an [121].

Jod ist vor allem für den Aufbau von Schilddrüsenhormonen unentbehrlich [9,122]. Diese Hormone spielen bei der Steuerung vieler Stoffwechselprozessen eine wichtige Rolle und sind unter anderem für die Knochenbildung, die Entwicklung des Gehirns, sowie den Energiestoffwechsel notwendig [9,122]. Auch Jod gilt als potenziell kritischer Nährstoff einer veganen Ernährungsweise [8]. Hierbei muss jedoch betont werden, dass eine geringe Zufuhr von Jod kein spezifisches Problem einer pflanzenbasierten Ernährung darstellt, sondern auch die deutsche Durchschnittsbevölkerung betrifft [9]. Jod ist in pflanzlichen und tierischen Lebensmitteln enthalten, wobei der Jodgehalt in Lebensmitteln variieren kann [9,122]. Der Jodgehalt in Lebensmitteln wird beispielsweise durch den Jodgehalte im Boden und Wasser,

sowie Produktionsbedingungen z.B. Jodgehalt im Tierfutter beeinflusst [9,122]. Meeresfisch, sowie Meeresalgen sind eine gute natürliche Jodquelle, aber auch Milch und Milchprodukte, Gemüse, Pilze und Hülsenfrüchte [9,122]. Darüber hinaus wird Jod aber vor allem über jodiertes Speisesalz und damit hergestellte Lebensmittel aufgenommen [9,122]. Bei Verwendung von Jodsalz in Lebensmitteln sind Fleisch, Wurst und Brot die Hauptquellen für Jod [9,122]. Die Daten der DEGS I Studie (Studie zur Gesundheit Erwachsener in Deutschland) zeigten, dass die geschätzte mediane Jodzufuhr bei Männern bei 126 µg/Tag und bei Frauen bei 125 µg/Tag liegen [9,123,124]. Ca. 30 % der Bevölkerung weisen eine Zufuhr unterhalb der Empfehlungen (Erwachsene: 200 µg/Tag) auf [9,123,124]. Über die Ausscheidung von Jod im Urin wird die Versorgung beurteilt, dabei signalisiert nach WHO-Empfehlungen eine Ausscheidung von 100-199 µg /l Urin eine adäquate Versorgung [125]. In der RBVD-Studie zeigte sich eine geringe Jodaufnahme [71], aber auch die Jodausscheidung im Urin lag bei 3/4 der Mischköstler:innen und bei nahezu allen Veganer:innen weit unter dem WHO-Grenzwert [46,71]. Die Jodausscheidung lag bei 1/3 der Veganer:innen [46,71] sogar unterhalb des WHO-Grenzwertes für eine schwere Unterversorgung (<20 µg/l) [125]. Wie die systematische Übersichtsarbeit von Bakaloudi et al. berichtete, sind die Ergebnisse der RBVD-Studie deckungsgleich mit weiteren aktuellen Studien, die ebenso geringere Jodaufnahmen und eine unzureichende Jodunterversorgung bei Veganer:innen aufzeigen [10]. Besteht über einen längere Zeitraum eine unzureichende Zufuhr, produziert die Schilddrüse zu wenig Hormone, wodurch es zu schwerwiegenden gesundheitlichen Folgen kommen kann [122]. In Folge eines chronisch alimentären Jodmangels ist die Blutkonzentration der Schilddrüsenhormone verringert und die Konzentration von TSH erhöht [9]. In der Literatur wird jedoch beschrieben, dass sich der TSH-Wert als wenig sensitiver Marker für die Jodzufuhr aus der Nahrung eignet [126]. Das ist in Übereinstimmung mit der RBVD-Studie, auch hier hatten nur vier Studienteilnehmer:innen bei einer deutlich verminderten Jodausscheidung einen erhöhten TSH-Wert [46,71]. Dennoch wurde neben Jod, auch TSH in der RRR als ein Biomarker mit Einfluss auf die Knochengesundheit identifiziert [46]. Tatsächlich zeigte eine dänische Kohortenstudie bei Teilnehmenden ohne bekannte Schilddrüsenerkrankung (n=222.138) einen Zusammenhang zwischen niedrigen TSH-Konzentrationen und einem erhöhten Frakturrisiko [127]. In ähnlicher Weise zeigte auch Murphy et al. (n=2374 euthyreoten postmenopausalen Frauen) ein um 35 % reduzierte nicht-vertebrales Frakturrisiko bei hohen TSH-Werten [128]. Zudem wurde berichtet, dass der Jodstatus bei Frauen mit postmenopausaler Osteoporose signifikant niedriger war und mit dem T-Score der Lendenwirbelsäule korreliert [129].

Auch das lebenswichtige Spurenelement Selen [130] gilt als potenziell kritischer Nährstoff einer veganen Ernährung [8]. Selen ist in pflanzlichen und tierischen Lebensmitteln enthalten, wobei der Selengehalt in pflanzlichen Lebensmitteln vom Selengehalt des Bodens abhängt [9]. In

Mittel- und Nordeuropa sind die Böden selenarm, daher sind auch die Selengehalte in den angebauten Nahrungspflanzen gering [9]. Lebensmittel tierischen Ursprunges wie Fleisch und Eier sowie Fisch stellen in Europa eine konstante Selenquelle dar [9]. Wissenschaftliche Studien zeigten, dass Veganer:innen eine geringere Selenzufuhr als Mischköstler:innen haben [9,10]. Dies spiegelt sich in den meisten Studien auch im niedrigen Selenstatus wieder [9]. Dabei gilt Selenoprotein P als bester Parameter für die Bestimmung für den Selenstatus [9]. Selenoprotein P ist für den Selentansport im Blut verantwortlich [9]. Deckungsgleich hatten die Veganer:innen der RBVD-Studie einen niedrigen Selenstatus (niedrigere Selenoprotein P Konzentrationen) im Vergleich zur Mischkostgruppe [46]. In Hinblick auf die Knochengesundheit wurde beobachtet, dass Selen und Selenoprotein P positiv mit der Knochendichte korreliert waren [131,132]. Dabei könnte vor allem Selenoprotein P aufgrund seiner möglichen Funktion als essentieller Selentransporter für die Knochen eine wichtigere Rolle spielen [133].

Laut der DGE sind potenzielle kritische Nährstoffe bei einer veganen Ernährung auch Proteine bzw. unentbehrliche Aminosäuren [8]. Tatsächlich zeigte sich wissenschaftliche Evidenz für eine niedrige Proteinzufuhr von Veganer:innen im Vergleich zur Mischköstler:innen [9]. Hierbei betonen Leitzmann und Keller jedoch auch, dass verschiedene Untersuchungen bei Veganer:innen eine durchschnittliche bedarfsdeckende Proteinzufuhr aufgezeigt haben (D-A-CH Referenzwert: 0.8 g Protein/kg Körpergewicht/ Tag) [9]. Diese Ergebnisse sind deckungsgleich mit der RBVD-Studie, auch hier wurde eine geringere, aber bedarfsdeckende Proteinzufuhr bei Veganer:innen im Vergleich zur Mischkostgruppe gesehen [134]. Interessanterweise konnte die RBVD-Studie hier auch zeigen, dass Veganer:innen im Vergleich zu Mischköstler:innen tatsächlich eine geringere mittlere Aufnahme vieler unentbehrlicher Aminosäuren (Isoleucin, Leucin, Lysin, Methionin, Threonin, Valin und Histidin) hatten [134]. Die Aufnahme in beiden Gruppen spiegelt jedoch eine durchschnittliche bedarfsdeckende Zufuhr von verschiedenen Aminosäuren wieder [134]. Durch die homöostatische Regulierung freier Aminosäuren im Blut [135,136] spiegeln sich die beobachteten Unterschiede verschiedener Aminosäuren über die Nahrung kaum in den Plasmakonzentrationen wieder [134]. Es zeigten sich niedrige Plasmakonzentrationen von Lysin und mit Tendenz für Tryptophan bei den Veganer:innen im Vergleich zur Mischkostgruppe in der RBVD-Studie [46,134]. Dagegen waren die Plasmakonzentrationen für Glutamin und Glycin bei den Veganer:innen in der RBVD-Studie höher als in der Mischkostgruppe [46,134]. Diese Ergebnisse stimmen in weiten Teilen mit den Ergebnissen der EPIC-Oxford-Studie (n=392 Männer) überein [137]. In Hinblick auf die Knochengesundheit identifizierte die RRR die Aminosäuren Leucin und Lysin als wichtige Biomarker im Biomarkermuster [46]. Mechanistische Hinweise deuten darauf hin, dass Leucin und Lysin (zusätzlich zu Arginin, Alanin, Prolin und Glutamin) die Insulinsekretion In-vitro stimulieren,

was vermutlich das Wachstum und die Differenzierung von Osteoblasten fördert [138-140]. Darüber hinaus hat sich gezeigt, dass Leucin die wirksamste der verzweigtkettigen Aminosäuren für die Stimulierung der Muskelproteinsynthese ist, was für die Aufrechterhaltung einer angemessenen Knochenstärke und -dichte entscheidend ist [140,141]. Jennings et al. zeigte in monozygoten Zwillingspaaren ebenso (n=135), dass die Zufuhr von Lysin, Leucin (zusätzlich zu Arginin, Alanin, Prolin und Glutaminsäure) mit einer höheren Knochendichte assoziiert war [140].

Auch die FGF23-α-Klotho-Achse wurde als Einflussfaktor für das Biomarkermuster identifiziert. FGF23 ist ein phosphotropes Hormon, das überwiegend im Knochen von Osteoblasten und Osteozyten produziert wird [142,143]. FGF23 spielt eine Schlüsselrolle im Gleichgewicht der Mineralhomöostase und der Knochenmineralisierung [144]. FGF23 ist ein wichtiger Regulator des Phosphat- und Vitamin D-Stoffwechsels [142,143,145]. α-Klotho ist ein Transmembranprotein, welches v. a. in der Niere, der Nebenschilddrüse und im Plexus chorioideus der Hirnventrikel exprimiert wird [143]. Zusammen mit dem FGF-Rezeptor 1 fungiert es als Rezeptorkomplex für FGF23 [142,143]. In der Niere hemmt der FGF-Rezeptor-1/α-Klotho-Rezeptor-Komplex im proximalen Tubulus die Phosphatrückresorption, was eine vermehrte Phosphatausscheidung zur Folge hat, während im distalen Tubulus die Calcium- und Natriumrückresorption stimuliert wird [142,143,145]. Zudem wird durch Hemmung der 1-alpha-Hydroxylase die Calcitriolsynthese (1,25-Dihydroxyvitamin D3) herabgesetzt, das führt u.a. zu einer reduzierten Phosphatresorption im Darm [142,143,145]. Die entscheidende Rolle von FGF23 in der Mineralhomöostase wurde erstmals bei genetisch bedingten und erworbenen rachitischen Erkrankungen des Menschen festgestellt [144]. Hier konnte gezeigt werden, dass ein Überschuss an FGF23 verschiedene Arten von hypophosphatämischen Rachitis/Osteomalazie verursacht, die durch eine gestörte Mineralisierung der Knochenmatrix gekennzeichnet sind [142,144]. Grundlegend stimmt das auch mit dem Ergebnis der RBVD-Studie überein, denn FGF23 hatte die stärkste negative Faktorladung in der explorativen RRR in Hinblick auf die Knochengesundheit [46]. Dennoch sind weitere wissenschaftliche Studien erforderlich, da bisher nur wenige Studien den Zusammenhang zwischen FGF23/ α-Klotho und der Knochengesundheit in gesunden Studienpopulationen untersucht haben [146-151].

