Die Mikroalge *Phaeodactylum tricornutum* – Bioverfügbarkeit, Sicherheit und potenzieller gesundheitlicher Nutzen für die humane Ernährung

Dissertation zur Erlangung des Doktorgrades

der Naturwissenschaften (Dr. rer. nat.)

Fakultät Naturwissenschaften

Universität Hohenheim

Institut für Ernährungsmedizin

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2023

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Eingereicht am: 03.03.2023

Datum der mündlichen Prüfung: 10.07.2023

Die vorliegende Arbeit wurde am 17.05.2023 von der Fakultät Naturwissenschaften der Universität Hohenheim als „Dissertation zur Erlangung des Doktorgrades der Naturwissenschaften“ angenommen.

Inhaltsverzeichnis

Übersicht über die eingeschlossenen Originalarbeiten

Als Erstautor

- **Stiefvatter, L.**; Lehnert, K.; Frick, K.; Montoya-Arroyo, A.; Frank, J.; Vetter, W.; Schmid-Staiger, U.; Bischoff, S.C. Oral Bioavailability of Omega-3 Fatty Acids and Carotenoids from the Microalgae *Phaeodactylum tricornutum* in Healthy Young Adults. Mar. Drugs 2021, 19, 700.
- **Stiefvatter, L.**; Neumann, U.; Rings, A.; Frick, K.; Schmid-Staiger, U.; Bischoff, S.C. The Microalgae *Phaeodactylum tricornutum* Is Well Suited as a Food with Positive Effects on the Intestinal Microbiota and the Generation of SCFA: Results from a Pre-Clinical Study. Nutrients 2022, 14, 2504.
- **Stiefvatter, L**.; Frick, K.; Lehnert, K.; Vetter, W.; Montoya-Arroyo, A.; Frank, J.; Schmid-Staiger, U.; Bischoff, S.C. Potentially Beneficial Effects on Healthy Aging by Supplementation of the EPA-Rich Microalgae *Phaeodactylum Tricornutum* or Its Supernatant—A Randomized Controlled Pilot Trial in Elderly Individuals. Marine Drugs 2022, 20, 716.

Erläuterung der Mitwirkung von Lena Kopp an den Publikationen

- Experimentelle Arbeit; statistische Auswertung; Dateninterpretation, Erstellung der Abbildungen und des Manuskripts

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Ich bestätige hiermit die Erläuterung über die Beiträge der Kandidatin zur Dissertation

Weitere, im Zuge des Promotionsprojektes entstandene Publikationen

- Tingö, L.; Hutchinson, A.N.; Bergh, C.; **Stiefvatter, L.**; Schweinlin, A.; Jensen, M.G.; Krüger, K.; Bischoff, S.C.; Brummer, R.J. Potential Modulation of Inflammation by Probiotic and Omega-3 Supplementation in Elderly with Chronic Low-Grade Inflammation—A Randomized, Placebo-Controlled Trial. Nutrients 2022, 14, 3998.

- **Kopp L**, Schweinlin A, Tingö L, Hutchinson AN, Feit V, Jähnichen T, et al. Potential Modulation of Inflammation and Physical Function by Combined Probiotics, Omega-3 Supplementation and Vitamin D Supplementation in Overweight/Obese Patients with Chronic Low-Grade Inflammation: A Randomized, Placebo-Controlled Trial. IJMS 2023;24:8567.

Kongressbeiträge

- Frick, K.; **Stiefvatter, L**.; Ramsch, T. MIATEST-BW Microalgae for food and plant fortifiers–two new value chains of Bioeconomy in Baden-Württemberg. Eingelandener Vortrag bei dem 3. Bio-Economy Congress 2020.
- **Stiefvatter, L** The microalgae *Phaeodactylum tricornutum* as an omega-3 fatty acid and carotenoid source- High bioavailability of nutrients in healthy volunteers. Eingelandener Vortrag bei der AlgaeEurope 2021.
- **Stiefvatter, L**. Mikroalgen in der Ernährung- die Bioverfügbarkeit von Omega-3-Fettsäuren und Carotinoiden aus der Mikroalge *Phaeodactylum tricornutum* bei gesunden jungen Erwachsenen. Posterpräsentation beim Kongress Ernährung 2022 mit Abstractpreis.
- **Kopp L,** Bischoff SC. Mikroalgen als alternative Nährstoffquelle in der Humanernährung und die Herausforderungen-am Beispiel von Phaeodacylum tricornutum. Aktuelle Ernährungsmedizin, vol. 48, Georg Thieme Verlag; 2023, p. Abstract 30.1.

Zusammenfassung

Mikroalgen sind Photosynthese betreibende Mikroorganismen und die Nahrungsgrundlage für viele Meeresorganismen. Aufgrund ihrer Diversität haben sie unterschiedliche Nährstoffzusammensetzungen, die für die menschliche Ernährung von großem Interesse sind. Heute sind nur wenige Mikroalgen, wie *Chlorella vulgaris*, auf dem Lebensmittelmarkt zu finden, da sie unter das Novel-Food Gesetz fallen. Eine neue und nicht zugelassene Mikroalge ist *Phaeodactylum tricornutum* (PT), die aufgrund ihrer Nährstoffzusammensetzung für die menschliche Ernährung von Bedeutung sein kann. Sie hat große Mengen der langkettigen Omega-3-Fettsäure (n-3-FS) Eicosapentaensäure (EPA), die sonst hauptsächlich in Fischen vorkommt. Zudem enthält PT einen hohen Anteil weiterer Nährstoffe, wie Proteine, Carotinoide, insbesondere Fucoxanthin (Fx), Vitamine und β-Glucane, die ein nutritives und therapeutisches Potenzial haben.

Daher war das Ziel der Promotion, die Eignung der Mikroalge PT für die menschliche Ernährung zu untersuchen. Dafür wurde die sichere tägliche Einnahme, die Bioverfügbarkeit von Nährstoffen und potenzielle gesundheitsfördernde Effekte von PT *in vivo* in drei Projekten bewertet. Dafür wurde die Mikroalge PT in einem Photobioreaktor kultiviert und durch die Veränderung der Bedingungen konnten zwei Biomassen generiert werden. Zum einen eine EPA- und Fx-reich und zum anderen eine Chrysolaminarin(Chrl)-reiche (ein β-1,3-1,6-Glucan) Biomasse. Beide PT-Biomassen wurden nach der Kultivierung geerntet, die Zellen mechanisch aufgeschlossen, gefriergetrocknet und anschließend als Pulver verarbeitet und in den Studien verwendet.

Im ersten Projekt konnte zum ersten Mal in einer klinischen Pilotstudie die sichere täglich Einnahme der EPA/Fx-reichen PT-Biomasse als Art „Smoothie“ zum Trinken über 14 Tage bei gesunden Probanden (Alter 25.7 ± 6 Jahre) nachgewiesen werden. Es zeigten sich keine Veränderungen von Laborparametern und gemessenen Marker der Darmbarriere. Jedoch wurden leichte Nebenwirkungen wie Blähungen während der Einnahme dokumentiert. Bezüglich der Bioverfügbarkeit führte die PT-Einnahme zu einem Anstieg der EPA-Plasma-Level, sowie einem vergleichbaren Anstieg der n-3-FS im Blutplasma wie nach der Fischöl-Einnahme, die in einem Cross-over-Design getestet wurde. Überdies erhielt eine reduzierte Anzahl von Teilnehmern 14 Tage lang zwei normale Fischportionen pro Woche für den Vergleich des Plasma Fettsäure (FS)-Anstiegs. Ferner konnten höhere Plasmaspiegel des Carotinoid Fx und seiner Metaboliten sowie des β-Carotin nach der PT-Aufnahme nachgewiesen werden. Die Ergebnisse deuten darauf hin, dass die Nährstoffe vom Menschen aufgenommen und PT als Lebensmittel betrachtet werden sollte.

Im zweiten Promotionsprojekt wurden die generierten EPA/Fx- und Chrl-reichen PT-Biomassen an Mäusen in einem 14-tägigen Fütterungsversuch getestet und bezüglich der potenziellen darmgesundheitlichen Vorteile untersucht. Beide Biomassen wurden dem Futter zu 5 %, 15 % und 25 % zugesetzt und mit einem Kontrollfutter verglichen. Die Mikroalgen-Diäten führten zu einem Anstieg von kurzkettigen FS in den Fäzes und zu einer Veränderung des Mikrobioms, was auf eine präbiotische Wirkung schließen lässt. Darüber hinaus wurden bis auf einen Anstieg des Tumornekrosefaktor-alpha (TNF-a) im Ileum nach der höchsten Dosierung beider PT-Diäten keine negativen Veränderungen im Gastrointestinaltrakt oder histologischen Untersuchungen gemessen. Zudem führten beide PT-Diäten zu einer Zunahme des Körpergewichts und zu einem gleichen oder geringeren Energieverlust in den Fäzes im Vergleich zur Kontrolldiät. Die Ergebnisse lassen daher auf eine sichere Einnahme und Energieabsorption bis zu einer Dosierung von mindestens 15 % schließen und zeigen mögliche präbiotische Wirkung nach der Supplementierung beider PT-Biomassen.

Das dritte Promotionsprojekt zielte darauf ab, die möglichen gesundheitlichen Vorteile der Einnahme von PT bei älteren Menschen zu untersuchen und die sichere Einnahme und Bioverfügbarkeit von Nährstoffen nachzuweisen. Zu diesem Zweck wurde eine randomisierte Pilotstudie mit älteren Probanden (67 ± 7 Jahre) durchgeführt. Die Probanden wurden in vier Interventionsgruppen aufgeteilt und erhielten I. die EPA-/Fx-reiche PT-Biomasse, II. den Chrl-reichen Überstand von PT, der aus der Chrl-reichen Biomasse durch Zentrifugieren generiert wird, III. eine Kombination aus beidem I. und II. und IV. eine Kontrollgruppe. Die Ergebnisse lassen auf eine mögliche entzündungshemmende und antioxidative Wirkungen nach der PT-Einnahme schließen. Zudem wurden Mobilitätsmarker verbessert und ein Marker für die Darmbarriere nach der Einnahme des Chrl-reichen Überstandes reduziert. Auch bestätigte die Studie die Bioverfügbarkeit von Fx und β-Carotin, demgegenüber wurde kein n-3-FS Plasma-Anstieg gemessen. Die Ergebnisse der Studie zeigen erneut die Eignung von verschiedener PT-Präparationen für die menschliche Ernährung und dass eine mögliche zusätzliche Zufuhr von PT potenzielle gesundheitliche Vorteile für ältere Menschen haben kann.

Zusammenfassend konnte im Rahmen des Promotionsprojektes erstmals die sichere Einnahme zweier PT-Biomassen und Präparationen *in vivo* an Mäusen und in zwei Humanstudien gezeigt werden. Es wurden neue Erkenntnisse über ernährungsphysiologisch relevante Bestandteile wie EPA, Fx und β-Carotin sowie über die Bioverfügbarkeit und potenzielle gesundheitliche Wirkungen, wie entzündungshemmende und präbiotische gewonnen. Die Ergebnisse lassen daher auf die Eignung der Mikroalge PT als Lebensmittel für die menschliche Ernährung mit möglichen gesundheitsfördernden Wirkungen schließen.

Abstract

Microalgae are photosynthesizing microorganisms and the nutritional basis for many marine organisms. Due to their diversity, they have different nutrient compositions that greatly interest human nutrition. Today, only a few microalgae, such as *Chlorella vulgaris*, are found in the food market. One microalga that has not yet been approved is *Phaeodactylum tricornutum* (PT). The microalgae could be important for human nutrition because it contains large amounts of the long-chain omega-3 fatty acid (n-3-FS) eicosapentaenoic acid (EPA), which is otherwise mainly found in fish. PT also contains large amounts of proteins, carotenoids such as fucoxanthin (Fx), vitamins and β-glucans, which could not only be used nutritively but also have health-promoting effects.

So far, little is known about the suitability of PT for human nutrition. Therefore, the PhD aimed to demonstrate the safe intake of the microalgae PT, the bioavailability of the nutrients and potential health-promoting effects in three projects. The microalgae PT was cultivated in a photobioreactor, and two biomasses could be generated due to different cultivation conditions. One was an EPA- and Fx-rich biomass; the other was a chrysolaminarin (Chrl)-rich biomass, a water-soluble β-1,3-1,6-glucan. After cultivation, the biomasses were harvested, the cells mechanically disrupted, freeze-dried, processed into a powder, and used in the studies.