Das Ergebnis der explorativen RRR verstärkt die Annahme, dass verschiedene knochenrelevante und ernährungsbedingte Biomarker eher in einem komplexen synergetischen Zusammenspiel die Knochengesundheit beeinflussen, als ein einzelner Biomarker allein. Eine Varianzanalyse über Tertile des Biomarkermuster-Scores zeigte, dass die Teilnehmer:innen in der dritten Tertile (T3) im Mittel 11% höhere BUA-Werte hatten, im Vergleich zur ersten Tertile (T1) (p für Trend<0.0001), während der prozentuale Anteil der Veganer:innen mit steigender Tertile niedriger wurde (T1: 70%, T2: 61%, T3: 26%) [46]. So finden sich auch wenige Veganer:innen im T3 des Biomarkermuster-Scores (mit hohen QUS-

Werte) und auch wenige Mischköstler:innen im T1 des Biomarkermuster-Scores (mit niedrigen QUS-Werte) [46]. Das Ergebnis macht deutlich, dass eine vegane Ernährungsform nicht per se mit einer niedrigen Knochengesundheit assoziiert sein muss. Vielmehr wird die Bedeutung einer abwechslungsreichen und ausgewogenen Ernährung deutlich, ggf. auch mit Ergänzung durch Nahrungsergänzungsmittel oder angereichten Lebensmitteln.

Dennoch muss auch betont werden, dass es sich hierbei um ein exploratives Biomarkermuster handelt [46]. Die Originalpublikation 4 hat erstmals eine RRR angewandt, um ein exploratives Biomarkermuster zu erkennen, dass möglicherweise eine Kombination von Biomarkern aufdeckt, die für die Knochengesundheit relevant sind [46]. Normalerweise wird die Methode der RRR in der Ernährungsepidemiologie zur Identifizierung von Ernährungsmustern eingesetzt [152]. Da es sich bei der RBVD-Studie jedoch um eine kleine Studie handelte, ist eine Wiederholung und Validierung des abgeleiteten Musters in einer unabhängigen Studienpopulation dringend empfohlen [152].

3.2.2. Einfluss der Säurelast auf den Knochen

Die Säurelast kann durch verschiedene Ansätze bestimmt werden, auf Basis der Nahrungsaufnahme oder Urinausscheidung [74]. Ein wesentlicher Vorteil der uPRAL gegenüber ernährungsbasierten Ansätzen ist dabei die Fähigkeit die tatsächliche Aufnahme und die individuelle Bioverfügbarkeit von säure- und basischrelevanten Anionen und Kationen widerzuspiegeln [153]. Daher kann uPRAL als ein direkter Biomarker für die ernährungsabhängige Säurelast gesehen werden [153]. Bisher wurde uPRAL nicht verwendet, um den vermuteten Unterschied in der ernährungsabhängigen Säurelast zwischen Veganer:innen und Mischköstler:innen zu untersuchen. In der RBVD-Studie zeigte sich ein deutlicher Unterschied in der uPRAL bei Veganer:innen und Mischköstler:innen auf der Grundlage von Urinausscheidungsdaten [74]. Dieses Ergebnis bestätigt andere wissenschaftliche Publikationen, dass die vegane Ernährungsform mit einer niedrigen Säurelast der Nahrung assoziiert ist [49,57-60]. Es gilt als bekannt, dass ein typisches Anzeichen einer latenten Azidose eine Abnahme des Harn-pH-Wertes darstellt [50]. Die RBVD-Studie zeigte in Übereinstimmung mit früheren wissenschaftlichen Studien, dass Veganer:innen einen höheren durchschnittlichen Urin-pH-Wert haben, im Vergleich zu Mischköstler:innen, was auf die geringere Säurebelastung ihrer Ernährung zurückzuführen sein könnte [38,59,154].

In Hinblick auf die Knochengesundheit wird berichtet, dass eine ernährungsbedingte latente Azidose physikalisch zu einer Ablösung von Mineralstoffen von der Knochenmatrix führen kann, zudem kann die Aktivität der Osteoklasten erhöht und die Aktivität der Osteoblasten gehemmt werden [155]. Somit wird vermutet, dass die geringere Säurelast der vegetarischen

und veganen Ernährungsweise ihrer potentiellen knochenschädigenden Wirkung entgegenwirkt [61]. Trotz der biologischen Plausibilität der möglichen knochenschädigenden Wirkung der Säurelast durch die Nahrung, sind die Ergebnisse wissenschaftlicher Studien nicht eindeutig und somit scheint der Einfluss der Säurelast auf den Knochen komplexer [74]. Auch in der RBVD-Studie wurde kein Einfluss der uPRAL auf die Parameter der QUS festgestellt [74]. Nähere Betrachtung unserer Ergebnisse sind dabei von großer Bedeutung [74]. Die Studienpopulation der RBVD-Studie war gesund, zudem hatten die Teilnehmenden ein eher geringes Spektrum, das von stark negativen (d. h. basischen) bis zu nur mäßig positiven (d. h. sauren) uPRAL-Werten reichte [74]. Die Nullbefunde in diesem kleinen Spektrum von uPRAL könnten als indirekte Unterstützung für die Ergebnisse interpretiert werden, die darauf hindeuten, dass die Vorteile einer Alkalisierung der Ernährung für die Knochengesundheit nur auf bestimmte Menschen beschränkt sein könnte [62,63,74], wie z.B. Menschen die gewöhnlich eine Ernährung mit hohem PRAL Gehalt zu sich nehmen, mit verminderter Säureausscheidungsfähigkeit der Nieren und/oder Menschen mit Prädispositionen z.B. (altersbedingte) eigeschränkte Nierenfunktion, metabolisches Syndrom [62,63].

3.3. PFAS in der veganen Ernährung

Die RBVD-Studie ist die erste Studie, die Unterschiede in PFAS-Konzentrationen zwischen Veganer:innen im Vergleich zu Mischköstler:innen untersucht hat [75]. Dabei wurden signifikant niedrigere Konzentrationen von PFOS und PFNA bei Veganer:innen im Vergleich zur Mischkostgruppe gesehen [75].

Aus den letzten Jahren sind nur sehr wenige Daten zur internen Exposition gegenüber PFAS für die deutsche Bevölkerung verfügbar. Die besten Vergleiche innerhalb Deutschlands sind mit Daten von Blutspendern aus München (n=158) [156] oder Daten aus der Deutschen Umweltprobenbank aus Münster möglich [157]. Die Daten zeigen ähnliche interne Expositionen gegenüber PFAS. Der Hauptunterschied zwischen diesen Untersuchungen und der RBVD-Studie sind die überraschend hohen Werte von PFHxS mit einem 3.7-fach höheren Median in Berlin [75]. Mögliche Ursachen können regionale unterschiedliche Ernährungsgewohnheiten, höhere Konzentrationen im Trinkwasser oder Unterschiede im sozioökonomischen Status sein [75]. Auch ein Zufallsbefund kann nicht ausgeschlossen werden kann, da Studienpopulationen aller Studien eher klein waren [75].

In der RBVD-Studie wurden keine Unterschiede für PFOA und PFHxS zwischen Veganer:innen und Mischköstler:innen festgestellt [75]. Im Falle von PFOA ist dies überraschend [75], da die EFSA in ihrer Bewertung zeigte, dass die Lebensmittelhauptgruppen "Fisch und andere Meeresfrüchte", "Eier und Eiprodukte", "Fleisch und Fleischerzeugnisse",

und "Obst und Fruchterzeugnisse" maßgeblich zur Exposition gegenüber PFAS (insbesondere für PFOS und PFOA) beitragen [67]. Interessanterweise wurden neben den oben genannten Lebensmittelkategorien auch "Gemüse und Gemüseerzeugnisse" und "Trinkwasser" als weitere wichtige Faktoren für die Exposition gegenüber PFOA aufgeführt [67]. In der Tat zeigte die RBVD-Studie starke Korrelationen zwischen den PFOA-Konzentrationen und dem Wasserkonsum (gesamte Studienpopulation, n=72) [75].

Die RBVD-Studie zeigte außerdem 54% bzw. 240% höhere mediane Konzentrationen in PFOS- und PFNA-Konzentrationen bei Mischköstler:innen im Vergleich zu Veganer:innen [75]. Daher kann vermutet werden, dass die interne Exposition dieser PFAS möglicherweise durch eine vegane Ernährungsweise durch den Verzicht tierischer Lebensmittel beeinflusst werden könnte [75]. Denn wie die Bewertung der EFSA zeigte, enthalten vor allem tierische Lebensmittel relativ hohe PFAS-Konzentrationen [64,67]. Auch die RBVD-Studie zeigte bei den Mischköstler:innen (n=36) die stärksten Korrelationen von PFOS und PFNA für "Fleisch und Fleischprodukte" und "Fisch und andere Meeresfrüchte" [75]. Unsere Ergebnisse stimmen mit den Studienergebnissen von Lin et al. aus dem Jahr 2020 (n=941 Erwachsenen mit Prädiabetes) überein [158]. Diese Studie zeigte ebenfalls, dass Teilnehmer:innen mit hohem Konsum von Fleisch, gebratenem Fisch und anderen Fischen/Schalentieren (aber nicht Omega-3-reichen Fischen) höhere Plasmakonzentrationen von PFOS, PFHxS und PFNA hatten [158]. Bei der Interpretation sollte jedoch berücksichtigt werden, dass es sich in der RBVD-Studie um eine kleine Stichprobe handelt [75]. Solche Zusammenhänge sollten in größeren Studien umfassender untersucht werden [75]. Andere Expositionswege neben der Ernährung, wie die Exposition über Hausstaub, das Einatmen von Innenraumluft und die dermale Absorption wurden nicht berücksichtigt [159,160].

Die EFSA sieht als einen möglichen Gesundheitseffekt der PFAS die überzeugendsten Evidenz, einen Anstieg des Gesamt- und LDL-Cholesterins [67]. Auch ein aktuelles systematisches Review zeigte, dass PFAS Exposition mit höheren Konzentrationen von Gesamt- und LDL-Cholesterins assoziiert waren [69]. Die RBVD-Studie konnte neben den Unterschieden in PFOS und PFNA beider Ernährungsgruppen auch zeigen, dass Veganer:innen niedrigere Konzentrationen von Gesamt- und LDL-Cholesterin im Vergleich zu Mischköstler:innen hatten [75]. Dennoch konnten bei der derzeitigen internen PFAS-Belastung in der RBVD-Studie keine relevanten Zusammenhänge zwischen PFAS und Blutfetten aufzeigt werden [75]. Die niedrigeren Werte von LDL-Cholesterin und Gesamtcholesterin bei Veganer:innen, bei gleichzeitig niedrigeren PFOS- und PFNA-Werten, wirft die Frage nach Confounding durch die Ernährung bei dieser Fragestellung in epidemiologischen Studien auf [75]. Nur wenige epidemiologische Studien nutzten bisher Ernährungsvariablen als mögliche Confounder dieser Fragestellung, z.B. Aufnahme von gesättigten oder tierischen Fetten

[161,162], Lebensmittelgruppen wie Fleisch- oder Fischkonsum [158,163,164]. Dieser Aspekt sollte in zukünftiger Forschung weiterführend berücksichtigt werden [75].