In the first project, a clinical pilot study with healthy volunteers (age 25.7 ± 6 years) was conducted and they received daily the EPA/Fx-rich PT biomass as a type of "smoothie" to drink for 14 days. We demonstrated for the first time the safe intake of PT as food. There were no changes in laboratory parameters and measured markers of the intestinal barrier. However, mild side effects such as flatulence were documented during intake. In terms of bioavailability, PT intake increased in plasma EPA levels, as well as a comparable increase in plasma n-3 FS after fish oil intake was tested in a cross-over design. In addition, a reduced number of participants received two normal fish servings per week for 14 days for the comparison of the plasma fatty acid (FS) increase. Furthermore, higher plasma levels of the carotenoid Fx and its metabolites as well as β-carotene were detected after PT intake. The results suggest that the nutrients can be absorbed by humans and PT should be considered as food.

In the second PhD project, the generated EPA/Fx and Chrl-rich PT biomasses were tested on mice in a 14-day feeding trial and investigated for potential gut health benefits. The two biomasses were added to the diet at 5%, 15% and 25% and compared to a control diet. Both microalgae diets resulted in an increase in short-chain FS in the feces and a change in the microbiome, suggesting a prebiotic effect. Furthermore, apart from an increase in tumour necrosis factor-alpha (TNF-a) in the ileum, no negative changes in the gastrointestinal tract or

histological examinations were measured after the highest dosage of both PT diets. In addition, both PT diets increased body weight and equal or lower energy loss in the feces compared to the control diet. The results suggest safe intake and energy absorption of both PT biomasses up to a dosage of at least 15 % and show possible positive effects on gut health after supplementation of both PT biomasses.

The third PhD project aimed to investigate the potential health benefits of PT intake in older people and to demonstrate safe nutrient intake and bioavailability. For this purpose, a randomized pilot study was conducted with elderly subjects (67 ± 7 years). Subjects were divided into four intervention groups and received I. the EPA/Fx-rich PT biomass, II. the Chrl-rich supernatant of PT generated from the Chrl-rich biomass by centrifugation, III. a combination of both I. and II., and IV. a control group. The results suggest possible anti-inflammatory and antioxidant effects following PT ingestion. In addition, mobility markers improved, and an intestinal barrier marker was improved after ingestion of the Chrl-rich supernatant. The study also confirmed the bioavailability of Fx and β-carotene, in contrast to which no n-3-FS plasma increase was measured. The results of the study again demonstrate the suitability of different PT preparations for human nutrition and that a possible additional intake of PT may have potential health benefits for the elderly.

In summary, the PhD project demonstrated for the first time the safe intake of two different PT biomasses and preparations *in vivo* in mice and in two human studies. New insights were gained into nutritionally relevant components such as EPA, Fx and β-carotene as well as bioavailability and potential health effects such as anti-inflammatory and prebiotic. The results suggest the suitability of the microalga PT as a food for human nutrition with possible health-promoting effects.

1. Einleitung

Aufgrund des Anstiegs der Weltbevölkerung und einer Nahrungsmittelbedarfssteigerung von weltweit etwa 70 % (gemessen an der Kalorienzahl) [1] steht das Ernährungssystem vor großen Herausforderungen, um eine gesunde und bedarfsgerechte Ernährung zu gewährleisten [2]. Das zukünftige Ernährungsverhalten wird auch von anderen globalen Transformationstreibern wie dem Klimawandel und der Ressourcenverknappung beeinflusst werden. Diese Veränderungen können zu ernsthaften Einschränkungen bei der Quantität und Qualität von Lebensmitteln führen [3]. Die Überfischung der Meere, Bodendegenerationen und hohe Treibhausgasemissionen der Massentierhaltung sind bereits aktuelle Probleme die uns zeigen, dass Konsum- und Produktionsmuster weltweit angepasst und auf nachhaltige Weise gesteigert werden müssen [4]. Daher ist es notwendig neue und alternative Ressourcen in Betracht zu ziehen, um die Weltbevölkerung ernähren zu können [5].

Mikroalgen sind eine neue Lebensmittelquelle und können zu einer gesunden und ausgewogenen Ernährung beitragen. Sie enthalten vielfältige bioaktive Verbindungen, wie Proteine, langkettige Fettsäuren (FS), Carotinoide, Vitamine und Polysaccharide [6–8]. Die Integrierung von Mikroalgen in die menschliche Ernährung steht allerdings noch an ihren Anfängen [9]. Mikroalgen als neues Lebensmittel bieten jedoch ein großes Potenzial für die Versorgung mit Makro- und Mikronährstoffen und könnten eine Alternative zu limitierten Ressourcen wie Fleisch und Fisch sein [10,11]. Auch steigt das Interesse aufgrund der enthaltenen bioaktiven Verbindungen, die bei einer Einnahme potenzielle gesundheitliche und präventive Vorteile haben können [9,12]. Da Lebensmittel häufig nicht an die menschlichen Bedürfnisse angepasst, und die Prävalenz für ernährungsbedingte Krankheiten, wie Herz-Kreislauf-Erkrankungen steigt [13], ist die Bereitstellung von ernährungsphysiologisch hochwertigen Lebensmitteln notwendig. Der Einbringung von Mikroalgen in die Ernährung stehen jedoch weitere Herausforderungen gegenüber, wie der Zulassung als neuartiges Lebensmittel, teure Produktionskosten und die Akzeptanz der Verbraucher [14]. Ein allgemeiner Wandel im Ernährungsverhalten hin zu weniger Fleischkonsum und einer veganen oder vegetarischen Ernährung [15] könnte Mikroalgen als nachhaltiges Lebensmittel weiter in den Vordergrund rücken. Daher ist die Untersuchung und Eignung von Mikroalgen als Lebensmittel notwendig.

1.1. Mikroalgen

Algen sind autotrophe aquatischen Organismen, die innerhalb ihrer Gruppe eine große morphologische Vielfalt aufweisen. Sie werden aufgrund ihrer Größe in Makro- und Mikroalgen (Abbildung 1A, B) oder aufgrund ihrer Pigmentzusammensetzung in Grün-, Rot-, Braunalgen und Cyanobakterien unterteilt. Makroalgen sind bis zu 65 Meter lang und können mit dem

bloßen Auge gesehen werden, wohingegen Mikroalgen mikroskopisch kleine Organismen sind [16,17].

Mikroalgen leben in Seen, Flüssen und Ozeanen und stehen am Anfang der Nahrungskette für Zooplankton und Fisch [17]. Auch für die menschliche Ernährung sind Mikroalgen von großem Interesse, da sie viele Vorteile im Vergleich zu Landpflanzen bieten. Für den Anbau von Mikroalgen müssen keine landwirtschaftlichen Flächen genutzt werden. Sie können in OpenPonds-Systemen (offene Becken) oder Photobioreaktoren kultiviert werden. Photobioreaktoren sind geschlossene und transparente Röhren oder Panele, die eine naturähnliche Wachstumsumgebung mit Licht und Nährstoffen für Mikroalgen bieten (Abbildung 1C, D) [17]. Durch das exponentielle Wachstum von Mikroalgen können Erntezyklen auf acht bis zehn Tage verkürzt [18,19] und ganzjährig geerntet werden [20]. Auch ist in kühleren Breitengraden, wie Deutschland, ein nachhaltiger Anbau von Mikroalgen möglich [21,22]. Ein weiterer Vorteil von Mikroalgen ist, dass sich die Zusammensetzungen der Mikroalgenbiomassen durch die Anpassung von Kultivierungsbedingungen verändern lassen. Dies kann genutzt werden, um den Gehalt an gewünschten (bioaktiven) Inhaltsstoffen in der Biomasse zu erhöhen. Unter Nährstoffmangel produzieren Kieselalgen z.B. vermehrt Polysaccharide als Energiespeicher [23,24].

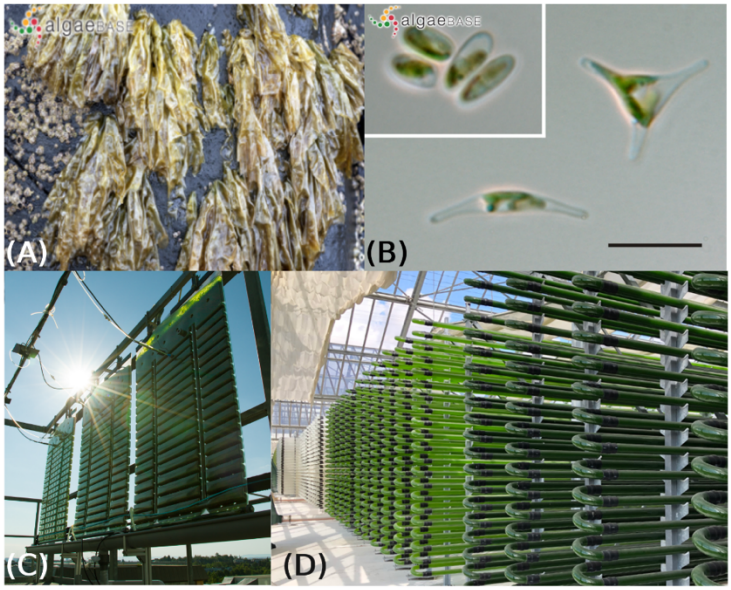


Abbildung 1. Makroalgen sind größere Algen, die mit dem bloßen Auge sichtbar sind (*Porphyra umbilicalis*) **(A)**, Mikroalgen sind dagegen nur mit einem Mikroskop sichtbar (*Phaeodactylum tricorntum*) **(B)**. Für den Anbau werden verschiedene Photobioreaktoren verwendet, wie der Flat panel airlift Photobioreaktor **(C)** oder ein Röhren-Photobioreaktor **(D)**. Bildquellen: A+ B: AlgaeBase.org, C: Fraunhofer IGB, D: Roquette Klötze GmbH.

Nach der Kultivierung der Mikroalgen entsteht eine verdünnte Suspension, auch Biomasse bezeichnet, die geerntet werden kann. Durch einen anschließenden mechanischen oder nicht-

mechanischen Zellaufschluss kann die Verdaulichkeit und Bioverfügbarkeit der Nährstoffe verbessert werden, da die Zellwand der Mikroalgen meist aus unverdaulichen Polysacchariden besteht [25–27]. Im nächsten Schritt werden die Mikroalgen gefriergetrocknet und können in Form von Tabletten, Presslingen, Pulver oder Flocken konsumiert werden [28]. Eine weitere Möglichkeit ist die weitere Extraktion oder Separation von Wertstoffen, wie EPA oder DHA aus einer Biomasse (Downstream Prozess). Es werden bereits n-3-FS-reiche Mikroalgenöle als Nahrungsergänzungsmittel verkauft [29].

1.2. Novel Food, Sicherheit und Bioverfügbarkeit von Mikroalgen

Der hervorragenden Zukunftsperspektive von Mikroalgen als Lebensmittel steht die Novel-Food-Verordnung VO (EU) 2015/228 und ein Zulassungsverfahren gegenüber. Diese gilt nur für Mikroalgen, die vor dem 15. Mai 1997 nicht in nennenswerten Mengen konsumiert wurden [30]. Die wohl bekanntesten Arten sind *Spirulina* und *Chlorella*. Sie dominieren derzeit den Markt und fallen nicht unter die Novel-Food-Verordnung, da sie bereits vor dem genannten Stichtag konsumiert wurden. Neuartige Mikroalgen müssen für die Eignung als Lebensmittel getestet werden. Neben einer Sicherheitsbewertung werden ernährungsphysiologische Eigenschaften und nutritive Wirkungen, sowie toxikologische Untersuchungen und gesundheitsverträgliche Verwendungsmengen untersucht [30]. Eine weitere Grundvoraussetzung für einen ernährungsphysiologischen Mehrwert von Mikroalgen ist die Bioverfügbarkeit der Nährstoff. Die Bioverfügbarkeit umfasst sowohl die Biozugänglichkeit als auch den Nachweis einer Bioaktivität, die eine positive Funktion im Körper auslösen könnte [31]. Bioaktive Verbindungen werden durch Verdauungsenzyme aus der Nahrung freigesetzt und stehen anschließend für die Nutzung in physiologischen Funktionen und für die Speicherung zur Verfügung [32]. Mithilfe von *In-vivo*- und *In-vitro*-Methoden können Bioverfügbarkeiten untersucht werden [33]. Im Hinblick auf Mikroalgen ist der Nachweis der Bioverfügbarkeit von einzelnen Nährstoffen eine Voraussetzung für die Zulassung und den Mehrwert als neues Lebensmittel. Nach einer umfassenden Bewertung und Eignung der untersuchten Mikroalge kann ein Novel Food Antrag eingereicht werden. Dieser wird von der Europäischen Behörde für Lebensmittelsicherheit (EFSA) bewertet und kann durch den anschließenden Beschluss der Europäischen Kommission zugelassen werden [34]. Nach der Zulassung eines Novel Foods wird die genaue Einnahmemenge und Verarbeitung in Lebensmitteln in einer Unionsliste festgehalten [35]. Nur wenige Mikroalgen sind bereits auf dem europäischen Lebensmittelmarkt zugelassen, ausgenommen *Spirulina* und *Chlorella.* Dazu gehören *Odontella aurita* und *Tetraselmis chuii*, die als ganze Mikroalgen hauptsächlich zum Würzen und Aromatisieren verwendet werden. Ebenso sind ein astaxanthinreiches Oleoresin aus der Mikroalge *Haematococcus pluvialis* sowie n-3-FS-reiche Öle aus den Arten *Schizochytrium sp.* und *Ulkenia sp*. zugelassen. In anderen Ländern, wie den USA, gelten andere Regularien und es sind im Vergleich zu Europa andere Mikroalgen zugelassen [36].

1.3. Ernährungsphysiologische Einschätzung von Mikroalgeninhaltsstoffen und deren potenzieller gesundheitlicher Vorteil

Makroalgen sind bereits Teil der Ernährung und werden in Sushi oder zum Würzen vor allem in asiatischen Ländern konsumiert. Sie enthalten vielfältige Mineralien, jedoch müssen mögliche Gesundheitsrisiken, die mit dem Verzehr übermäßiger Mengen verbunden sind, weiter untersucht werden [37]. Auch Mikroalgen sind aufgrund ihrer zahlreichen bioaktiven Verbindungen für die menschliche Ernährung von großem Interesse und höhere Einnahmemengen könnten nicht nur Nährstofflücken füllen, sondern auch potenzielle gesundheitliche Vorteile bewirken.

1.3.1. Proteine

Aufgrund der hohen Nachfrage von Nahrungsproteinen können Mikroalgen eine pflanzliche Alternative zu Fleisch und Fisch darstellen- auch in Ländern, in denen tierische Proteine unzureichend sind [38]. Mikroalgen haben einen Proteingehalt von 40 bis 70 % und eine gute Aminosäureverteilung [39–41]. Daher könnten sie als Proteinquelle für Futter- und [39,42] Lebensmittel eingesetzt werden [43,44]. Für die Verwendung von Mikroalgen als neuartige Proteinquelle muss jedoch zunächst die ernährungsphysiologische Qualität und Verwertbarkeit untersucht werden [45]. Die Bioverfügbarkeit von Proteinen aus den Mikroalgen *Chlorella vulgaris*, *Nannochloropsis oceanica* and PT konnte bereits *in vivo* bei Mäusen nachgewiesen werden [46]. Zur Beurteilung der Proteinverfügbarkeit wird die biologische Wertigkeit *in vivo* bei Tieren mithilfe der Netto-Proteinverwertung gemessen, die eine Kombination aus biologischer Wertigkeit und Verdaulichkeit angibt [39,46]. Beim Menschen ist die Untersuchung der Bioverfügbarkeit von Proteinen aufwendiger, wie invasive Verfahren [47]. Mikroalgen bietet daher ein großes Potenzial, um in Zukunft als alternative Proteinquelle genutzt zu werden.

1.3.2. Omega-3-Fettsäuren

Mikroalgen sind die Primärproduzenten der mehrfach ungesättigten n-3-FS, vor allem EPA und Docosahexaensäure (DHA), die für die menschliche Ernährung besonders relevant sind. Der Gesamtlipidgehalt und die FS-zusammensetzung variiert innerhalb von Mikroalgenspezien aufgrund von verschiedenen Umweltfaktoren und Lichtintensität [7,48]. In die menschliche Ernährung gelangen EPA und DHA hauptsächlich über den Verzehr von Fisch und Krustentieren. Fische produzieren diese FS jedoch nicht selbst, sondern nehmen sie durch Mikroalgen als Teil ihrer Nahrung auf [49,50]. Aufgrund der weltweiten Überfischung der Meere und der globalen Erwärmung gehen die Bestände von DHA und EPA in den Ozeanen zurück. Daraus ergibt sich schätzungsweise eine Bedarfslücke von über einer Million Tonnen [51,52]. Der Mensch kann EPA und DHA durch Umwandlung der essenziellen Alpha-Linolsäure (ALA) herstellen, allerdings in begrenzten und unzureichenden Mengen [53]. Daher

ist ein direkter Verzehr erforderlich. Empfohlen wird eine Aufnahme von 250 bis 500 mg EPA und DHA pro Tag [54], was in der Regel durch ein bis zwei Portionen fettreichem Fisch pro Woche gedeckt werden kann. Würde jedoch jeder diesem nachgehen, wären unsere Gewässer leer gefischt. Daher sind Mikroalgen nicht nur eine alternative Quelle für Vegetarier und Veganer, die keinen Fisch essen, sondern könnten auch dazu beitragen, Bedarfslücken nachhaltig zu schließen [22,51]. Auch bei der Untersuchung neuer Mikroalgen ist der Nachweis der Bioverfügbarkeit der FS erforderlich. Die Bioverfügbarkeit von FS kann durch die Konzentrationsmessung im Serum oder Plasma bei Humanstudien gemessen werden, was eine kurz- bis mittelfristige Zufuhr über die Nahrung widerspiegelt [55] und für die Forschung nützlich ist [56]. Bei mehrfacher täglicher oraler Zufuhr von n-3-FS kann innerhalb von sieben bis zehn Tagen ein Höchstwert oder eine Steady-State-Konzentration erreicht werden [57,58]. Die Messung von FS in der Erythrozytenmembran ist dagegen ein Indikator für eine langfristige Versorgung mit n-3-FS. Häufig wird der Omega-3-Index berechnet, der ein guter Indikator ist, um zu überprüfen, ob eine ausreichende Versorgung mit EPA und DHA gegeben ist [56,59]. Studien zeigen bereits, dass DHA aus Mikroalgenöl in ähnlichem Maße aufgenommen wird wie durch eine klassische Einnahme von Fischölkapseln oder Fisch [60,61]. Ein weiterer Vorteil bei der Verwendung von Algen als n-3-FS Lieferant ist ein gutes Verhältnis der Omega-6 zu n-3-FS (n-6:n-3) [62,63]. Die westliche Ernährung ist meist durch ein Ungleichgewicht beider FS und einer übermäßigen Aufnahme von n-6-FS gekennzeichnet. Ein Gleichgewicht beider FS ist von großer Bedeutung, da für die Produktion von Eicosanoiden beider FS gleiche Enzyme mit entgegengesetzten Wirkungen verwendet werden. EPA und DHA sind Ausgangsstoff für die Bildung von entzündungshemmenden Eicosanoiden, die als hormonähnliche Substanzen, Immunmodulatoren und Neurotransmitter wirken können, wohingegen n-6-FS entzündungsfördernd wirken [64,65]. Ein niedriges Verhältnis beider FS ist daher mit einem verringerten Risiko für Herz-Kreislauf-Erkrankungen verbunden, wohingegen erhöhte Verhältnisse das Krebsrisiko, Entzündungserkrankungen, kardiovaskuläre Erkrankungen [66] und Darmdysbiosen fördern [67]. Daher hat eine ausreichende Versorgung mit EPA und DHA viele positive Auswirkungen und könnte bei der Prävention verschiedener Erkrankungen helfen [68]. Indes liegen auch widersprüchliche Ergebnisse für die Wirkungen von n-3-FS vor, die durch eine Standardisierung von Messmethoden und weitere Interventionsstudien überprüft werden müssen [69]. Insgesamt sind Mikroalgen eine nachhaltige Quelle für EPA und DHA und eine ausreichende Einnahme dieser FS kann gesundheitliche Vorteile haben [68,52].

1.3.3. Carotinoide

Ein weiterer Bestandteil von Mikroalgen sind Pigmente [70], zu denen Chlorophyll, Phycobiliproteine und Carotinoide gezählt werden [71,72]. Diese bilden Proteinkomplexe in der Thylakoidmembran und sind für die Absorption von Licht während der Photosynthese

verantwortlich [70]. Pigmente aus Mikroalgen haben ein großes Potenzial für die Anwendung in der Lebensmittelindustrie [73]. Chlorophyll wird bereits in der Lebensmittelpigmentierung und in der Nahrungsergänzungsmittelindustrie als färbender Zusatzstoff verwendet [74]. Phycobiliproteine kommen in Cyanobakterien vor, z. B. Phycocyanin in *Spirulina*. Diesen Pigmenten werden gesundheitliche Eigenschaften zugeschrieben und sie werden in Kosmetik und als natürlicher Farbstoff in Lebensmitteln verwendet [75]. Carotinoide werden in Carotine (α-, β-Carotin und Lycopin) und sauerstoffhaltige Xanthophylle unterteilt (Astaxanthin, Fx, Lutein und Zeaxanthin) [76]. Mikroalgen enthalten vor allem große Menge des β-Carotin, das eine wichtige Vorstufe von Vitamin A und ein Antioxidans ist [73,77]. Bereits heute wird die Mikroalge *Dunaliella salina* zur Produktion von β-Carotin verwendet [78]. Xanthophylle haben eine höhere antioxidative Kapazität, indem sie freie Radikale fangen und Singulett-Sauerstoff löschen [79]. Die Mikroalge *Haematococcus pluvialis* wird bereits für die kommerzielle Produktion von Astaxanthin verwendet [80]. In braunen Mikro- und Makroalgen ist Fx das wichtigste Xanthophyll [81], dem viele potenzielle gesundheitliche Vorteile wie neuroprotektive, antioxidative, antiproliferative, fettleibigkeitshemmende und krebsbekämpfende Eigenschaften zugesprochen werden [81,82]. Gesundheitsbezogene Angaben im Zusammenhang mit Fx sind von der EFSA und der US-Food and Drug Administration jedoch noch nicht bewertet worden. Für Fx ist bekannt, dass es im Magen-Darm-Trakt innerhalb von zwei Stunden durch die Verdauungsenzyme Lipase und Cholesterinesterase zu Fucoxanthinol (FxOH) hydrolysiert, in den Darmzellen absorbiert und anschließend in der Leber in Amarouciaxanthin A (AxA) umgewandelt wird [81]. Die Metabolisierung von Fx aus Makroalgen konnte bereits nachgewiesen werden [83], sowie von Mikroalgen in Mäusen [84]. Eine gute Bioverfügbarkeit von Carotinoiden hängt jedoch von mehreren Faktoren ab, wie der Lebensmittelmatrix, der Dauer des Kochens, den beteiligten Carotinoiden sowie dem Vorhandensein von Fetten, Fasern, Proteinen und anderen Nährstoffen in der Nahrung [76,85]. Durch *in vitro* Verdauungsstudien oder durch die Bestimmung von Konzentrationen in verschiedenen Geweben bei Tieren oder Plasmakonzentrationen beim Menschen nach Supplementierung kann die Bioverfügbarkeit untersucht werden. Dabei ist der richtige Zeitpunkt für die Probenentnahme entscheidend [86,87]. Viele gesundheitliche Vorteile, wie antioxidative Wirkungen, sind nach der Einnahme von Carotinoiden aus Mikroalgen zu erwarten, jedoch müssen diese in klinischen Studien nachgewiesen werden. Insgesamt ist die weitere Untersuchung der Bioverfügbarkeit von Carotinoiden aus Mikroalgen notwendig, um gesundheitliche Vorteile für die menschliche Ernährung zu erzielen [88].

1.3.4. Vitamine

Algen produzieren eine Vielzahl von Vitaminen zur Regulierung lebenswichtiger Funktionen. Die Variabilität der Vitamine steht im Zusammenhang mit einer physiologischen Reaktion auf verschiedene Umweltreize [89]. Mikroalgen enthalten die Vitamine A, B, C, D, E und K [90].

Da Tiere und Menschen Vitamine kontinuierlich über die Nahrung aufnehmen müssen, können Mikroalgen eine zusätzliche Vitaminquelle darstellen. Gerade das Vitamin B12 in Mikroalgen ist speziell für Vegetarier und Veganer von großer Bedeutung, da es sonst nur in tierischen Lebensmitteln vorkommt. Jedoch muss dieses in einer biologisch aktiven Form vorliegen, um für den Menschen bioverfügbar zu sein. *Spirulina* enthält große Mengen des Pseudovitamins B12, wohingegen die Grünalge *Chlorella vulgaris* das aktive und bioverfügbare B12 enthält [91,92]. Daher muss die biologische Verwertbarkeit der Vitamine für den Menschen überprüft werden. Weiter ist das Vitamin E in Mikroalgen für die menschliche Ernährung von Interesse [93]. Mikroalgen können hohe Vitamin E Konzentrationen von bis zu 6,32 mg/g Trockenmasse enthalten. Durch den zusätzlichen Verzehr von Mikroalgen könnte nicht nur der tägliche Bedarf von bis zu 15mg/Tag gedeckt werden [94], sondern eine Einnahme auch positive gesundheitliche Effekte wie antioxidative und entzündungshemmende Eigenschaften haben [93]. Vitamin E ist für künftige therapeutische Anwendungen, wie Alzheimererkrankungen von großer Relevanz [95]. Die Absorption im Darm und die Sekretion in den Blutkreislauf sind bereits gut erforscht [96,97]. Jedoch ist die schlechte Bioverfügbarkeit von Vitamin E eine Herausforderung für die Untersuchung in klinischen Studien [98,99]. Insgesamt Stellen Mikroalgen eine Alternative Vitaminquelle für die menschliche Ernährung da, jedoch muss die Bioverfügbarkeit und klinische Anwendung weiter untersucht werden [18,50,90].