In der RBVD-Studie fanden verschiedene Instrumente der Ernährungserhebung Anwendung. Aufgrund der langen HWZ von PFAS wurden für die Originalpublikation 5 die Daten des eingesetzten FFQ genutzt, um die vergangene langfristige Ernährung abbilden zu können [75]. Dabei erfasste EPIC-FFQ die Zeitspanne des vergangenes Jahres [165]. Jedoch wurden für pflanzenbasierte Ernährungsformen Grenzen für den vorliegenden FFQ gesehen, da er nicht die gesamte Vielfalt einer veganen Ernährung abdeckt [75]. Einige Lebensmittelgruppen werden nicht oder nicht vollständig erfasst, z.B. vegane Alternativprodukte, sodass die Ernährung von Veganer:innen und auch anderen pflanzenbasierten Ernährungsformen unterschätzt werden könnten [75].

3.4. Limitationen

Alle Publikationen mit Ausnahme von Originalpublikation 2 basieren auf der RBVD-Studie. Es handelt sich hierbei um eine Querschnittsstudie mit insgesamt 72 Teilnehmer:innen mit einer lokalen Datenerhebung im Raum Berlin [46,71,72,74,105,134]. Als Limitation wird das Querschnittsdesign gesehen, welches keine kausalen Schlüsse zulässt [46,72,105,134]. Zudem umfasst die Studie mit vergleichsweise kleiner Stichprobe Männer und Frauen mittleren Alters aus einem kleinen lokalen Gebiet (Berlin, Deutschland) [46,72,105,134]. Daher sind die Ergebnisse möglicherweise nicht auf andere Bevölkerungsgruppen übertragbar [46,72,74,105,134]. Dennoch liefert die RBVD-Studie umfassende, qualitativ hochwertige Daten aufgrund standardisierter Datenerhebung [46]. Dabei erfolgte die Datenerhebung nicht nur mittels computergestützter Fragebögen für verschiedene Lebensstilfaktoren, sondern es wurden auch klinische Parameter wie anthropometrische Maße, Blutdruck oder Knochengesundheit erhoben. Zudem wurden mannigfaltige Bioproben gesammelt und verschiedene Instrumente der Ernährungserhebung angewandt.

Als Limitation kann ferner auch gesehen werden, dass die Teilnehmenden vor allem über Aushänge rekrutiert wurden, ein besonderes Gesundheitsbewusstsein als Motivation für die Studienteilnahme kann dabei nicht ausgeschlossen werden [71]. Allerdings kann aufgrund der gleichen Rekrutierungsstrategie für Veganer:innen und Mischköstler:innen ein ähnliches Gesundheitsbewusstsein angenommen werden [71]. Es wurden zudem keine relevanten Unterschiede in den Lebensstilcharakteristika beider Gruppen festgestellt [71].

3.5. Public Health Relevanz

Pflanzenbasierte Ernährungsformen wie Vegetarismus und Veganismus erleben wachsendes Interesse in der Bevölkerung [2]. Zudem wird berichtet, dass auch Mischköstler:innen in Deutschland ganz bewusst den Konsum von Fleisch- und Fischkonsum einschränken [3]. In Hinblick auf langfristige Gesundheitseffekte könnten vegane oder vegetarische Ernährungsformen eine hohe Public Health Relevanz haben. Bisher ist die wissenschaftliche Studienlage zum Gesundheitsstatus oder langfristige Gesundheitseffekte einer pflanzenbasierten Kostform eingeschränkt [166]. Aber dennoch wird vermutet, dass vegane und/oder vegetarische Ernährungsformen das Risiko für verschiedene Erkrankungen [12], wie T2D [13], Herz-Kreislauferkrankungen [14] und für einige Krebserkrankungen [15] senken können. Neben den vermuteten Vorteilen für die individuelle Gesundheit, könnte die Umstellung auf eine pflanzliche Ernährung auch die Gesundheitssysteme entlasten [167]. So wird geschätzt, dass auf den übermäßigen Verzehr von rotem und verarbeitetem Fleisch ca. 244 Millionen Todesfälle weltweit und etwa 240 Millionen Euro an Gesundheitskosten im Jahr 2020 zurückzuführen sind [167].

Umfassender betrachtet ist bekannt, dass die Erzeugung von Lebensmitteln negative Umweltauswirkungen haben, z.B. durch den Verbrauch von Energie und Ressourcen, Zerstörung von Boden und Biotop durch Schadstoffemission etc. [168]. Die Folgen dieser Eingriffe in die Umwelt sind vielfältig, wie z.B. Reduktion der Artenvielfalt, Beeinträchtigung der humanen Gesundheit und globaler Klimawandel [168]. Die WHO sieht den Klimawandel als die größte Gesundheitsbedrohung für die Menschheit, denn durch den Klimawandel erhöhen sich klimabedingte Gesundheitsrisiken [169]. Dabei trägt die Produktion von tierischen Lebensmitteln überdurchschnittlich zur Umweltschädigung bei, besondere zur Klimaerhitzung durch die erheblichen Mengen an klimaschädlichen Treibhausgasen [168]. Tatsächlich wird beschrieben, dass die Treibhausgasemission bei veganer Kostform um ca. 50% und bei einer ovo-lakto-vegetarischen Ernährungsform um ca. 35% niedriger ist, als bei einer Mischkost, zusätzlich zum geringeren Verbrauch natürlicher Ressourcen [170]. Leitzmann und Keller fassen zusammen, dass sich durch Verringerung oder Verzicht von tierischen Lebensmitteln zugunsten von pflanzlichen Lebensmitteln die negativen Umweltwirkungen des globalen Ernährungssystems am effektivsten reduzieren lassen [168].

3.6. Ausblick

Die in Deutschland durchgeführten Studien mit erwachsenen Veganer:innen und Vegetarier:innen stammen überwiegend aus den 1980er und 1990er Jahren [171,172] und repräsentieren durch die Vielfalt an veganen und vegetarischen Alternativprodukten keine pflanzenbasierte Ernährung aus heutiger Sicht. Zum jetzigen Zeitpunkt gibt es zum Ernährungsstatus bei Erwachsenen mit pflanzenbasierten Ernährungsformen derzeit in Deutschland lediglich zwei Querschnittsstudien. Zum einen die o.g. RBVD-Studie (n=36 Veganer:innen und n=36 Mischköstler:innen) [71] und die NuEva Studie (Nutritional Evaluation Studie: n=58 Veganer:innen, n=65 Vegetarier:innen, n=70 Flexitarier:innen, n=65 Mischköstler:innen) [102]. Diese beiden Studien im Querschnittsdesign lassen jedoch keine Rückschlüsse auf langfristige Gesundheitseffekte pflanzenbasierter Ernährungsformen zu. Auch international gibt es bislang nur wenige Studien (zum Teil mit kleinen Studienpopulationen) zu pflanzenbasierten Ernährungsweisen, insbesondere zu veganen Ernährungsformen [43,173-177]. Weitere Studien, insbesondere prospektive Studien sind daher notwendig, um bestehende Datenlücken zu schließen und auch evidenzbasierte Ernährungsempfehlungen zu pflanzenbasierten Ernährungsformen zu ermöglichen.

Ein geplantes Projekt ist hierbei die COhort on PLANT-based diets (COPLANT)-Studie [178]. Die COPLANT-Studie (Studienstart: 2023) ist die bisher größte geplante Kohortenstudie zu pflanzenbasierten Ernährungsformen (n=1500 Veganer:innen, n=1500 Vegetarier:innen, n=1500 Flexitarier:innen, n=1500 Mischköstler:innen) im deutschsprachigen Raum [178]. Dabei sollen ca. 6.000 Frauen und Männer im Alter von 18-69 Jahren in sieben Studienzentren in Deutschland und einem weiteren Studienzentrum in Wien umfassend untersucht werden [178]. Eine Nachbeobachtung der Studienteilnehmenden von mindestens 20 Jahren ist geplant [178]. Im Rahmen der COPLANT-Studie soll eine detaillierte Ernährungserhebung erfolgen, sowie die Erhebung einer Vielzahl von anderen relevanten Daten, wie z.B. Körperzusammensetzung, körperlicher Aktivität und andere Lebensstilfaktoren etc. [178]. Außerdem ist es geplant, Bioproben (Blut, Urin, Stuhl) für verschiedene Biomarkeranalysen zu sammeln [178]. Die Studie plant die Vor- und Nachteile von pflanzenbasierten Ernährungsformen für die Gesundheit zu untersuchen [178]. Zudem werden aber auch soziale, ökologische und ökonomische Auswirkungen dieser Ernährungsweisen untersucht und im Rahmen einer Nachhaltigkeitsanalyse gemeinsam mit den Erkenntnissen zu den Gesundheitseffekten betrachtet [178].

4. Zusammenfassung

Pflanzenbasierte Ernährungsformen, insbesondere vegane Ernährungsweisen werden in Deutschland immer beliebter, aber vegane Kostformen sind in wissenschaftlichen Studien bisher wenig umfangreich untersucht.

Daher wurde die RBVD-Studie mit umfangreicher Datenerhebung durchgeführt, die den aktuellen Versorgungsstatus von Veganer:innen im Vergleich zu Mischköstler:innen in der deutschen Bevölkerung darstellt und auch andere gesundheitsrelevante Fragestellung untersucht.

Es wird ein vorteilhaftes inflammatorisches Biomarkerprofil von vegan lebenden Menschen vermutet, welches als intermediärer Risikofaktor einen Einfluss auf das bekanntermaßen verringerte Erkrankungsrisiko von T2D, Herz-Kreislauferkrankungen oder für bestimmte Krebserkrankungen haben könnte. Auf Basis der RBVD-Studie wurde im Rahmen der vorliegenden Arbeit aufgezeigt, dass Veganer:innen sich hinsichtlich inflammatorischer Biomarker nicht von Mischköstler:innen unterscheiden. In der Betrachtung gepoolter Ergebnisse durch eine Metaanalyse zeigte sich jedoch, dass eine vegane Ernährungsweise mit niedrigeren CRP-Werten im Vergleich zu Mischköstler:innen assoziiert waren.

Zudem gilt als bekannt, dass die Knochengesundheit im Rahmen einer veganen Ernährungsweise negativ beeinflusst wird. Das zeigten auch die Daten der RBVD-Studie. Überdies konnte erstmals ein exploratives Biomarkermuster aus knochenrelevanten und ernährungsbedingten Biomarkern abgeleitet werden, welches einen Erklärungsansatz bietet, warum Veganer:innen im Vergleich zu Mischköstler:innen eine niedrige Knochengesundheit haben könnten. In der RBVD-Studie zeigte sich ein deutlicher Unterschied in der uPRAL zwischen Veganer:innen und der Mischkostgruppe, uPRAL hatte aber keinen Einfluss auf die Knochengesundheit in der RBVD-Studie.