1.3.5. Polysaccharide

Des Weiteren enthalten Algen Polysaccharide, die meist im Cytosol als Energie- und Kohlenhydratspeicher gelöst sind. Polysaccharide aus Makroalgen sind bereits bekannt [100] und werden als Verdickungs- und Geliermittel in der Kosmetik- und Lebensmittelindustrie verwendet [101]. Viele Polysaccharide aus Mikroalgen werden zu den β-Glucanen, welches D-Glucose-Monomere sind, die über β-glycosidische Bindungen verbunden sind gezählt. β-Glucane sind bereits aus anderen Quellen wie Getreide, Pilzen, Bakterien und Hefe bekannt und werden in Lebensmitteln, Medizin und Kosmetika verwendet [102,103]. Ihnen werden nutrazeutische Funktionen wie antitumorale, entzündungshemmende, antioxidative, gerinnungshemmende, immunstimulierende, sowie präbiotische Wirkungen zugesprochen [103–105]. Viele dieser gesundheitlichen Vorteile werden auch Polysacchariden aus Makroalgen und [50,106,107] Mikroalgen zugeschrieben, die für biomedizinische Anwendungen Interessant sind [108,109]. Ein neuartiges β-Glucanen aus Mikroalgen ist Chrl, ein wasserlösliches β-1,3-1,6-Glucan, was aufgrund seiner anti-oxidativen und immunmodulatorischen Eigenschaften für die menschliche Ernährung von Interesse sein kann [110–112]. Die Struktur des Chrl ist dem Laminarin aus Braunalgen sehr ähnlich, welches bereits in Tierfutter verwendet wird [113] und dem β-1,3-1,6-Glucan der Bäckerhefe *Saccharomyces cerevisiae,* welches bereits von der EFSA als Novel Food zugelassen wurde [114]. Daher ist die Anwendung auch von Chrl aus Mikroalgen denkbar. Die präbiotischen

Wirkungen entstehen durch die Verstoffwechselung des Darmmikrobioms und den dadurch entstehenden kurzkettigen FS wie Acetat, Butyrat und Propionat. Kurzkettigen FS sind an der Regulierung der Glukosehomöostase und Immunfunktionen beteiligt. Eine Einnahme von β-Glucanen kann daher die Prävention von Krankheiten wie Krebs, Diabetes, Hypercholesterinämie und Herz-Kreislauf-Erkrankungen fördern [103]. Diese gesundheitsfördernden Effekte sind auch durch die Einnahme von Mikroalgen-Polysacchariden denkbar und die Wirkmechanismen müssen weiter untersucht werden, da nur wenige klinische Studien zu finden sind [108,115].

1.4. Die Mikroalge *Phaeodactylum tricornutum* und ihr Potenzial als neuer Nahrungsstoff

Die Mikroalge PT gehört zu den Kieselalgen (Diatomeen), die in marinen Ökosystemen vorkommt. Sie wird auch als Modellorganismus bezeichnet und ist bereits gut charakterisiert und kann in größerem Maßstab kultiviert werden. PT hat vielfältige ernährungsphysiologisch bioaktive Moleküle wie Proteine, FS, Carotinoide, und β-Glucane. Daher könnte sie eine nachhaltige Zutat für die Entwicklung funktioneller Lebensmittel sein [116–118]. Dabei gewinnen insbesondere die großen Mengen der FS EPA, Fx, sowie Chrl, an Relevanz [23,24,119]. Aufgrund von unzureichenden Studien und begrenzter Kenntnissen ist PT bis heute nicht für die menschliche Ernährung zugelassen [120]. Jedoch wird in den USA indes schon ein EPA-reiches Öl aus PT verkauft [121] und ein Antrag für eine Zulassung in Irland wurde bereits eingereicht. Überdies entwickelt das Unternehmen Microphyt verschiedene Produkte zur Verbesserung der kognitiven Fähigkeiten auf PT-Basis, sowie ein Produkt zum Abnehmen [122]. Darüber hinaus wurde ein Medikament „FucoVital“ entwickelt, das für die Lebergesundheit eingesetzt wird, aber in Europa noch nicht zugelassen ist [123]. Ein neuer Ansatz ist es die ganze PT-Biomasse zu verwenden, ohne einen nachgeschalteten Downstream-Prozess durchzuführen. Für die Fischzucht kann PT bis zu 6 % des Fischmehls bereits ersetzen und zu einer Verbesserung des Fettsäureprofils beitragen [124]. Dies ist auch für die menschliche Ernährung denkbar. Nur die dicke Zellwand muss mechanisch aufgeschlossen werden, um die Nährstoffe bioverfügbar zu machen [26]. Durch die Anpassung und Veränderung von Kultivierungsbedingungen von PT können zudem gewünschte bioaktive Verbindungen erhöht werden. Unter Nährstoffsättigung enthält PT höhere Mengen der Nährstoffe EPA und Fx und unter Nährstoffreduzierung größere Mengen des Chrl (Abbildung 2) [23,24,119]. Zudem kann durch Zentrifugieren ein Chrl-reicher Überstand generiert werden, da das Chrl wasserlöslich ist [24]. Die verschiedenen PT-Biomassen bieten aufgrund ihrer unterschiedlichen Nährstoffzusammensetzung daher ein großes therapeutisches Potenzial und könnten zusätzlich für die Deckung des Nährstoffbedarfs verwendet werden [125]. Bislang ist nur wenig über die Eignung der Mikroalge PT für die menschliche Ernährung bekannt. *In vivo* an Mäusen konnte bereits die

sichere Einnahme der EPA-/Fx-reichen PT-Biomasse nachgewiesen werden, die dem Futter gefriergetrocknet in einer Menge von 5, 15 oder 25 wt% (Gewichtsprozent) zugesetzt wurde. Die 14-tägige Einnahme führte zu keinen histologischen Veränderungen und es konnten keine Auswirkungen auf toxische Marker in Herz, Leber und Niere gemessen werden, was auf eine sichere Einnahme schließen lässt [46]. Zudem wurde bereits die Bioverfügbarkeit von FS, Proteinen [46] und Carotinoiden in Mäusen gezeigt [84]. Auch konnte neben der EPA-/Fx-reichen PT-Biomasse die sichere Einnahme der Chrl-reichen Biomasse und des Chrl-reichen Überstandes bei Fischen nachgewiesen werden (Abbildung 2) [112,126].

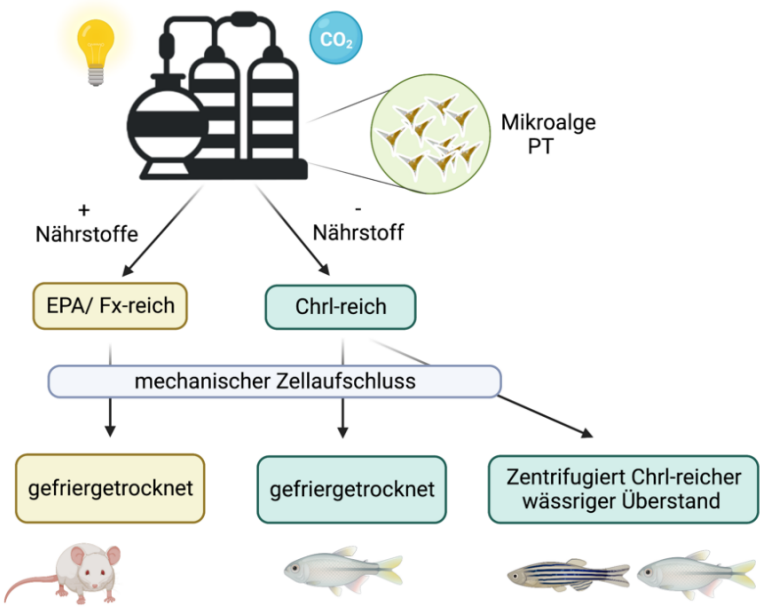
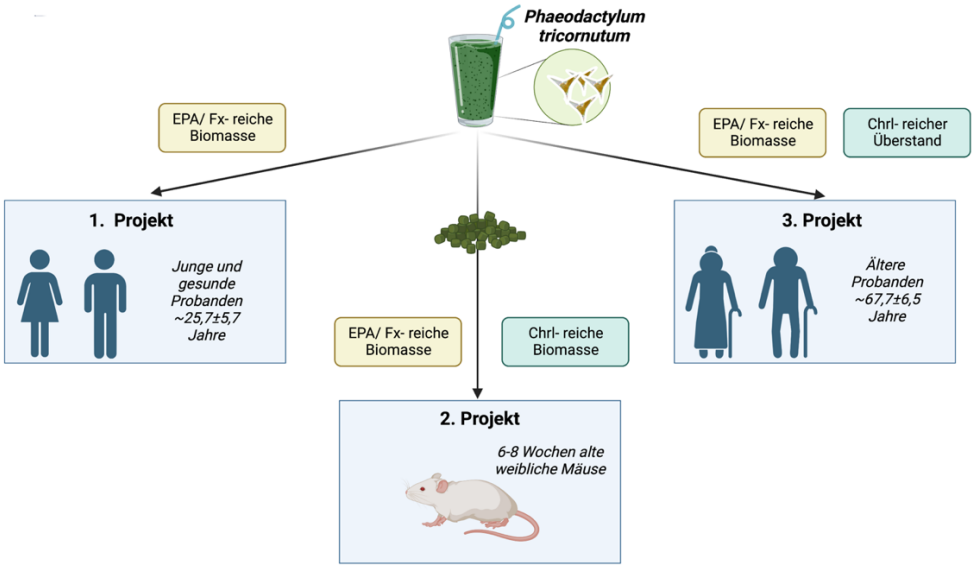


Abbildung 2. Übersicht von zwei Kultivierungsbedingungen der Mikroalge PT im Photobioreaktor, was zu einer Generierung einer EPA- und Fx-reichen Biomasse und einer Chrl-reichen Biomasse führt. Anschließend erfolgt ein mechanischer Zellaufschluss. Durch Zentrifugieren der Chrl-reichen Biomasse kann ein Chrl-reicher Überstand generiert werden. Die Biomassen wurden bereits jeweils in unterschiedlichen *In-vivo*-Studien getestet, wie an Mäusen [46], Goldbrassen und Zebrafischen [112,126]. Das Bild wurde mit BioRender.com erstellt.

Neben der Bioverfügbarkeit und der Untersuchung von Sicherheitsparametern konnten zudem bereits mögliche gesundheitliche Vorteile durch die Einnahme von PT gezeigt werden. *In vitro* wurden entzündungshemmende, antioxidative und antiproliferative Wirkungen auf menschliche Blutzellen nachgewiesen [127,128]. Zudem werden Fx aus PT antioxidative [129] und positiven Effekte gegen Fettleibigkeit zugeschrieben [130–132]. I*n vivo* in Goldbrassen konnte gezeigt werden, dass die Supplementierung des Chrl-reichen Überstandes als Gegenmaßnahme bei Darmentzündungen aufgrund von immunmodulatorischen und antioxidativen Wirkungen eingesetzt werden könnte [112]. Ferner wurde bei Zebrafischen durch die Supplementierung des Chrl-reichen Überstandes Störungen des Lipidstoffwechsels, die durch Cholesterin in der Nahrung verursachte wurden, gelindert [126].

1.5. Zielsetzung

Die Studien zur Mikroalge PT sind vielversprechend. Daher war das Ziel des Promotionsprojektes, die Eignung von PT für die menschliche Ernährung zu untersuchen. Dabei wurden Sicherheitsparameter und die Bioverfügbarkeit ausgewählter Nährstoffe nach der Einnahme der Mikroalge untersucht. Zudem sollten mögliche Bioaktivitäten von PT aufgezeigt werden, die eine positive Funktion im Körper auslösen könnten. Während der Promotion konnten drei Projekte dazu durchgeführt werden und beide kultivierten PT-Biomassen, die zum einen EPA-/Fx-reiche und Chrl-reiche, wurden dabei berücksichtigt (Abbildung 3). Im ersten Projekt wurde eine humane Pilotstudie konzipiert und die tägliche Einnahme der EPA-/Fx-reichen Biomasse von PT über 14 Tage an gesunden Probanden untersucht. Das Hauptziel der Studie war die erstmalige Untersuchung der Eignung der Mikroalge für die humane Ernährung durch die Messung von Sicherheitsparametern und der Nachweise der Bioverfügbarkeit von FS und Carotinoiden nach einer Einnahme. Im zweiten Projekt wurden beide generierten PT-Biomassen an Mäusen in einem 14-tägigen Fütterungsversuch getestet und bezüglich einer unbedenklichen Einnahme überprüft, sowie auf potenzielle darmgesundheitliche Vorteile untersucht. Im dritten Projekt wurde eine weitere Humanstudie mit älteren Probanden mit vier Interventionsgruppen durchgeführt. Das Ziel hierbei war es, mögliche gesundheitliche Vorteile für ein gesundes Altern nach dem Verzehr der EPA-/Fx-reichen Biomasse und des Chrl-reichen Überstandes, sowie einer Kombination beider zu untersuchen.



(BioRender.com)

Abbildung 3. Arbeitsübersicht der drei Promotionsprojekte und verwendeten PT-Biomassen. Im ersten und dritten Projekt wurde jeweils eine Humanstudie durchgeführt und im zweiten Projekt eine Studie mit Mäusen. In allen drei Studien wurde eine 14-tägige PT-Einnahme untersucht und Sicherheitsparameter, die Bioverfügbarkeit ausgewählter Nährstoffe sowie potenziell gesundheitliche Vorteile untersucht.

2. Publizierte Manuskripte

2.1. Humane Interventionsstudie zur Untersuchung der sicheren Einnahme und Bioverfügbarkeit von Inhaltsstoffen der Mikroalge PT

Die weltweite Überfischung der Meere ist eines der größten Umweltprobleme der Gegenwart. Fischereipraktiken müssen verändert und die Meere nachhaltig genutzt werden [133]. Viele Menschen supplementieren zum Teil EPA und DHA in Form von Fischölkapseln, jedoch sind für die Produktion von 1 kg Fischöl zwischen 20 und 25 kg Futterfisch erforderlich. Insofern können diese Kapseln keine nachhaltige Alternative darstellen [134]. Bereits heute werden Mikroalgenöle verkauft, die meist große Mengen der FS DHA, aber nur geringe Mengen an EPA enthalten. Da PT große Mengen der FS EPA produziert, könnte die Mikroalge eine alternative EPA-Quelle darstellen und sowohl für die kommerzielle Produktion als auch für die menschliche Ernährung geeignet sein [50]. Da die Mikroalge jedoch nicht nur EPA enthält, ist die Einnahme der gesamten Biomasse von großer Relevanz, um weitere nachgeschaltete Prozesse zu vermeiden. Auf Grundlage dieser Überlegungen wurde die unbedenkliche Einnahme der EPA/Fx-reichen PT-Biomasse in der ersten Phase der Promotionsarbeit in einer Pilotstudie mit gesunden Probanden untersucht. Die Mikroalge PT wurde nach dem mechanischen Zellaufschluss gefriergetrocknet, in mit Wasser verdünnter Gemüsebrühe eingerührt und den Probanden als Art „Smoothie“ zum Trinken über 14 Tage täglich verabreicht. Schwerpunkt der Studie war die Bewertung der sicheren Einnahme der Mikroalge im Rahmen der humanen Ernährung durch die Dokumentierung von Nebenwirkungen, die Messung von Laborparametern inklusive Darmbarrieremarkern und die Sequenzierung des Darmmikrobiom. Zur Überprüfung der Nährstoffverfügbarkeit für den Menschen wurden FS, Carotinoide und Vitamin E gemessen. Um eine Vergleichbarkeit der Nährstoffverfügbarkeit von n-3-FS und EPA aus der PT-Aufnahme zu erhalten, wurde parallel die Verfügbarkeit von n-3-FS und EPA aus Fischölkapseln in einem Cross-over-Design und in einer weiteren Follow-up-Phase aus dem Verzehr von Lachs untersucht. Außer Nährstoffverfügbarkeit und Sicherheit wurden auch erste Daten zu den möglichen gesundheitlichen Vorteilen durch die Einnahme von PT erhoben. In unserer Arbeit konnten wir zeigen, dass die Einnahme von PT zu einem ähnlichen Anstieg der n-3-FS und EPA-Gehalts im Plasma führt und in einer Abnahme des n-6: n-3-Verhältnisses resultiert. Außerdem wurde Fx aufgenommen, das zu FxOH und AxA metabolisiert wird. Nach der Einnahme von PT traten keine relevanten Nebenwirkungen auf. Die Studie zeigte, dass PT eine sichere und effektive Quelle für EPA und FX – und wahrscheinlich andere Nährstoffe – ist und daher als zukünftiges nachhaltiges Lebensmittel in Betracht gezogen werden kann. Der Artikel wurde im Dezember 2021 von der Fachzeitschrift Marine Drugs akzeptiert und publiziert [135].

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Article

Oral Bioavailability of Omega-3 Fatty Acids and Carotenoids from the Microalgae *Phaeodactylum tricornutum* in Healthy Young Adults

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Citation: Stiefvatter, L.; Lehnert, K.; Frick, K.; Montoya-Arroyo, A.; Frank, J.; Vetter, W.; Schmid-Staiger, U.; Bischoff, S.C. Oral Bioavailability of Omega-3 Fatty Acids and Carotenoids from the Microalgae *Phaeodactylum tricornutum* in Healthy Young Adults. *Mar. Drugs* **2021**, *19*, 700. https://doi.org/10.3390/md19120700

Academic Editor: Marialuisa Menna

Received: 17 November 2021
Accepted: 8 December 2021
Published: 10 December 2021

Abstract: The microalgae *Phaeodactylum tricornutum* (PT) contains valuable nutrients such as proteins, polyunsaturated omega-3 fatty acids (*n*-3 PUFA), particularly eicosapentaenoic acid (EPA) and some docosahexaenoic acid (DHA), carotenoids such as fucoxanthin (FX), and beta-glucans, which may confer health benefits. In a randomized intervention trial involving 22 healthy individuals, we administered for two weeks in a crossover manner the whole biomass of PT (5.3 g/day), or fish oil (FO) containing equal amounts of EPA and DHA (together 300 mg/day). In an additional experiment, sea fish at 185 g/week resulting in a similar EPA and DHA intake was administered in nine individuals. We determined the bioavailability of fatty acids and carotenoids and assessed safety parameters. The intake of PT resulted in a similar increase in the *n*-3 PUFA and EPA content and a decrease in the PUFA *n*-6:*n*-3 ratio in plasma. PT intake caused an uptake of FX that is metabolized to fucoxanthinol (FXOH) and amarouciaxanthin A (AxA). No relevant adverse effects occurred following PT consumption. The study shows that PT is a safe and effective source of EPA and FX—and likely other nutrients—and therefore should be considered as a future sustainable food item.

Keywords: microalgae; bioavailability; fatty acids; omega-3 fatty acids; eicosapentaenoic acid; fucoxanthin

1. Introduction

The world population is growing, while food sources are limited and become eventually further limited because of climate changes, resulting in serious restrictions and a need for novel food sources [1]. Nutritional protein from meat and fish will be limited in the future, and microalgae have been proposed as a possible and sustainable alternative protein source. Microalgae can be harvested from the oceans but also grown in open ponds or photobioreactors [2] and thus, no farmland is needed to grow them. The composition of microalgae varies and includes—besides proteins—also lipids, especially *n*-3 PUFA, and antioxidants (e.g., carotenoids and vitamin E), which have potential physiological and health-beneficial effects in humans [3–5]. Carotenoids, which cannot be synthesized by humans and animals, are of particular interest here, because they must be ingested through food or supplements [6]. Relevant sources for carotenoids are egg yolks since animals accumulate lutein and zeaxanthin there [7], milk, salmon, fish, or crustaceans, and in the future

possibly also selected microalgae [8]. A prominent carotenoid, β-carotene, is an antioxidant, thus protecting from reactive oxygen species (ROS) and free radical-induced damage, and a precursor of vitamin A [9,10]. Xanthophylls, another type of carotenoids, exhibit higher antioxidant capacity compared to β-carotene by scavenging free radicals and quenching singlet oxygen [11]. Fucoxanthin (FX), is the major xanthophyll in brown-colored micro-and macroalgae (seaweeds) [12,13]. FX acts as an antioxidant, causes a reduction of plasma and liver triglycerides, and has a positive effect on cholesterol regulating enzymes in preclinical studies [14]. The brown color comes from the combination of the green chlorophyll and the red xanthophyll FX.

Microalgae are primary producers of *n*-3 PUFA, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are essential for human nutrition because they cannot be synthesized in the human body [4]. Microalgae are not currently used as a primary food source of *n*-3 PUFA, even though they are at the beginning of the food chain, since fish, our major *n*-3 PUFA source, take the fatty acids up from microalgae. Since microalgae provide both *n*-3 PUFA and protein, they could replace to some extent fish as recommended food. This is of particular interest since the demand for EPA and DHA continues to rise and at the moment there is a global gap of 1.1 million tons, as fish currently accounts for only 15% and krill for 0.3% of the calculated amounts needed by humans daily [15].

Precursors such as alpha-linolenic acid (ALA) are found e.g., in green leafy vegetables, nuts, and flaxseed, but they cannot be converted to EPA and DHA in the body at high rates [16]. For a healthy diet, eating fish for 1–2 servings per week is recommended by the DGE (German Nutrition Society) and EFSA (European Food Safety Authority) to ensure a daily intake of 250 mg EPA + DHA [17], to improve markers or risk factors associated with cardiovascular diseases [18]. If the world population would follow these recommendations, our waters would be fished dry.

The amount of EPA and DHA is varying depending on fish species and farming conditions. For supplementation of EPA + DHA, some people consume fish oil (FO) capsules. However, FO capsules are not sustainable either, since about 20–25 kg of fish is needed to produce 1 kg FO [19] and two-third of the current FO production is used for fish feed [20]. In this context, EPA- and DHA-rich microalgae represent an innovative food source capable of at least partially replacing fish and FO. Prominent *n*-3 PUFA producers are *Phaeodactylum tricornutum* (PT), *Nannochloropsis*, *Thraustochytrium*, *Ulkenia*, *Schizochytrium*, and *Crypthecodinium cohnii* sp., of which the last three in the list have been classified as oil as generally recognized as safe (GRAS) [21]. *Odontella aurita* as a whole alga, *Ulkenia* sp.-oil, and *Schizochytrium* sp.-oil are approved under the EU Commission [22].

The microalgae PT, which belongs to the diatom family, has received attention in recent years because it is a particularly rich source of EPA [23,24]. In addition to proteins and *n*-3 PUFA, PT contains large amounts of β-carotene and FX [25]. PT is not yet approved as whole biomass, yet an EPA-rich oil made of PT is on the market in the U.S.A [26] and a 2% FX-rich extract of PT has GRAS status ("BrainPhyt-PhaeoSOL") [27]. We have previously shown a good bioavailability of fatty acids from microalgae in preclinical studies [28–30].

This study demonstrates the bioavailability of fatty acids, carotenoids, and vitamins from PT and the safety of ingestion of the microalgae as whole biomass in humans. In a randomized intervention trial involving healthy individuals, the whole biomass of PT, or commercial FO capsules containing equal amounts of EPA + DHA were administered for two weeks in a cross-over manner. For a detailed safety assessment, intestinal health was investigated during the study by measuring intestinal barrier markers, as well as microbiota composition and function. In a subset of study participants, PT effects were also compared with eating fish providing similar amounts of EPA + DHA as the study products.

2. Results

2.1. Adverse Effects during the Intervention

PT dissolved in water after ball milling was generally well accepted by the study participants, although taste could be improved. Adverse effects following administration of PT and other study products were monitored by a diary, in which the participants documented their complaints, and by a questionnaire conducted at each study visit by the personnel. No serious side effects were reported, neither by the study participants in their diaries nor by the personnel in the questionnaires (Table 1). In detail, 15 participants did not mention any adverse events while taking PT or FO and no one had a side effect from eating fish. Most side effects occurred to a minimal or mild extent after PT consumption, e.g., headache, and feeling of thirst, skin problems, and reduced appetite. Gastrointestinal problems such as bloating were described for both treatments, PT and FO. Other side effects were reported occasionally, such as flatulence, stomach pain, constipation, and diarrhea.