Auch hinsichtlich toxikologischer Fragestellungen sind Veganer:innen eine interessante Bevölkerungsgruppe. Durch den vollständigen Verzicht tierischer Produkte, kann vermutet werden, dass Veganer:innen niedrigere PFAS-Konzentrationen im Blut im Vergleich zu Mischköstler:innen haben könnten. Tatsächlich zeigte die RBVD-Studie, dass Veganer:innen im Vergleich zur Mischkostgruppe niedrigere Plasmakonzentrationen für PFOS und für PFNA hatten. Keine signifikanten Unterschiede wurden für PFOA und PFHxS festgestellt. Zudem bestätigte sich niedrige Konzentrationen von Gesamtcholesterin und LDL-Cholesterin bei Veganer:innen im Vergleich zu Mischköstler:innen. Jedoch konnten keine Zusammenhänge zwischen den untersuchten PFAS und diesen Unterschieden festgestellt werden.

5. Literaturverzeichnis

1. Leitzmann C.; Keller M. Charakteristika vegetarischer Ernährungs- und Lebensformen. In Vegetarische und vegane Ernährung, Eugen Ulmer KG: 2020; Vol. 4, pp. 20-32.
2. Leitzmann C.; Keller M. Einführung in die vegetarische und vegane Ernährung. In Vegetarische und vegane Ernährung, Eugen Ulmer KG: 2020; Vol. 4, pp. 15-19.
3. Bundesministerium für Ernährung und Landwirtschaft. Deutschland, wie es isst. Der BMEL-Ernährungsreport 2022. https://www.bmel.de/SharedDocs/Downloads/DE/Broschueren/ernaehrungsreport-2022.pdf?__blob=publicationFile&v=9 (letzter Zugriff: 19.12.2022) 2022.
4. Institut für Demoskopie Allensbach. Umfrage in Deutschland zur Anzahl der Vegetarier bis 2022 https://de.statista.com/statistik/daten/studie/173636/umfrage/lebenseinstellung-anzahl-vegetarier/ (letzter Zugriff: 18.11.2022) 2022.
5. Institut für Demoskopie Allensbach. Umfrage in Deutschland zur Anzahl der Veganer bis 2022 https://de.statista.com/statistik/daten/studie/445155/umfrage/umfrage-in-deutschland-zur-anzahl-der-veganer/ (letzter Zugriff: 18.11.2022) 2022.
6. Bundesinstitut für Riskobewertung. Vegane Ernährung als Lebensstil: Motive und Praktizierung (Abschlussbericht). https://www.bfr.bund.de/cm/350/vegane-ernaehrung-als-lebensstil-motive-und-praktizierung.pdf (letzter Zugriff: 18.11.2022) 2017.
7. Leitzmann C.; Keller M. Ernährungsphysiologische Bewertung einer Kostform. In Vegetarische und vegane Ernährung, Eugen Ulmer KG: 2020; Vol. 4, pp. 80-88.
8. Richter M; Boeing H; Grünewald-Funk D; Heseker H; Kroke A; Leschik-Bonnet E; Oberritter H; Strohm D; Watzl B for the German Nutrition Society (DGE). Vegan diet. Position of the German Nutrition Society (DGE). Ernahrungs Umschau 2016, 63, M262.
9. Leitzmann C.; Keller M. Potenziell kritische Nährstoffe bei vegatarischer Ernährung. In Vegetarische und vegane Ernährung, Eugen Ulmer KG: 2020; Vol. 4, pp. 309-360.
10. Bakaloudi, D.R.; Halloran, A.; Rippin, H.L.; Oikonomidou, A.C.; Dardavesis, T.I.; Williams, J.; Wickramasinghe, K.; Breda, J.; Chourdakis, M. Intake and adequacy of the vegan diet. A systematic review of the evidence. Clin Nutr 2021, 40, 3503-3521, doi:10.1016/j.clnu.2020.11.035.
11. Leitzmann C.; Keller M. Vegetarismus in Prävention und Therapie chronischer Erkrankungen. In Vegetarische und vegane Ernährung, Eugen Ulmer KG: 2020; Vol. 6, pp. 89-283.
12. Melina, V.; Craig, W.; Levin, S. Position of the Academy of Nutrition and Dietetics: Vegetarian Diets. J Acad Nutr Diet 2016, 116, 1970-1980, doi:10.1016/j.jand.2016.09.025.
13. Lee, Y.; Park, K. Adherence to a Vegetarian Diet and Diabetes Risk: A Systematic Review and Meta-Analysis of Observational Studies. Nutrients 2017, 9, doi:10.3390/nu9060603.
14. Kahleova, H.; Levin, S.; Barnard, N.D. Vegetarian Dietary Patterns and Cardiovascular Disease. Prog Cardiovasc Dis 2018, 61, 54-61, doi:10.1016/j.pcad.2018.05.002.
15. Dinu, M.; Abbate, R.; Gensini, G.F.; Casini, A.; Sofi, F. Vegetarian, vegan diets and multiple health outcomes: A systematic review with meta-analysis of observational studies. Crit Rev Food Sci Nutr 2017, 57, 3640-3649, doi:10.1080/10408398.2016.1138447.
16. Fachgesellschaft für Ernährungstherapie und Prävention (FET) e.V. Vegane Ernährung: Auswirkugen auf die Gesundheit. https://fet-ev.eu/produkt/vegane-ernaehrung-auswirkungen-auf-gesundheit/ (letzter Zugriff: 29.11.2022) 2020.
17. Hofseth, L.J.; Hébert, J.R. Chapter 3 - Diet and acute and chronic, systemic, low-grade inflammation. In Diet, Inflammation, and Health, Hébert, J.R., Hofseth, L.J., Eds. Academic Press: 2022; https://doi.org/10.1016/B978-0-12-822130-3.00011-9pp. 85-111.
18. Noe, S.; Heldwein, S.; Tiller, F.W. Entzündungszeichen richtig interpretieren. CME 2019, 16, 49-57, doi:10.1007/s11298-019-6909-0.
19. Leuti, A.; Fazio, D.; Fava, M.; Piccoli, A.; Oddi, S.; Maccarrone, M. Bioactive lipids, inflammation and chronic diseases. Adv Drug Deliv Rev 2020, 159, 133-169, doi:10.1016/j.addr.2020.06.028.
20. Furman, D.; Campisi, J.; Verdin, E.; Carrera-Bastos, P.; Targ, S.; Franceschi, C.; Ferrucci, L.; Gilroy, D.W.; Fasano, A.; Miller, G.W., et al. Chronic inflammation in the etiology of disease across the life span. Nat Med 2019, 25, 1822-1832, doi:10.1038/s41591-019-0675-0.
21. Liu, C.; Feng, X.; Li, Q.; Wang, Y.; Li, Q.; Hua, M. Adiponectin, TNF-alpha and inflammatory cytokines and risk of type 2 diabetes: A systematic review and meta-analysis. Cytokine 2016, 86, 100-109, doi:10.1016/j.cyto.2016.06.028.
22. Jefferis, B.J.; Papacosta, O.; Owen, C.G.; Wannamethee, S.G.; Humphries, S.E.; Woodward, M.; Lennon, L.T.; Thomson, A.; Welsh, P.; Rumley, A., et al. Interleukin 18 and coronary heart disease:

prospective study and systematic review. Atherosclerosis 2011, 217, 227-233, doi:10.1016/j.atherosclerosis.2011.03.015.
23. Pearson, T.A.; Mensah, G.A.; Alexander, R.W.; Anderson, J.L.; Cannon, R.O., 3rd; Criqui, M.; Fadl, Y.Y.; Fortmann, S.P.; Hong, Y.; Myers, G.L., et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003, 107, 499-511, doi:10.1161/01.cir.0000052939.59093.45.
24. Shibata, R.; Ouchi, N.; Murohara, T. Adiponectin and cardiovascular disease. Circ J 2009, 73, 608-614, doi:10.1253/circj.cj-09-0057.
25. Li, S.; Shin, H.J.; Ding, E.L.; van Dam, R.M. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 2009, 302, 179-188, doi:10.1001/jama.2009.976.
26. Eichelmann, F.; Schwingshackl, L.; Fedirko, V.; Aleksandrova, K. Effect of plant-based diets on obesity-related inflammatory profiles: a systematic review and meta-analysis of intervention trials. Obes Rev 2016, 17, 1067-1079, doi:10.1111/obr.12439.
27. Neale, E.P.; Batterham, M.J.; Tapsell, L.C. Consumption of a healthy dietary pattern results in significant reductions in C-reactive protein levels in adults: a meta-analysis. Nutr Res 2016, 36, 391-401, doi:10.1016/j.nutres.2016.02.009.
28. Haghighatdoost, F.; Bellissimo, N.; Totosy de Zepetnek, J.O.; Rouhani, M.H. Association of vegetarian diet with inflammatory biomarkers: a systematic review and meta-analysis of observational studies. Public Health Nutr 2017, 20, 2713-2721, doi:10.1017/S1368980017001768.
29. Craddock, J.C.; Neale, E.P.; Peoples, G.E.; Probst, Y.C. Vegetarian-Based Dietary Patterns and their Relation with Inflammatory and Immune Biomarkers: A Systematic Review and Meta-Analysis. Adv Nutr 2019, 10, 433-451, doi:10.1093/advances/nmy103.
30. Franco-de-Moraes, A.C.; de Almeida-Pititto, B.; Fernandes, G.D.; Gomes, E.P.; Pereira, A.D.; Ferreira, S.R.G. Worse inflammatory profile in omnivores than in vegetarians associates with the gut microbiota composition. Diabetol Metab Syndr 2017, 9, doi:ARTN 6210.1186/s13098-017-0261-x.
31. Sebekova, K.; Krajcovicova-Kudlackova, M.; Schinzel, R.; Faist, V.; Klvanova, J.; Heidland, A. Plasma levels of advanced glycation end products in healthy, long-term vegetarians and subjects on a western mixed diet. Eur J Nutr 2001, 40, 275-281, doi:DOI 10.1007/s394-001-8356-3.
32. Ogilvie, A.R.; McGuire, B.D.; Meng, L.; Shapses, S.A. Fracture Risk in Vegetarians and Vegans: the Role of Diet and Metabolic Factors. Curr Osteoporos Rep 2022, 20, 442-452, doi:10.1007/s11914-022-00754-7.
33. Galchenko, A.; Gapparova, K.; Sidorova, E. The influence of vegetarian and vegan diets on the state of bone mineral density in humans. Crit Rev Food Sci Nutr 2021, 10.1080/10408398.2021.1996330, 1-17, doi:10.1080/10408398.2021.1996330.
34. Li, T.; Li, Y.; Wu, S. Comparison of human bone mineral densities in subjects on plant-based and omnivorous diets: a systematic review and meta-analysis. Arch Osteoporos 2021, 16, 95, doi:10.1007/s11657-021-00955-0.
35. Ho-Pham, L.T.; Nguyen, N.D.; Nguyen, T.V. Effect of vegetarian diets on bone mineral density: a Bayesian meta-analysis. Am J Clin Nutr 2009, 90, 943-950, doi:10.3945/ajcn.2009.27521.
36. Iguacel, I.; Miguel-Berges, M.L.; Gomez-Bruton, A.; Moreno, L.A.; Julian, C. Veganism, vegetarianism, bone mineral density, and fracture risk: a systematic review and meta-analysis. Nutr Rev 2019, 77, 1-18, doi:10.1093/nutrit/nuy045.
37. Tong, T.Y.N.; Appleby, P.N.; Armstrong, M.E.G.; Fensom, G.K.; Knuppel, A.; Papier, K.; Perez-Cornago, A.; Travis, R.C.; Key, T.J. Vegetarian and vegan diets and risks of total and site-specific fractures: results from the prospective EPIC-Oxford study. BMC Med 2020, 18, 353, doi:10.1186/s12916-020-01815-3.
38. Knurick, J.R.; Johnston, C.S.; Wherry, S.J.; Aguayo, I. Comparison of correlates of bone mineral density in individuals adhering to lacto-ovo, vegan, or omnivore diets: a cross-sectional investigation. Nutrients 2015, 7, 3416-3426, doi:10.3390/nu7053416.
39. Tucker, K.L. Vegetarian diets and bone status. Am J Clin Nutr 2014, 100 Suppl 1, 329S-335S, doi:10.3945/ajcn.113.071621.
40. Office of the Surgeon General. Determinants of Bone Health. In Bone Health and Osteoporosis: A Report of the Surgeon General, Office of the Surgeon General (US): Rockville (MD), 2004.
41. Rittenau, N. Kalzium und andere Stoffe für die Knochengesundheit. In Vegan-Klischee ade! Wissenschaftliche Antworten auf kritische Fragen zu pflanzlicher Ernährung, Ventil Verlag: 2020; p. 169.

42. Palermo, A.; Tuccinardi, D.; D'Onofrio, L.; Watanabe, M.; Maggi, D.; Maurizi, A.R.; Greto, V.; Buzzetti, R.; Napoli, N.; Pozzilli, P., et al. Vitamin K and osteoporosis: Myth or reality? Metabolism 2017, 70, 57-71, doi:10.1016/j.metabol.2017.01.032.
43. Davey, G.K.; Spencer, E.A.; Appleby, P.N.; Allen, N.E.; Knox, K.H.; Key, T.J. EPIC-Oxford: lifestyle characteristics and nutrient intakes in a cohort of 33 883 meat-eaters and 31 546 non meat-eaters in the UK. Public Health Nutr 2003, 6, 259-269, doi:10.1079/PHN2002430.
44. Dai, Z.; Koh, W.P. B-vitamins and bone health--a review of the current evidence. Nutrients 2015, 7, 3322-3346, doi:10.3390/nu7053322.
45. Kalimeri, M.; Leek, F.; Wang, N.X.; Koh, H.R.; Roy, N.C.; Cameron-Smith, D.; Kruger, M.C.; Henry, C.J.; Totman, J.J. Folate and Vitamin B-12 Status Is Associated With Bone Mineral Density and Hip Strength of Postmenopausal Chinese-Singaporean Women. Jbmr Plus 2020, 4, doi:ARTN e1039910.1002/jbm4.10399.
46. Menzel, J.; Abraham, K.; Stangl, G.I.; Ueland, P.M.; Obeid, R.; Schulze, M.B.; Herter-Aeberli, I.; Schwerdtle, T.; Weikert, C. Vegan Diet and Bone Health-Results from the Cross-Sectional RBVD Study. Nutrients 2021, 13, doi:10.3390/nu13020685.
47. Siener, R. Einfluss der Ernährung auf den Säure-Basen-Haushalt. Ernährungs-Umschau 2006, 53, 168-173.
48. König, D.; Berg, A. Säure-Basen-Haushalt und Knochengesundheit. Schweitzer Zeitschrift für Ernährungsmedizin 2011, 2, 33-38.
49. Muller, A.; Zimmermann-Klemd, A.M.; Lederer, A.K.; Hannibal, L.; Kowarschik, S.; Huber, R.; Storz, M.A. A Vegan Diet Is Associated with a Significant Reduction in Dietary Acid Load: Post Hoc Analysis of a Randomized Controlled Trial in Healthy Individuals. Int J Environ Res Public Health 2021, 18, doi:10.3390/ijerph18199998.
50. Martin, H. Säure-Basen-Haushalt: Besser basisch essen. UGBforum https://www.ugb.de/ernaehrungsplan-praevention/saeure-basen-haushalt/druckansicht.pdf (letzter Zugriff: 17.12.2022) 2017, 2, 86-89.
51. Carnauba, R.A.; Baptistella, A.B.; Paschoal, V.; Hubscher, G.H. Diet-Induced Low-Grade Metabolic Acidosis and Clinical Outcomes: A Review. Nutrients 2017, 9, doi:10.3390/nu9060538.
52. de Jonge, E.A.L.; Koromani, F.; Hofman, A.; Uitterlinden, A.G.; Franco, O.H.; Rivadeneira, F.; Kiefte-de Jong, J.C. Dietary acid load, trabecular bone integrity, and mineral density in an ageing population: the Rotterdam study. Osteoporos Int 2017, 28, 2357-2365, doi:10.1007/s00198-017-4037-9.
53. Hayhoe, R.P.G.; Abdelhamid, A.; Luben, R.N.; Khaw, K.T.; Welch, A.A. Dietary acid-base load and its association with risk of osteoporotic fractures and low estimated skeletal muscle mass. Eur J Clin Nutr 2020, 74, 33-42, doi:10.1038/s41430-020-0686-4.
54. New, S.A.; MacDonald, H.M.; Campbell, M.K.; Martin, J.C.; Garton, M.J.; Robins, S.P.; Reid, D.M. Lower estimates of net endogenous non-carbonic acid production are positively associated with indexes of bone health in premenopausal and perimenopausal women. Am J Clin Nutr 2004, 79, 131-138, doi:10.1093/ajcn/79.1.131.
55. Welch, A.A.; Bingham, S.A.; Reeve, J.; Khaw, K.T. More acidic dietary acid-base load is associated with reduced calcaneal broadband ultrasound attenuation in women but not in men: results from the EPIC-Norfolk cohort study. American Journal of Clinical Nutrition 2007, 85, 1134-1141, doi:DOI 10.1093/ajcn/85.4.1134.
56. Gholami, F.; Naghshi, S.; Samadi, M.; Rasaei, N.; Mirzaei, K. Dietary Acid Load and Bone Health: A Systematic Review and Meta-Analysis of Observational Studies. Front Nutr 2022, 9, 869132, doi:10.3389/fnut.2022.869132.
57. Kahleova, H.; McCann, J.; Alwarith, J.; Rembert, E.; Tura, A.; Holubkov, R.; Barnard, N.D. A plant-based diet in overweight adults in a 16-week randomized clinical trial: The role of dietary acid load. Clin Nutr ESPEN 2021, 44, 150-158, doi:10.1016/j.clnesp.2021.05.015.
58. Cosgrove, K.; Johnston, C.S. Examining the Impact of Adherence to a Vegan Diet on Acid-Base Balance in Healthy Adults. Plant Foods Hum Nutr 2017, 72, 308-313, doi:10.1007/s11130-017-0620-7.
59. Johnston, C.S.; Bliss, C.; Knurick, J.R.; Scholtz, C. Rapid Eating Assessment for Participants [shortened version] scores are associated with Healthy Eating Index-2010 scores and other indices of diet quality in healthy adult omnivores and vegetarians. Nutr J 2018, 17, 89, doi:10.1186/s12937-018-0399-x.

60. Cupisti, A.; D'Alessandro, C.; Gesualdo, L.; Cosola, C.; Gallieni, M.; Egidi, M.F.; Fusaro, M. Non-Traditional Aspects of Renal Diets: Focus on Fiber, Alkali and Vitamin K1 Intake. Nutrients 2017, 9, doi:10.3390/nu9050444.
61. Burckhardt, P. The role of low acid load in vegetarian diet on bone health: a narrative review. Swiss Med Wkly 2016, 146, w14277, doi:10.4414/smw.2016.14277.
62. Frassetto, L.; Banerjee, T.; Powe, N.; Sebastian, A. Acid Balance, Dietary Acid Load, and Bone Effects-A Controversial Subject. Nutrients 2018, 10, doi:10.3390/nu10040517.
63. Frassetto, L.A.; Hardcastle, A.C.; Sebastian, A.; Aucott, L.; Fraser, W.D.; Reid, D.M.; Macdonald, H.M. No evidence that the skeletal non-response to potassium alkali supplements in healthy postmenopausal women depends on blood pressure or sodium chloride intake. European Journal of Clinical Nutrition 2012, 66, 1315-1322, doi:10.1038/ejcn.2012.151.
64. Bundesinstitut für Riskobewertung. PFAS in Lebensmitteln: BfR bestätigt kritische Exposition gegenüber Industriechemikalien. https://www.bfr.bund.de/cm/343/pfas-in-lebensmitteln-bfr-bestaetigt-kritische-exposition-gegenueber-industriechemikalien.pdf (letzter Zugriff: 17.12.2022) 2021.
65. Sunderland, E.M.; Hu, X.C.; Dassuncao, C.; Tokranov, A.K.; Wagner, C.C.; Allen, J.G. A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. J Expo Sci Environ Epidemiol 2019, 29, 131-147, doi:10.1038/s41370-018-0094-1.
66. Umweltbundesamt. Schwerpunkt: PFAS - gekommen, um zu bleiben. https://www.umweltbundesamt.de/sites/default/files/medien/2546/publikationen/uba_sp_pfas_web_0.pdf (letzter Zugriff: 17.12.2022) 2020, 1.
67. EFSA Panel on Contaminants in the Food Chain; Schrenk, D.; Bignami, M.; Bodin, L.; Chipman, J.K.; Del Mazo, J.; Grasl-Kraupp, B.; Hogstrand, C.; Hoogenboom, L.R.; Leblanc, J.C., et al. Risk to human health related to the presence of perfluoroalkyl substances in food. Efsa j 2020, 18, e06223, doi:10.2903/j.efsa.2020.6223.
68. Evich, M.G.; Davis, M.J.B.; McCord, J.P.; Acrey, B.; Awkerman, J.A.; Knappe, D.R.U.; Lindstrom, A.B.; Speth, T.F.; Tebes-Stevens, C.; Strynar, M.J., et al. Per- and polyfluoroalkyl substances in the environment. Science 2022, 375, eabg9065, doi:10.1126/science.abg9065.
69. Ho, S.H.; Soh, S.X.H.; Wang, M.X.; Ong, J.; Seah, A.; Wong, Y.; Fang, Z.; Sim, S.; Lim, J.T. Perfluoroalkyl substances and lipid concentrations in the blood: A systematic review of epidemiological studies. Sci Total Environ 2022, 850, 158036, doi:10.1016/j.scitotenv.2022.158036.
70. Yokoyama, Y.; Levin, S.M.; Barnard, N.D. Association between plant-based diets and plasma lipids: a systematic review and meta-analysis. Nutrition Reviews 2017, 75, 683-698, doi:10.1093/nutrit/nux030.
71. Weikert, C.; Trefflich, I.; Menzel, J.; Obeid, R.; Longree, A.; Dierkes, J.; Meyer, K.; Herter-Aeberli, I.; Mai, K.; Stangl, G.I., et al. Vitamin and Mineral Status in a Vegan Diet. Dtsch Arztebl Int 2020, 117, 575-582, doi:10.3238/arztebl.2020.0575.
72. Menzel, J.; Biemann, R.; Longree, A.; Isermann, B.; Mai, K.; Schulze, M.B.; Abraham, K.; Weikert, C. Associations of a vegan diet with inflammatory biomarkers. Sci Rep 2020, 10, 1933, doi:10.1038/s41598-020-58875-x.
73. Menzel, J.; Jabakhanji, A.; Biemann, R.; Mai, K.; Abraham, K.; Weikert, C. Systematic review and meta-analysis of the associations of vegan and vegetarian diets with inflammatory biomarkers. Sci Rep 2020, 10, 21736, doi:10.1038/s41598-020-78426-8.
74. Penczynski, K.J.; Remer, T.; Menzel, J.; Abraham, K.; Weikert, C. Urinary Potential Renal Acid Load (uPRAL) among Vegans Versus Omnivores and Its Association with Bone Health in the Cross-Sectional Risks and Benefits of a Vegan Diet Study. Nutrients 2022, 14, doi:10.3390/nu14214468.
75. Menzel, J.; Abraham, K.; Dietrich, S.; Fromme, H.; Volkel, W.; Schwerdtle, T.; Weikert, C. Internal exposure to perfluoroalkyl substances (PFAS) in vegans and omnivores. Int J Hyg Environ Health 2021, 237, 113808, doi:10.1016/j.ijheh.2021.113808.
76. Franco-de-Moraes, A.C.; de Almeida-Pititto, B.; da Rocha Fernandes, G.; Gomes, E.P.; da Costa Pereira, A.; Ferreira, S.R.G. Worse inflammatory profile in omnivores than in vegetarians associates with the gut microbiota composition. Diabetol Metab Syndr 2017, 9, 62, doi:10.1186/s13098-017-0261-x.
77. Šebeková, K.; Krajčovičová-Kudláčková, M.; Schinzel, R.; Faist, V.; Klvanová, J.; Heidland, A. Plasma levels of advanced glycation end products in healthy, long-term vegetarians and subjects on a western mixed diet. Eur J Nutr 2001, 40, 275-281, doi:10.1007/s394-001-8356-3.