Table 1. Adverse effects during the intervention.

Side Effects	PT (*n* = 22) Diary | Protocol			FO (*n* = 22) Diary | Protocol			Fish (*n* = 9) Diary | Protocol		
	Minimal	**Mild**	**Severe**	**Minimal**	**Mild**	**Severe**	**Minimal**	**Mild**	**Severe**
Bowel problems	2 | 6	-	-	1 | 6	-	-	-	-	-
Bloating	1 | 6	.	-		1 | 1	-	-	-	-
Stomach pain	2 | 2	-	-	-	-	-	-	-	-
Constipation	1 | 2	-	-	-	-	-	-	-	-
Stool discoloration	0 | 2	-	-	-	-	-	-	-	-
Increased bowel movements	1 | 2	-	-	-	1 | 2	-	-	-	-
Belching (at least 1×)	1 | 2	-	-	-	7 | 7	-	-	-	-
Headache	0 | 2	-	-	1 | 0	-	-	-	-	-
Increased skin impurities	0 | 1	-	-	-	-	-	-	-	-
Increased feeling of thirst	0 | 1	-	-	-	-	-	-	-	-
Reduced appetite	0 | 1	-	-	-	-	-	-	-	-

Adverse effects were documented in a diary by the participants and recorded upon questionnaire by the study personnel at the visits. Values from completers are expressed as absolute numbers. Minimal, transient symptoms with no impairment of the patient's daily activities; Mild, consistent symptoms with moderate impairment of the patient's daily activities; Severe, significant impairment of the patient's daily activities. Abbreviations: PT, *Phaeodactylum tricornutum*; FO, fish oil.

2.2. Laboratory Parameters

Different laboratory parameters were determined at 11 time points (V1–V11, see Figure 1 to examine the safety of PT consumption compared to FO and fish consumption (Table 2). During the PT intervention, uric acid concentrations increased from pre- to post-intervention ($p = 0.004$) but never differed to the baseline. In addition, PT intake was associated with an increase in high-density lipoprotein (HDL)-cholesterol (Chol) compared to the baseline ($p = 0.01$). Comparing laboratory parameters after the different interventions, we found differences between PTpost and FOpost for Chol ($p = 0.003$), HDL cholesterol ($p = 0.003$), low density lipoprotein (LDL) Chol ($p = 0.003$), as well triacylglycerols (TAG) ($p = 0.009$). As well, the LDL/HDL ratio was higher at PTpost compared to FOpost ($p = 0.04$). There was no significant difference between PT and fish intervention.

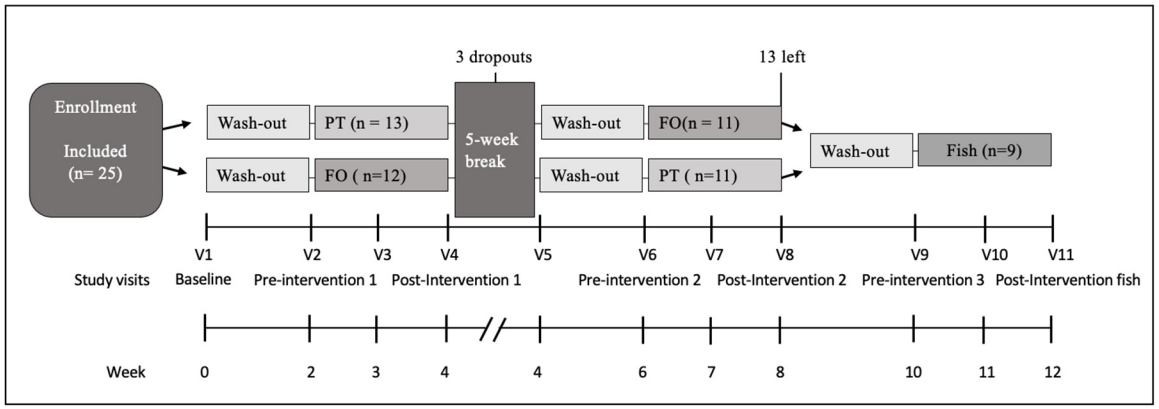

Figure 1. Study flow diagram. A total of 25 participants were randomized into two groups at V1 (baseline). The first and second part of the study (PT (and FO exposure, cross-over design) was completed by 22 individuals and were included for analysis. Of them, nine individuals volunteered to participate in the third part (fish exposure). Abbreviations: PT, *Phaeodactylum tricornutum*; FO, fish oil. For further details see text.

Table 2. Effect of interventions on laboratory parameters.

Blood Count	Baseline (V1)	PT_{pre} (V2/V6)	PT_{post} (V4/V8)	FO_{pre} (V2/V6)	FO_{post} (V4/V8)	$Fish_{pre}$ (V9)	$Fish_{post}$ (V11)
γ-GT [mg/L]	14.4 ± 4.9	14.1 ± 4.4	13.6 ± 4.0	14.0 ± 4.4	14.4 ± 5.2	11.4 ± 3.2	12.4 ± 2.7 *
AST [mg/L]	21.7 ± 5.0	21.5 ± 3.9	25.0 ± 12.5	21.6 ± 4.8	24.0 ± 10.3	23.6 ± 4.2	23.3 ± 7.8
ALT [mg/L]	19.3 ± 12.8	16.8 ± 5.5	17.0 ± 7.8	15.5 ± 5.8	21.1 ± 21	14.8 ± 5.9	12.9 ± 3.3 #
CRP [mg/L]	1.1 ± 1.4	1.0 ± 0.9	1.1 ± 1.0	0.8 ± 0.8	0.8 ± 0.8	0.8 ± 0.7	1.1 ± 1.0
Glucose [mg/dL]	81.5 ± 8.9	83.0 ± 7.0	81.1 ± 6.0	83.8 ± 6.0	81.1 ± 5.7	77.8 ± 3.7	81.9 ± 3.1
Uric acid [mg/dL]	4.5 ± 0.9	4.4 ± 0.8	4.8 ± 1.1 **	4.6 ± 1.1	4.7 ± 1.1	4.4 ± 0.5	4.3 ± 0.6
Chol [mg/dL]	187 ± 37	186 ± 40	195 ± 31	188 ± 37	180 ± 30 §§	191 ± 38	192 ± 36
TAG [mg/dL]	78.2 ± 23.3	91.2 ± 49.5	94.8 ± 43.7	84.2 ± 27.5	75.0 ± 28.6 §§	91.3 ± 69.9	78.3 ± 36.7
HDL Chol [mg/dL]	58.7 ± 12.3	59.3 ± 11.1	62.3 ± 12.0 #	61.5 ± 13.7	58.9 ± 11.9 §§	63.9 ± 10.6	66.3 ± 8.7
LDL Chol [mg/dL]	115 ± 26.2	106 ± 27.2	115 ± 22.7	110 ± 26.6	104 ± 21.3 #§§	116 ± 26.4	115 ± 24.0
LDL/HDL-ratio	2.1 ± 0.7	1.9 ± 0.6 #	2.0 ± 0.6	1.9 ± 0.6 ##	1.9 ± 0.5 ##§	1.9 ± 0.3	1.8 ± 0.3 *
Chol/HDL-ratio	3.3 ± 0.8	3.3 ± 0.8	3.2 ± 0.8	3.2 ± 0.7	3.2 ± 0.7	3.1 ± 0.4	2.9 ± 0.4 **

Values are expressed as arithmetic mean ± SD from 22 (PT, FO), or 9 (Fish intervention) participants. Analyses were measured at different time points determined before ("pre") and after ("post") intervention. Abbreviations: PT, Interventions with *Phaeodactylum tricornutum*; FO, Intervention with fish oil; Fish, Intervention with salmon; γ-GT, gamma-glutamyl transferase; AST, aspartate aminotransferase; ALT, alanine transaminase; CRP, c-reactive protein; Chol, cholesterol; TAG, Triacylglycerols; HDL, High-density lipoprotein; LDL, Low-density lipoprotein. Statistics: * indicate differences to "pre", # indicate differences to baseline, § indicate differences between PTpost and FOpost. */#/§ $p < 0.05$, **/##/§§ $p < 0.01$.

2.3. Change of Plasma Fatty Acids upon Intervention

In order to determine the bioavailability of *n*-3 PUFA plasma fatty acids were measured for each intervention at baseline as well as pre- and post-intervention (Figure 2, and Supplementary Table S2). The *n*-3 PUFA plasma concentrations always decreased during the washout period from baseline to pre-intervention for all interventions (PT, $p = 0.003$; FO, $p = 0.02$; fish, $p = 0.04$), confirming that no *n*-3 PUFA was consumed at that time

(Figure 2A). All *n*-3 PUFA-rich interventions increased plasma *n*-3 PUFA levels from pre- to post-intervention (PT, FO, $p < 0.001$; fish, $p = 0.008$), the increase being maximal after fish consumption (Fishpost compared to PTpost, $p = 0.03$; and Fishpost compared to FOpost, $p = 0.03$). The higher daily *n*-3 PUFA concentration of fish (554 mg/day) increased *n*-3 PUFA plasma concentrations from 8.8% to 11.3% (Δ2.5%) over two weeks. The increase was less pronounced for PT 8.0% to 9.25% (Δ1.24%) and FO 8.1% to 9.4% (Δ1.29%).

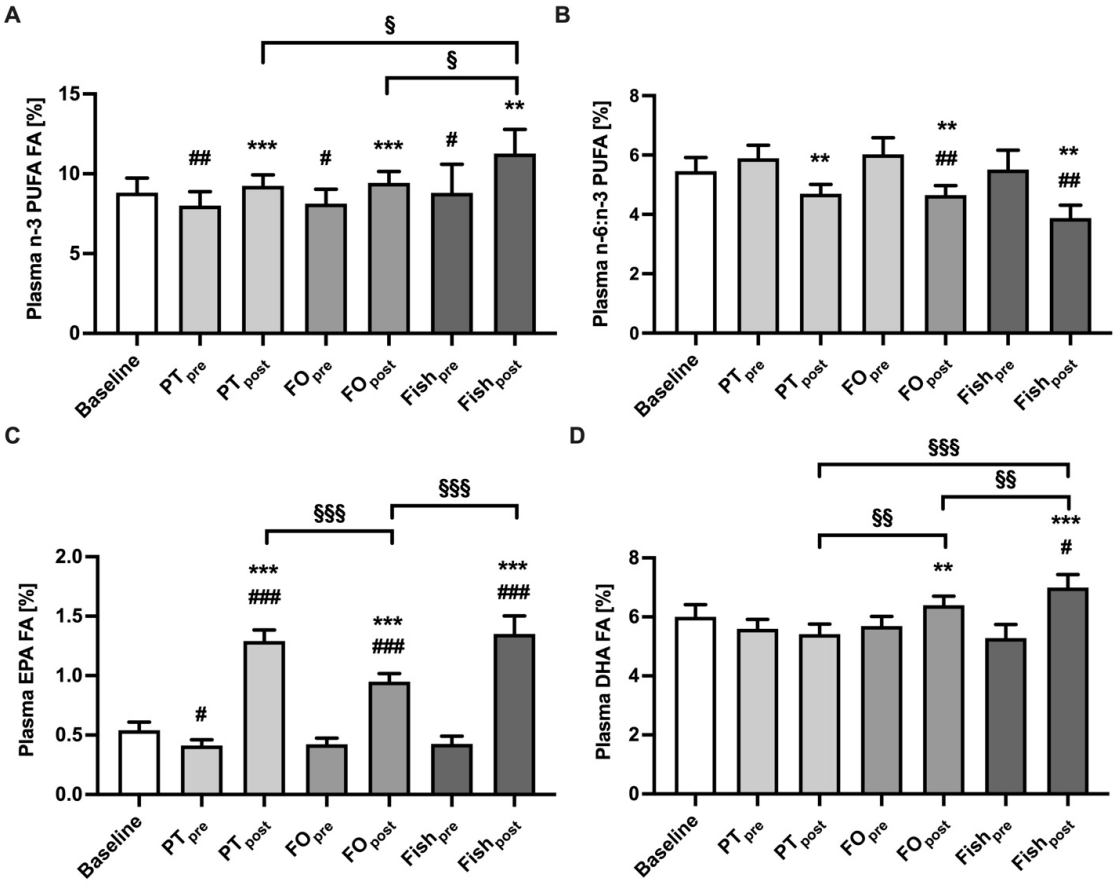

Figure 2. Change of plasma fatty acids concentrations upon intervention. *n*-3 PUFA (panel (**A**)), *n*-6 PUFA to *n*-3 PUFA ratio (**B**), EPA (**C**), and DHA (**D**) in plasma were determined before ("pre") and after two weeks ("post") intervention. Values are expressed in percent as mean ± SEM from 22 (PT, FO), or nine (Fish) participants. Statistics: * indicate differences to "pre", # indicate differences to baseline, § indicate differences between different interventions. $^{**}/^{\#\#}/^{\S\S}$ $p < 0.01$, $^{***}/^{\#\#\#}/^{\S\S\S}$ $p < 0.001$. Abbreviations: PT, Interventions with *Phaeodactylum tricornutum*; FO, Intervention with fish oil; Fish, Intervention with salmon; *n*-3 PUFA, polyunsaturated omega-3 fatty acids; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid.