78. Mezzano, D.; Munoz, X.; Martinez, C.; Cuevas, A.; Panes, O.; Aranda, E.; Guasch, V.; Strobel, P.; Munoz, B.; Rodriguez, S., et al. Vegetarians and cardiovascular risk factors: hemostasis, inflammatory markers and plasma homocysteine. Thromb Haemost 1999, 81, 913-917.
79. Krajcovicova-Kudlackova, M.; Blazicek, P. C-reactive protein and nutrition. Bratisl Lek Listy 2005, 106, 345-347.
80. Szeto, Y.T.; Kwok, T.C.Y.; Benzie, I.F.F. Effects of a long-term vegetarian diet on biomarkers of antioxidant status and cardiovascular disease risk. Nutrition 2004, 20, 863-866, doi:https://doi.org/10.1016/j.nut.2004.06.006.
81. Sebekova, K.; Boor, P.; Valachovicova, M.; Blazicek, P.; Parrak, V.; Babinska, K.; Heidland, A.; Krajcovicova-Kudlackova, M. Association of metabolic syndrome risk factors with selected markers of oxidative status and microinflammation in healthy omnivores and vegetarians. Mol Nutr Food Res 2006, 50, 858-868, doi:10.1002/mnfr.200500170.
82. Hung, K.-C.; Pei, D.; Kuo, H.-J.; Chen, T.-H.; Lin, C.-H.; Wu, C.-Z.; Hsia, T.-L.; Su, C.-C.; Hsiao, F.-C.; Lu, C.-H. The comparison of the metabolic syndrome between Chinese vegetarians and omnivores. Diabetes & Metabolic Syndrome: Clinical Research & Reviews 2008, 2, 99-104, doi:https://doi.org/10.1016/j.dsx.2008.02.002.
83. Chen, C.W.; Lin, Y.L.; Lin, T.K.; Lin, C.T.; Chen, B.C.; Lin, C.L. Total cardiovascular risk profile of Taiwanese vegetarians. European Journal of Clinical Nutrition 2008, 62, 138-144, doi:10.1038/sj.ejcn.1602689.
84. Chen, C.W.; Lin, C.T.; Lin, Y.L.; Lin, T.K.; Lin, C.L. Taiwanese Female Vegetarians Have Lower Lipoprotein-Associated Phospholipase A2 Compared with Omnivores. Yonsei Med J 2011, 52, 13-19, doi:10.3349/ymj.2011.52.1.13.
85. Lee, C.G.; Hahn, S.J.; Song, M.K.; Lee, J.K.; Kim, J.H.; Lim, Y.J.; Koh, M.-S.; Lee, J.H.; Kang, H.W. Vegetarianism as a Protective Factor for Colorectal Adenoma and Advanced Adenoma in Asians. Digestive Diseases and Sciences 2014, 59, 1025-1035, doi:10.1007/s10620-013-2974-5.
86. Chuang, S.Y.; Chiu, T.H.T.; Lee, C.Y.; Liu, T.T.; Tsao, C.K.; Hsiung, C.A.; Chiu, Y.F. Vegetarian diet reduces the risk of hypertension independent of abdominal obesity and inflammation: a prospective study. J Hypertens 2016, 34, 2164-2171, doi:10.1097/Hjh.0000000000001068.
87. Acosta-Navarro, J.; Antoniazzi, L.; Oki, A.M.; Bonfim, M.C.; Hong, V.; Acosta-Cardenas, P.; Strunz, C.; Brunoro, E.; Miname, M.H.; Filho, W.S., et al. Reduced subclinical carotid vascular disease and arterial stiffness in vegetarian men: The CARVOS Study. International Journal of Cardiology 2017, 230, 562-566, doi:https://doi.org/10.1016/j.ijcard.2016.12.058.
88. Su, T.C.; Torng, P.L.; Jeng, J.S.; Chen, M.F.; Liau, C.S. Arterial function of carotid and brachial arteries in postmenopausal vegetarians. Vasc Health Risk Manag 2011, 7, 517-523, doi:10.2147/VHRM.S18881.
89. Zorena, K.; Jachimowicz-Duda, O.; Ślęzak, D.; Robakowska, M.; Mrugacz, M. Adipokines and Obesity. Potential Link to Metabolic Disorders and Chronic Complications. Int J Mol Sci 2020, 21, doi:10.3390/ijms21103570.
90. Gluba-Brzózka, A.; Franczyk, B.; Rysz, J. Vegetarian Diet in Chronic Kidney Disease-A Friend or Foe. Nutrients 2017, 9, doi:10.3390/nu9040374.
91. Trapp, C.B.; Barnard, N.D. Usefulness of vegetarian and vegan diets for treating type 2 diabetes. Curr Diab Rep 2010, 10, 152-158, doi:10.1007/s11892-010-0093-7.
92. McMacken, M.; Shah, S. A plant-based diet for the prevention and treatment of type 2 diabetes. J Geriatr Cardiol 2017, 14, 342-354, doi:10.11909/j.issn.1671-5411.2017.05.009.
93. Morgan, S.L.; Prater, G.L. Quality in dual-energy X-ray absorptiometry scans. Bone 2017, 104, 13-28, doi:https://doi.org/10.1016/j.bone.2017.01.033.
94. van de Ven, A.C.; Erdtsieck, R.J. Changes of bone mineral density, quantitative ultrasound parameters and markers of bone turnover during treatment of hyperthyroidism. Neth J Med 2008, 66, 428-432.
95. Rufus-Membere, P.; Holloway-Kew, K.L.; Diez-Perez, A.; Kotowicz, M.A.; Pasco, J.A. Associations between Bone Material Strength Index, Calcaneal Quantitative Ultrasound, and Bone Mineral Density in Men. J Endocr Soc 2021, 5, bvaa179, doi:10.1210/jendso/bvaa179.
96. Pisani, P.; Renna, M.D.; Conversano, F.; Casciaro, E.; Muratore, M.; Quarta, E.; Paola, M.D.; Casciaro, S. Screening and early diagnosis of osteoporosis through X-ray and ultrasound based techniques. World J Radiol 2013, 5, 398-410, doi:10.4329/wjr.v5.i11.398.