Our data show that abstaining from *n*-3 PUFA-rich foods increased the PUFA *n*-6:*n*-3 ratio from baseline to PTpre ($p = 0.051$), FOpre ($p = 0.007$) and Fishpre ($p = 0.002$) groups, but following supplementation with PT, FO or fish resulted in a decrease of the ratio after two weeks (PT, $p = 0.001$; FO, $p = 0.005$; fish, $p = 0.002$). The washout phase resulted in a decrease of EPA from baseline to all interventions but only significant to PTpre ($p = 0.02$). After a two-week consumption of the study products, all plasma EPA concentrations increased from baseline to post-intervention ($p < 0.001$) and from pre- to post-intervention ($p < 0.001$). The value of FOpost was lower compared to PTpost ($p < 0.001$) and the Fishpost was higher compared to FOpost ($p = 0.03$). PT increased EPA plasma concentrations from 0.4% to 1.3%, (Δ0.9%) higher compared to FO increase by 0.4% to 1.0% (Δ0.5%, $p = 0.04$). Fish increased EPA in plasma from 0.4% to 1.4% (Δ0.9%) comparable to PT. Since PT is not a DHA supplier, only the FO and Fish interventions increased DHA from pre- to post-intervention (FO, $p = 0.004$; fish, $p = 0.0006$) and Fish from baseline to Fishpost ($p = 0.02$).

This results in higher DHA plasma concentrations for FOpost ($p = 0.008$) and Fishpost ($p < 0.001$) compared to PTpost. For further results see Supplementary Table S2.

2.4. Carotenoids and Tocopherol Concentrations before and after Intervention with PT

The carotenoids FX and β-carotene as well as other carotenoids were measured within the PT intervention at three time points after pre-intervention (PTpre), post-intervention one week (PTpost1), and post-intervention two weeks (PTpost2). FX was detected in rather small amounts in plasma after one week and two weeks of PT ingestion (Figure 3A). FX metabolites could be detected at higher concentrations and more consistently in the study participants. FXOH, to which FX is hydrolyzed by cholesterol esterase in the intestinal tract, was detected after one week of exposure to PT, and further increased after two weeks of exposure (Figure 3B). AxA, to which FXOH is converted in the liver [31] and which accumulates mainly in adipose tissue, could be also detected after one and two weeks of PT consumption (Figure 3C).

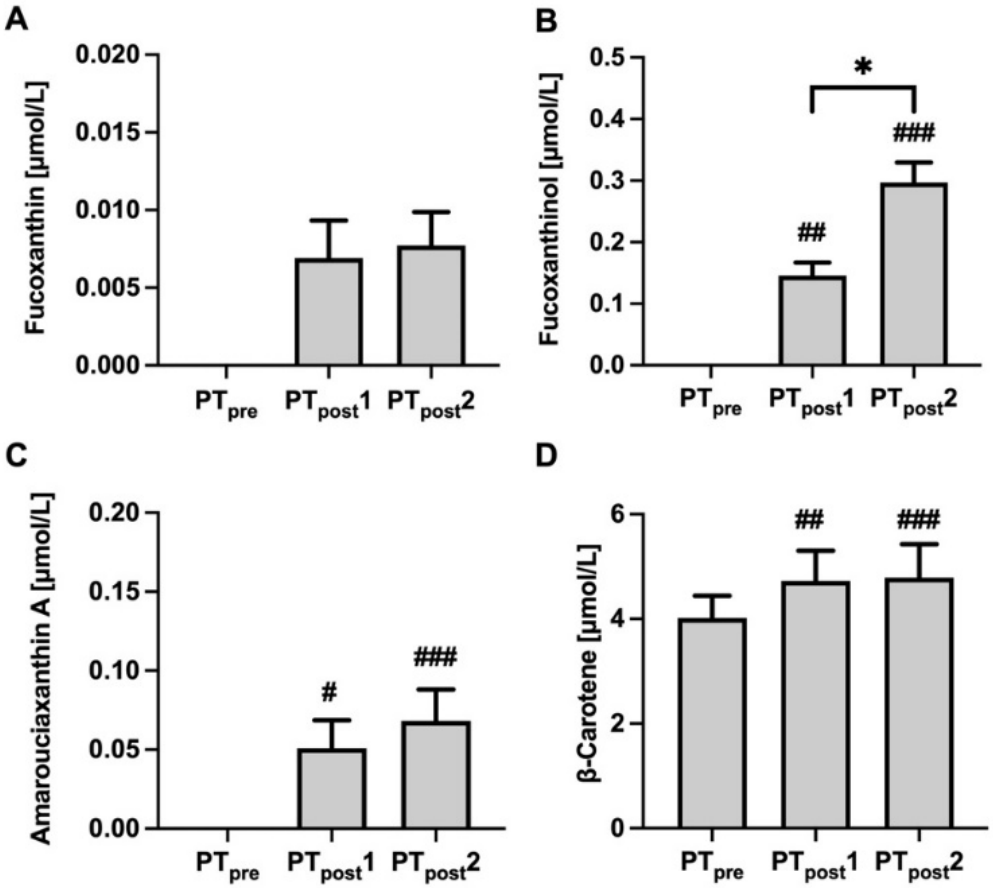

Figure 3. Plasma fucoxanthin (FX) and its metabolites as well as β-carotene concentrations before and after interventions with the microalgae *Phaeodactylum tricornutum* (PT). Plasma values of FX (**A**), fucoxanthinol (FXOH) (**B**), amarouciaxanthin (AxA) (**C**), and β-carotene (**D**) before intervention (PTpre) and one and two weeks after intervention (PTpost1, PTpost2) are shown as mean ± SEM from 22 participants. Statistics: # indicate difference to PTpre; * indicate difference between PTpost1 and PTpost2 */# $p < 0.5$, ## $p < 0.01$; ### $p < 0.001$.

Plasma β-carotene also increased after one and two weeks of PT consumption (Figure 3D). Due to the pro-vitamin A activity of β-carotene, we also measured retinol before and after PT exposure, but no increase was observed (data not shown). Other carotenoids, such as lutein/zeaxanthin, lycopene, β-cryptoxanthin, and α-carotene were detectable in plasma, but plasma concentrations did not change following PT consumption. Furthermore, plasma tocopherols did not change after two weeks of PT consumption (data not shown).

2.5. Gut Barrier Function and Gut Microbiome

Fecal zonulin and plasma LBP, two recently validated markers for intestinal permeability [32], were examined after consumption of PT, FO, and fish. Neither the fecal zonulin nor the plasma LBP were negatively affected by PT consumption or any of the other challenges. All values were above the normal ranges for fecal zonulin (61 ng/mg ± 46 ng/mL) or even higher in the healthy individuals who participated in the study. Plasma LBP concentrations were within the normal range (5–10 µg/mL) according to the kit manufactures information (Figure 4A,B). Measurement of the SCFA butyrate, iso-butyrate, acetate, and propionate in feces revealed no change following the consumption of the *n*-3 PUFA-rich study products (Figure 4C–F).

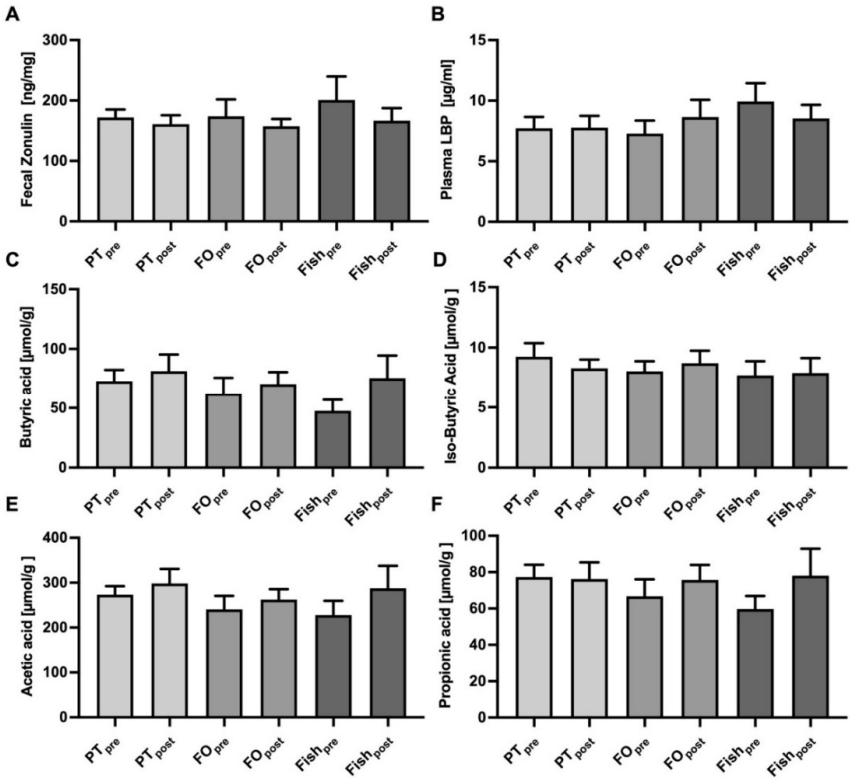

Figure 4. Assessment of gut barrier function and fecal short-chain fatty acid (SCFA) levels. Gut barrier function was analyzed by the validated biomarkers fecal zonulin (**A**), and plasma lipopolysaccharide-binding protein (LBP) (**B**). The SCFA butyric acid (**C**), iso-butyric acid (**D**), acetic acid (**E**), and propionic acid (**F**) were measured in feces before ("pre") and after ("post") intervention. Means ± SEM from 22 (PT, FO), or 9 (Fish) participants are shown. Abbreviations: see Figure 1.

Analysis of the gut microbiome revealed only minor changes following the consumption of the three study products. The β-diversity (bray-curtis distance) was not affected by the different interventions (data not shown). The α-diversity related to bacterial richness (observed OUT; fish, $p = 0.02$) and the Shannon index (variety; fish, $p = 0.04$) increased only after fish consumption but was not changed by PT or FO exposure (Figure 5A,B). Evenness (equal distribution) was not affected by all three interventions (not shown). We found no changes at the phylum level before and after PT consumption (Figure 5C). Additionally, the ratio of *firmicutes*/*bacteroides* (F/B ratio) was not affected, except for a trend of reduction by FO that could be seen ($p = 0.1$, Figure 5D). At the family level, *Rikenellaceae*, *Christensenellaceae*, *Lachnospiraceae*, *Oscillospiraceae*, *Ruminococcaceae*, *Akkermansiaceae* showed a trend of increasing, except *Lachnospiraceae* which decreased after FO and PT intake, the latter by trend (Supplementary Table S3). Only fish consumption resulted in some changes at the family and genus level. Fish consumption leads to a decrease of the *Oscillospiraceae* family and the UCG-002 genus belonging to *Oscillospiraceae* (Figure 5E,F), and to an increase in the

Lachnospiraceae family (Supplementary Table S3) and the *Akkermansia* genus (Figure 5G). Additionally, PT induced some increase in *Akkermansia* by trend and *Agathobacter* was higher after PT compared to FO administration (Figure 5H).