97. Chan, M.Y.; Nguyen, N.D.; Center, J.R.; Eisman, J.A.; Nguyen, T.V. Quantitative ultrasound and fracture risk prediction in non-osteoporotic men and women as defined by WHO criteria. Osteoporos Int 2013, 24, 1015-1022, doi:10.1007/s00198-012-2001-2.
98. Anna, U.M.; Maria, S.; Kerstin, B. Comparison of quantitative ultrasound of calcaneus and dual energy X-ray absorptiometry in measuring bone density and predicting fractures in patients with diabetic polyneuropathy: A prospective cohort study. Diabetes Res Clin Pract 2021, 180, 109064, doi:10.1016/j.diabres.2021.109064.
99. Tapsell, L.C.; Neale, E.P.; Satija, A.; Hu, F.B. Foods, Nutrients, and Dietary Patterns: Interconnections and Implications for Dietary Guidelines. Adv Nutr 2016, 7, 445-454, doi:10.3945/an.115.011718.
100. Waldmann, A.; Dorr, B.; Koschizke, J.W.; Leitzmann, C.; Hahn, A. Dietary intake of vitamin B6 and concentration of vitamin B6 in blood samples of German vegans. Public Health Nutr 2006, 9, 779-784, doi:10.1079/phn2005895.
101. Deutsche Gesellschaft für Ernährung e.V. Ausgewählte Fragen und Antworten zu Vitamin A. https://www.dge.de/fileadmin/public/doc/ws/faq/FAQs-Vitamin_A-DGE.pdf (letzter Zugriff: 24.01.2023) 2020.
102. Dawczynski, C.; Weidauer, T.; Richert, C.; Schlattmann, P.; Dawczynski, K.; Kiehntopf, M. Nutrient Intake and Nutrition Status in Vegetarians and Vegans in Comparison to Omnivores - the Nutritional Evaluation (NuEva) Study. Front Nutr 2022, 9, 819106, doi:10.3389/fnut.2022.819106.
103. Yee, M.M.F.; Chin, K.Y.; Ima-Nirwana, S.; Wong, S.K. Vitamin A and Bone Health: A Review on Current Evidence. Molecules 2021, 26, doi:10.3390/molecules26061757.
104. Khojah, Q.; AlRumaihi, S.; AlRajeh, G.; Aburas, A.; AlOthman, A.; Ferwana, M. Vitamin A and its dervatives effect on bone mineral density, a systematic review. J Family Med Prim Care 2021, 10, 4089-4095, doi:10.4103/jfmpc.jfmpc_663_21.
105. Menzel, J.; Longree, A.; Abraham, K.; Schulze, M.B.; Weikert, C. Dietary and Plasma Phospholipid Profiles in Vegans and Omnivores-Results from the RBVD Study. Nutrients 2022, 14, doi:ARTN 290010.3390/nu14142900.
106. Burns-Whitmore, B.; Froyen, E.; Heskey, C.; Parker, T.; San Pablo, G. Alpha-Linolenic and Linoleic Fatty Acids in the Vegan Diet: Do They Require Dietary Reference Intake/Adequate Intake Special Consideration? Nutrients 2019, 11, doi:10.3390/nu11102365.
107. Welch, A.A.; Shakya-Shrestha, S.; Lentjes, M.A.; Wareham, N.J.; Khaw, K.T. Dietary intake and status of n-3 polyunsaturated fatty acids in a population of fish-eating and non-fish-eating meat-eaters, vegetarians, and vegans and the product-precursor ratio [corrected] of alpha-linolenic acid to long-chain n-3 polyunsaturated fatty acids: results from the EPIC-Norfolk cohort. Am J Clin Nutr 2010, 92, 1040-1051, doi:10.3945/ajcn.2010.29457.
108. Bao, M.; Zhang, K.; Wei, Y.; Hua, W.; Gao, Y.; Li, X.; Ye, L. Therapeutic potentials and modulatory mechanisms of fatty acids in bone. Cell Prolif 2020, 53, e12735, doi:10.1111/cpr.12735.
109. Sadeghi, O.; Djafarian, K.; Ghorabi, S.; Khodadost, M.; Nasiri, M.; Shab-Bidar, S. Dietary intake of fish, n-3 polyunsaturated fatty acids and risk of hip fracture: A systematic review and meta-analysis on observational studies. Crit Rev Food Sci Nutr 2019, 59, 1320-1333, doi:10.1080/10408398.2017.1405908.
110. Abdelhamid, A.; Hooper, L.; Sivakaran, R.; Hayhoe, R.P.G.; Welch, A. The Relationship Between Omega-3, Omega-6 and Total Polyunsaturated Fat and Musculoskeletal Health and Functional Status in Adults: A Systematic Review and Meta-analysis of RCTs. Calcif Tissue Int 2019, 105, 353-372, doi:10.1007/s00223-019-00584-3.
111. Orchard, T.S.; Pan, X.; Cheek, F.; Ing, S.W.; Jackson, R.D. A systematic review of omega-3 fatty acids and osteoporosis. Br J Nutr 2012, 107 Suppl 2, S253-260, doi:10.1017/s0007114512001638.
112. Pepa, G.D.; Brandi, M.L. Microelements for bone boost: the last but not the least. Clin Cases Miner Bone Metab 2016, 13, 181-185, doi:10.11138/ccmbm/2016.13.3.181.
113. Jahnen-Dechent, W.; Ketteler, M. Magnesium basics. Clin Kidney J 2012, 5, i3-i14, doi:10.1093/ndtplus/sfr163.
114. Groenendijk, I.; van Delft, M.; Versloot, P.; van Loon, L.J.C.; de Groot, L. Impact of magnesium on bone health in older adults: A systematic review and meta-analysis. Bone 2022, 154, 116233, doi:10.1016/j.bone.2021.116233.
115. Rondanelli, M.; Faliva, M.A.; Tartara, A.; Gasparri, C.; Perna, S.; Infantino, V.; Riva, A.; Petrangolini, G.; Peroni, G. An update on magnesium and bone health. Biometals 2021, 34, 715-736, doi:10.1007/s10534-021-00305-0.

116. Foley, K.F.; Boccuzzi, L. Urine Calcium: Laboratory Measurement and Clinical Utility. Labmedicine 2010, 41, 683-686, doi:10.1309/Lm9so94znbhedntm.
117. Vannucci, L.; Masi, L.; Gronchi, G.; Fossi, C.; Carossino, A.M.; Brandi, M.L. Calcium intake, bone mineral density, and fragility fractures: evidence from an Italian outpatient population. Arch Osteoporos 2017, 12, 40, doi:10.1007/s11657-017-0333-4.
118. Tai, V.; Leung, W.; Grey, A.; Reid, I.R.; Bolland, M.J. Calcium intake and bone mineral density: systematic review and meta-analysis. BMJ 2015, 351, h4183, doi:10.1136/bmj.h4183.
119. Bolland, M.J.; Leung, W.; Tai, V.; Bastin, S.; Gamble, G.D.; Grey, A.; Reid, I.R. Calcium intake and risk of fracture: systematic review. BMJ 2015, 351, h4580, doi:10.1136/bmj.h4580.
120. Appleby, P.; Roddam, A.; Allen, N.; Key, T. Comparative fracture risk in vegetarians and nonvegetarians in EPIC-Oxford. Eur J Clin Nutr 2007, 61, 1400-1406, doi:10.1038/sj.ejcn.1602659.
121. Warensjo, E.; Byberg, L.; Melhus, H.; Gedeborg, R.; Mallmin, H.; Wolk, A.; Michaelsson, K. Dietary calcium intake and risk of fracture and osteoporosis: prospective longitudinal cohort study. BMJ 2011, 342, d1473, doi:10.1136/bmj.d1473.
122. Bundesinstitut für Riskobewertung. Jodversorgung in Deutschland wieder rückläufig - Tipps für eine gute Jodversorgung. https://www.bfr.bund.de/cm/343/jodversorgung-in-deutschland-wieder-ruecklaeufig-tipps-fuer-eine-gute-jodversorgung.pdf (letzter Zugriff: 02.02.2023) 2021.
123. Robert Koch-Institut. Gesundheit in Deutschland. https://www.rki.de/DE/Content/Gesundheitsmonitoring/Gesundheitsberichterstattung/GesInDtld/gesundheit_in_deutschland_2015.pdf?__blob=publicationFile (letzter Zugriff: 03.02.2023) 2015.
124. Bundesministerium für Ernährung und Landwirtschaft. Jodversorgung in Deutschland: Ergebnisse des Jodmonitorings. https://www.bmel.de/DE/themen/ernaehrung/gesunde-ernaehrung/degs-jod-studie.html (letzter Zugriff: 03.02.2023) 2020.
125. World Health Organization. Urinary iodine concentrations for determining iodine status deficiency in populations. Vitamin and Mineral Nutrition Information System. http://www.who.int/nutrition/vmnis/indicators/urinaryiodine (letzter Zugriff: 03.02.2023) 2013.
126. Zimmermann, M.B. Iodine deficiency. Endocr Rev 2009, 30, 376-408, doi:10.1210/er.2009-0011.
127. Abrahamsen, B.; Jørgensen, H.L.; Laulund, A.S.; Nybo, M.; Brix, T.H.; Hegedüs, L. Low Serum Thyrotropin Level and Duration of Suppression as a Predictor of Major Osteoporotic Fractures—The OPENTHYRO Register Cohort. Journal of Bone and Mineral Research 2014, 29, 2040-2050, doi:https://doi.org/10.1002/jbmr.2244.
128. Murphy, E.; Glüer, C.C.; Reid, D.M.; Felsenberg, D.; Roux, C.; Eastell, R.; Williams, G.R. Thyroid function within the upper normal range is associated with reduced bone mineral density and an increased risk of nonvertebral fractures in healthy euthyroid postmenopausal women. J Clin Endocrinol Metab 2010, 95, 3173-3181, doi:10.1210/jc.2009-2630.
129. Arslanca, T.; Korkmaz, V.; Arslanca, S.B.; Karadag, B.; Ergun, Y. Body iodine status in women with postmenopausal osteoporosis. Menopause 2018, 25, 320-323, doi:10.1097/GME.0000000000000987.
130. Deutsche Gesellschaft für Ernährung e.V. Ausgewählte Fragen und Antworten zu Selen. https://www.dge.de/fileadmin/public/doc/ws/faq/FAQs-Selen-DGE.pdf (letzter Zugriff: 06.02.2023) 2021.
131. Hoeg, A.; Gogakos, A.; Murphy, E.; Mueller, S.; Kohrle, J.; Reid, D.M.; Gluer, C.C.; Felsenberg, D.; Roux, C.; Eastell, R., et al. Bone turnover and bone mineral density are independently related to selenium status in healthy euthyroid postmenopausal women. J Clin Endocrinol Metab 2012, 97, 4061-4070, doi:10.1210/jc.2012-2121.
132. Beukhof, C.M.; Medici, M.; van den Beld, A.W.; Hollenbach, B.; Hoeg, A.; Visser, W.E.; de Herder, W.W.; Visser, T.J.; Schomburg, L.; Peeters, R.P. Selenium Status Is Positively Associated with Bone Mineral Density in Healthy Aging European Men. Plos One 2016, 11, doi:ARTN e015274810.1371/journal.pone.0152748.
133. Pietschmann, N.; Rijntjes, E.; Hoeg, A.; Stoedter, M.; Schweizer, U.; Seemann, P.; Schomburg, L. Selenoprotein P is the essential selenium transporter for bones. Metallomics 2014, 6, 1043-1049, doi:10.1039/c4mt00003j.
134. Dietrich, S.; Trefflich, I.; Ueland, P.M.; Menzel, J.; Penczynski, K.J.; Abraham, K.; Weikert, C. Amino acid intake and plasma concentrations and their interplay with gut microbiota in vegans and omnivores in Germany. Eur J Nutr 2022, 61, 2103-2114, doi:10.1007/s00394-021-02790-y.
135. Broer, S.; Broer, A. Amino acid homeostasis and signalling in mammalian cells and organisms. Biochem J 2017, 474, 1935-1963, doi:10.1042/BCJ20160822.

136. Cynober, L.A. Plasma amino acid levels with a note on membrane transport: characteristics, regulation, and metabolic significance. Nutrition 2002, 18, 761-766, doi:10.1016/s0899-9007(02)00780-3.
137. Schmidt, J.A.; Rinaldi, S.; Scalbert, A.; Ferrari, P.; Achaintre, D.; Gunter, M.J.; Appleby, P.N.; Key, T.J.; Travis, R.C. Plasma concentrations and intakes of amino acids in male meat-eaters, fish-eaters, vegetarians and vegans: a cross-sectional analysis in the EPIC-Oxford cohort. European Journal of Clinical Nutrition 2016, 70, 306-312, doi:10.1038/ejcn.2015.144.
138. Yang, J.; Zhang, X.; Wang, W.; Liu, J. Insulin stimulates osteoblast proliferation and differentiation through ERK and PI3K in MG-63 cells. Cell Biochemistry and Function 2010, 28, 334-341, doi:https://doi.org/10.1002/cbf.1668.
139. Liu, Z.; Jeppesen, P.B.; Gregersen, S.; Chen, X.; Hermansen, K. Dose- and Glucose-Dependent Effects of Amino Acids on Insulin Secretion from Isolated Mouse Islets and Clonal INS-1E Beta-Cells. Rev Diabet Stud 2008, 5, 232-244, doi:10.1900/rds.2008.5.232.
140. Jennings, A.; MacGregor, A.; Spector, T.; Cassidy, A. Amino Acid Intakes Are Associated With Bone Mineral Density and Prevalence of Low Bone Mass in Women: Evidence From Discordant Monozygotic Twins. J Bone Miner Res 2016, 31, 326-335, doi:10.1002/jbmr.2703.
141. Fujita, S.; Volpi, E. Amino acids and muscle loss with aging. J Nutr 2006, 136, 277s-280s, doi:10.1093/jn/136.1.277S.
142. Fukumoto, S. FGF23 and Bone and Mineral Metabolism. Handb Exp Pharmacol 2020, 262, 281-308, doi:10.1007/164_2019_330.
143. Erben, R.G. Physiologie und Pathophysiologie von FGF23 und Klotho. Nephrologe 2019, 4, 302-304.
144. Guo, Y.C.; Yuan, Q. Fibroblast growth factor 23 and bone mineralisation. Int J Oral Sci 2015, 7, 8-13, doi:10.1038/ijos.2015.1.
145. Van de Loo, I.; Harbeck, B. Knochen und Kalziumstoffwechsel In Facharztwissen Endokrinologie und Diabetologie, Springer: 2020; pp. 105 - 107.
146. Wang, Y.; Wang, H.; Chen, P. Higher Fibroblast Growth Factor 23 Levels Are Causally Associated With Lower Bone Mineral Density of Heel and Femoral Neck: Evidence From Two-Sample Mendelian Randomization Analysis. Front Public Health 2020, 8, 467, doi:10.3389/fpubh.2020.00467.
147. Isakova, T.; Cai, X.; Lee, J.; Katz, R.; Cauley, J.A.; Fried, L.F.; Hoofnagle, A.N.; Satterfield, S.; Harris, T.B.; Shlipak, M.G., et al. Associations of FGF23 With Change in Bone Mineral Density and Fracture Risk in Older Individuals. J Bone Miner Res 2016, 31, 742-748, doi:10.1002/jbmr.2750.
148. Chalhoub, D.; Marques, E.; Meirelles, O.; Semba, R.D.; Ferrucci, L.; Satterfield, S.; Nevitt, M.; Cauley, J.A.; Harris, T. Association of Serum Klotho with Loss of Bone Mineral Density and Fracture Risk in Older Adults. J Am Geriatr Soc 2016, 64, e304-e308, doi:10.1111/jgs.14661.
149. Jovanovich, A.; Buzkova, P.; Chonchol, M.; Robbins, J.; Fink, H.A.; de Boer, I.H.; Kestenbaum, B.; Katz, R.; Carbone, L.; Lee, J., et al. Fibroblast growth factor 23, bone mineral density, and risk of hip fracture among older adults: the cardiovascular health study. J Clin Endocrinol Metab 2013, 98, 3323-3331, doi:10.1210/jc.2013-1152.
150. Shen, J.; Fu, S.; Song, Y. Relationship of Fibroblast Growth Factor 23 (FGF-23) Serum Levels With Low Bone Mass in Postmenopausal Women. J Cell Biochem 2017, 118, 4454-4459, doi:10.1002/jcb.26101.
151. Han, W.; Bai, X.J.; Han, L.L.; Sun, X.F.; Chen, X.M. The relationship between serum fibroblast growth factor 23, Klotho, and lumbar spine bone mineral density in northern Chinese postmenopausal women. Menopause 2019, 26, 546-553, doi:10.1097/gme.0000000000001276.
152. Weikert, C.; Schulze, M.B. Evaluating dietary patterns: the role of reduced rank regression. Curr Opin Clin Nutr Metab Care 2016, 19, 341-346, doi:10.1097/MCO.0000000000000308.
153. Remer, T.; Krupp, D.; Shi, L. Dietary protein's and dietary acid load's influence on bone health. Crit Rev Food Sci Nutr 2014, 54, 1140-1150, doi:10.1080/10408398.2011.627519.
154. Ausman, L.M.; Oliver, L.M.; Goldin, B.R.; Woods, M.N.; Gorbach, S.L.; Dwyer, J.T. Estimated net acid excretion inversely correlates with urine pH in vegans, lacto-ovo vegetarians, and omnivores. J Ren Nutr 2008, 18, 456-465, doi:10.1053/j.jrn.2008.04.007.
155. Vormann, J. Säure-Basen-Haushalt: latente Azidose als Ursache chronischer Erkrankungen. In Säuren - Basen - Schlacken, Pro und Contra - eine wissenschaftliche Diskussion, Springer: 2007; pp. 25-37.

156. Fromme, H.; Wöckner, M.; Roscher, E.; Völkel, W. ADONA and perfluoroalkylated substances in plasma samples of German blood donors living in South Germany. International Journal of Hygiene and Environmental Health 2017, 220, 455-460, doi:https://doi.org/10.1016/j.ijheh.2016.12.014.
157. Göckener, B.; Weber, T.; Rüdel, H.; Bücking, M.; Kolossa-Gehring, M. Human biomonitoring of per- and polyfluoroalkyl substances in German blood plasma samples from 1982 to 2019. Environment International 2020, 145, 106123, doi:https://doi.org/10.1016/j.envint.2020.106123.
158. Lin, P.-I.D.; Cardenas, A.; Hauser, R.; Gold, D.R.; Kleinman, K.P.; Hivert, M.-F.; Fleisch, A.F.; Calafat, A.M.; Sanchez-Guerra, M.; Osorio-Yáñez, C., et al. Dietary characteristics associated with plasma concentrations of per- and polyfluoroalkyl substances among adults with pre-diabetes: Cross-sectional results from the Diabetes Prevention Program Trial. Environment International 2020, 137, 105217, doi:https://doi.org/10.1016/j.envint.2019.105217.
159. Abraham, K.; Monien, B.H. Transdermal absorption of (13)C(4)-perfluorooctanoic acid ((13)C(4)-PFOA) from a sunscreen in a male volunteer - What could be the contribution of cosmetics to the internal exposure of perfluoroalkyl substances (PFAS)? Environ Int 2022, 169, 107549, doi:10.1016/j.envint.2022.107549.
160. Poothong, S.; Papadopoulou, E.; Padilla-Sánchez, J.A.; Thomsen, C.; Haug, L.S. Multiple pathways of human exposure to poly- and perfluoroalkyl substances (PFASs): From external exposure to human blood. Environment International 2020, 134, 105244, doi:https://doi.org/10.1016/j.envint.2019.105244.
161. Nelson, J.W.; Hatch, E.E.; Webster, T.F. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population. Environ Health Perspect 2010, 118, 197-202, doi:10.1289/ehp.0901165.
162. Eriksen, K.T.; Raaschou-Nielsen, O.; McLaughlin, J.K.; Lipworth, L.; Tjonneland, A.; Overvad, K.; Sorensen, M. Association between Plasma PFOA and PFOS Levels and Total Cholesterol in a Middle-Aged Danish Population. Plos One 2013, 8, doi:ARTN e5696910.1371/journal.pone.0056969.
163. Skuladottir, M.; Ramel, A.; Rytter, D.; Haug, L.S.; Sabaredzovic, A.; Bech, B.H.; Henriksen, T.B.; Olsen, S.F.; Halldorsson, T.I. Examining confounding by diet in the association between perfluoroalkyl acids and serum cholesterol in pregnancy. Environ Res 2015, 143, 33-38, doi:10.1016/j.envres.2015.09.001.
164. Canova, C.; Barbieri, G.; Jeddi, M.Z.; Gion, M.; Fabricio, A.; Dapra, F.; Russo, F.; Fletcher, T.; Pitter, G. Associations between perfluoroalkyl substances and lipid profile in a highly exposed young adult population in the Veneto Region. Environment International 2020, 145, doi:ARTN 10611710.1016/j.envint.2020.106117.
165. Nöthlings, U.; Hoffmann, K.; Bergmann, M.M.; Boeing, H. Fitting Portion Sizes in a Self-Administered Food Frequency Questionnaire. The Journal of Nutrition 2007, 137, 2781-2786, doi:https://doi.org/10.1093/jn/137.12.2781.
166. Appleby, P.N.; Key, T.J. The long-term health of vegetarians and vegans. The Proceedings of the Nutrition Society 2016, 75, 287-293, doi:10.1017/s0029665115004334.
167. WHO European Office for the Prevention and Control of Noncommunicable Diseases. Plant-based diets and their impact on health, sustainability and the environment: a review of the evidence. https://apps.who.int/iris/bitstream/handle/10665/349086/WHO-EURO-2021-4007-43766-61591-eng.pdf?sequence=1&isAllowed=y (Letzter Zugriff: 11.02.2023) 2021.
168. Leitzmann C.; Keller M. Globale Aspekte des Vegetarismus. In Vegetarische und vegane Ernährung, Eugen Ulmer KG: 2020; Vol. 4, pp. 424-448.
169. World Health Organization. Climate change and health. https://www.who.int/news-room/fact-sheets/detail/climate-change-and-health (Letzter Zugriff: 12.02.2023) 2021.
170. Fresan, U.; Sabate, J. Vegetarian Diets: Planetary Health and Its Alignment with Human Health. Adv Nutr 2019, 10, S380-S388, doi:10.1093/advances/nmz019.
171. Leitzmann, C.; Schönhöfer-Rempt, R.; Boy, M. Ernährung und Gesundheit von Vegetariern. Die Giessener Vegetarier-Studie. Echo-Verlag Hannover 1988, 9-14.
172. Waldmann, A.; Koschizke, J.W.; Leitzmann, C.; Hahn, A. Dietary intakes and lifestyle factors of a vegan population in Germany: results from the German Vegan Study. Eur J Clin Nutr 2003, 57, 947-955, doi:10.1038/sj.ejcn.1601629.
173. Schüpbach, R.; Wegmüller, R.; Berguerand, C.; Bui, M.; Herter-Aeberli, I. Micronutrient status and intake in omnivores, vegetarians and vegans in Switzerland. Eur J Nutr 2017, 56, 283-293, doi:10.1007/s00394-015-1079-7.

174. Kristensen, N.B.; Madsen, M.L.; Hansen, T.H.; Allin, K.H.; Hoppe, C.; Fagt, S.; Lausten, M.S.; Gøbel, R.J.; Vestergaard, H.; Hansen, T., et al. Intake of macro- and micronutrients in Danish vegans. Nutr J 2015, 14, 115, doi:10.1186/s12937-015-0103-3.
175. Allès, B.; Baudry, J.; Méjean, C.; Touvier, M.; Péneau, S.; Hercberg, S.; Kesse-Guyot, E. Comparison of Sociodemographic and Nutritional Characteristics between Self-Reported Vegetarians, Vegans, and Meat-Eaters from the NutriNet-Santé Study. Nutrients 2017, 9, doi:10.3390/nu9091023.
176. Clarys, P.; Deliens, T.; Huybrechts, I.; Deriemaeker, P.; Vanaelst, B.; De Keyzer, W.; Hebbelinck, M.; Mullie, P. Comparison of nutritional quality of the vegan, vegetarian, semi-vegetarian, pesco-vegetarian and omnivorous diet. Nutrients 2014, 6, 1318-1332, doi:10.3390/nu6031318.
177. Elorinne, A.L.; Alfthan, G.; Erlund, I.; Kivimaki, H.; Paju, A.; Salminen, I.; Turpeinen, U.; Voutilainen, S.; Laakso, J. Food and Nutrient Intake and Nutritional Status of Finnish Vegans and Non-Vegetarians. Plos One 2016, 11, e0148235, doi:10.1371/journal.pone.0148235.
178. Bundesinstitut für Riskobewertung. Die COPLANT-Studie - Forschung zu pflanzenbasierter Ernährung. https://www.bfr.bund.de/de/coplant-studie.html (Letzer Zugriff: 12.02.2023) 2023.

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Erklärung

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,
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Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

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