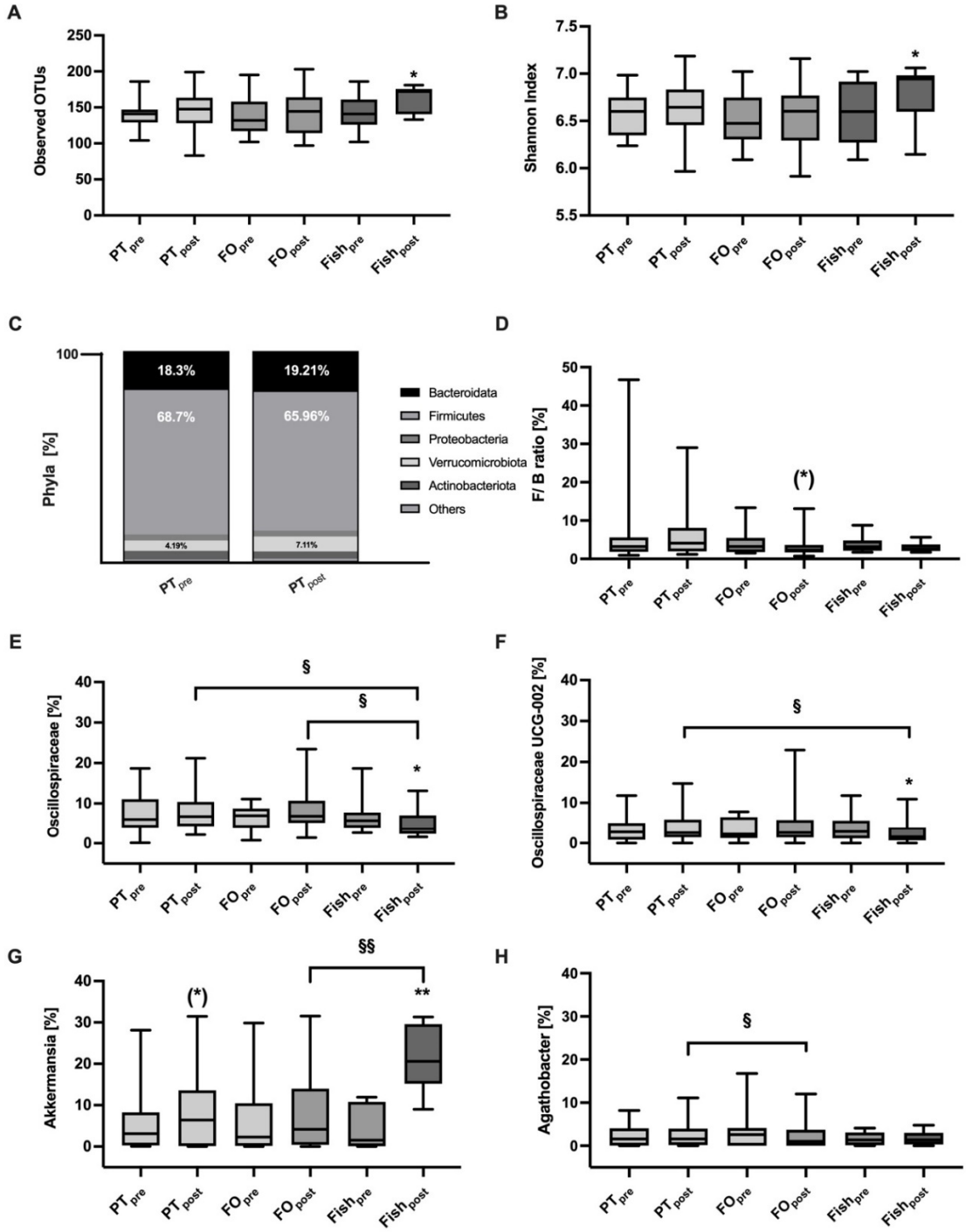

Figure 5. PT did not affect the gut microbiome. The α-diversity (Observed OTU, panel (**A**)) and the Shannon index (panel (**B**)) increase following the fish intervention. Relative bacterial abundancies at the phylum level (panel (**C**)) and the *Firmicutes*/*Bacteroides* (F/B) ratio (panel (**D**)) remained unchanged. Some changes of selected bacterial in family and genus level were found only after fish consumption (panel (**E**–**H**), see Supplementary Table S3). Data are shown as bar plots (panel (**C**)) or as box plots with median, 25% and 75% percentiles, min and max. * indicate differences to "pre", § indicate differences between different interventions. (*) $p = 0.1$, */§ $p < 0.05$, **/§§ $p < 0.01$.

3. Discussion

The present study is the first in which the unfractionated microalgae PT has been examined in humans. We focused in this pilot trial on acceptance of the product, safety issues, and bioavailability of nutritive components of PT. Our data revealed that the bioavailability of fatty acids such as *n*-3 PUFA and EPA is similar to PT and FO administered in a crossover design, indicating that such fatty acids can be absorbed equally well from milled microalgae biomass suspensions and a commercially available fish oil product. Moreover, we could show that selected carotenoids, such as FX and its metabolites, and β-carotene are bioavailable from PT. The data suggest that PT should be considered for human nutrition in the future. By consuming the entire biomass, which has been only processed by ball milling to disrupt the microalgae cells, the costs of nutrient-rich food made from PT could be kept low. Microalgae such as PT contain for example high amounts of protein and minerals, and we could show in a pre-clinical study that the bioavailability of this protein is high [28]. Based on this data, microalgae such as PT could become a valuable and sustainable new food source, especially for vegetarians and vegans, as well as for people in developing countries.

The present study confirms that PT is an *n*-3 PUFA rich microalgae that contains *n*-3 PUFA at a concentration of 58 mg/g, compared to 21 mg/g in fish. EPA is the main *n*-3 PUFA in PT (53 mg/g), whereas fish contains much less (7 mg/g). For this reason, PT is used at up to 6% in fish meals for feeding Atlantic salmon [33]. Previous studies showed that DHA is well absorbed from oil derived from the microalgae *Schizochytrium* sp. [34], or from *Crypthecodinium cohnii* [35] in a similar range as from classical FO or whole sea fish. Our study extends this finding by showing that EPA and the total *n*-3 PUFA are well absorbed from milled and resuspended PT. The fact that DHA did not increase following PT consumption might be explained due to the minor amounts of DHA in PT compared to FO or fish, and not necessarily because DHA bioavailability is low. However, this must be further evaluated in the future. The observation that EPA increased after fish and FO consumption to a similar degree as after PT consumption, even though fish and FO contained lower amounts of EPA, could be due to retro conversion of DHA to EPA after absorption [36].

Our study provides evidence that PT is a valuable source for EPA, as described before for another diatom, *Odontella aurita*, which has been already authorized as whole algae, although no nutritional human trials are available so far, in which *Odontella aurita* has been tested for safety or bioavailability of its ingredients. The nutritional composition of *Odontella aurita* is quite similar to that of PT [37] and the microalgae have been shown in pre-clinical studies to reduce risk factors for metabolic syndrome [38,39] similar to PT [23]. Although *Odontella aurita* has been approved as whole microalgae, only 0.5–1.5% of it is allowed in food, mainly for flavoring [22]. From such small amounts, no health benefits can be expected. Higher amounts need to be tested in prospective randomized trials.

EPA and DHA are both necessary to increase the total *n*-3 PUFA, therefore PT could be an alternative EPA source and serve together with DHA-rich microalgae like *Schizochytrium* sp, which is mainly used in supplements with *Ulkenia* sp. It has been shown that the regular intake of EPA and DHA leads to a reduction in the *n*-6:*n*-3 ratio of PUFA, which results in higher EPA + DHA concentrations in red blood cell membranes (omega-3 index), considered as beneficial, e.g., to lower inflammatory processes [40]. The Western diet is characterized by a high *n*-6:*n*-3 ratio thought to promote diet-related diseases such as obesity, type 2 diabetes, and cardiovascular disease [41]. The *n*-3 PUFA EPA and DHA not only have anti-inflammatory and anticoagulant effects but also promote the synthesis of specialized pro-resolving mediators crucial for the healing phase and termination of inflammation [42]. A low PUFA *n*-6:*n*-3 ratio is therefore associated with a reduced risk of cardiometabolic diseases [43] and possible prevention for death by COVID-19, as shown recently [44]. Our study shows that the *n*-6:*n*-3 ratio and total *n*-6 PUFA can be reduced not only by the consumption of fish and FO but also by PT consumption.

Another particular feature of diatoms is the high content of FX besides EPA. FX is found in PT, as well as in *Odontella aurita* [37] at concentrations of about 10–20 mg/g dry weight) depending on the culture condition. The potent antioxidant is also found in some brown algae such as *U. pinnatifida* found in Miso soup and Wakame, albeit at lower concentrations (2.7 mg/g) [45]. A good bioavailability is a requirement for beneficial health effects, e.g., anti-cancer and anti-obesity effects [46,47]. Our study demonstrates that FX is absorbed in the intestine and can be detected in plasma; however, only at low levels (Figure 2). A likely explanation for the low plasma concentrations of FX is that it is rapidly metabolized into FXOH and AxA. These two FX metabolites were detected at much higher concentrations compared to FX supporting our hypothesis of a rapid metabolization in the intestine and liver. This hypothesis is also supported by pharmacokinetic studies in rodents [48,49], and a few conflicting studies in humans. One human study reported a maximum concentration (Cmax) of 44 nmol/L FXOH in plasma 4 h after ingestion of 31 mg FX of Kombu extract [50]. Others found only a concentration of 2.7 nmol/L FXOH in normal and overweight humans after ingestion of 2 mg FX from Akamoku oil for eight weeks [51]. A third human trial revealed an increase of 0.8 nmol/L FXOH in plasma after one week of ingestion of 6.1 g FX from Wakame [52].

In our study, ingestion of PT at 30 mg/d FX increased FXOH plasma concentrations to 232 nmol/L after one week and 482 nmol/L after two weeks, suggesting the bioavailability of FX metabolites from PT. Moreover, we detected AxA, another FX metabolite that was not analyzed in the previous human trials, at up to 111 nmol/L. This indicates a further metabolization of FXOH in the liver. Our dosing of FX from PT, which contains about 1% of FX, corresponds to approximately 0.5 mg/kg body weight. In a rodent model, a dose between 1 and 10 mg/kg body weight has been administered for 28 days and no increase in mortality or toxic effects have been observed [53]. FX from macroalgae is approved for supplementation in the EU. For the PT-based oil, which contains about 2% FX, the recommended dose has been set to 437 mg, which corresponds to 10 mg FX because higher doses have not been studied so far. In our study, the higher dose of 30 mg FX and Chlorophyll caused some green discoloration of the feces, but almost no adverse effects and, if at all, only mild abdominal symptoms, such as bloating, stomach pain, belching, constipation, and diarrhea in a few participants. Moreover, we observed no toxic effects in our study, since none of the safety parameters included (γ-GT, AST, ALT, CRP, glucose, uric acid, cholesterol, and triacylglycerols) a change upon intervention. This confirms our previous toxicological studies in mice showing no toxic effects of PT administered at very high concentrations [28].

PT is also a source of lutein/zeaxanthin, which is otherwise found in green vegetables, yellow fruits, and egg yolk [54], and of β-carotene, which is present mainly in carrots. However, according to our data, the bioavailability of lutein/zeaxanthin seems to be low, but this might be a result of the suboptimal timing of our measurements. We measured one and two weeks after the start of the challenge to assess *n*-3 PUFA. For assessment of the lutein/zeaxanthin bioavailability, shorter time intervals might be needed, such as hours or a few days, as performed in classical pharmacokinetic studies. While lutein/zeaxanthin levels hardly changed, we observed an increase in β-carotene levels, but not of retinol, after one and two weeks. The low cleavage of β-carotene to retinol could result from the presence of retinol in the diet [55], but also due to an interaction with other carotenoids such as zeaxanthin [56]. Nevertheless, microalgae are generally good sources of carotenoids. Harvey et al. showed that the cost of producing β-carotene synthetically is 10 times higher than using the microalgae *Dunella salina* [57].

Another ingredient of PT is tocopherol, the amount of which is dependent on the microalgae cultivation conditions [58] and on the processing since it was added as a fat-soluble antioxidant during the cell disruption. The microalgae PT contains all tocopherols and γ-tocotrienol. During the study, the subjects consumed ~2.5 mg of total vitamin E daily just by PT intake, which covers 16.5% of the daily requirement. No increase of α- and γ-tocopherol levels were measured after two weeks of consumption of PT, which does not

exclude some tocopherol uptake at earlier time points. More detailed pharmacokinetic studies are required to assess bioavailability here since vitamin E concentrations are regulated by the hepatic α-tocopherol transfer protein and undergo metabolic degradation to short-chain metabolites within due time.

Gut-related parameters were carefully assessed in our study for two reasons. First, microalgae components such as fibers might induce some adverse effects, such as bloating, but also increase the production of SCFA. Secondly, gut barrier function or commensal microbiota could be affected. As reported before, PT induced some mild GI symptoms at slightly higher rates compared to FO or fish, but no effects on SCFA concentrations in feces. It is possible that the number of fibers (15 g fibers per 100 g PT, corresponding to <1 g fibers per day) and *n*-3 PUFA may not be enough to alter SCFA production by the commensal microbiota. Detailed microbiome analysis at the 16S RNA level revealed only minor changes that were statistically significant only after fish consumption for a few selected bacterial genera such as *Akkermansia*, which increased after fish and by trend after PT consumption, but not after FO consumption. Such results might become different if patients, e.g., with inflammatory bowel diseases [59], instead of healthy subjects are studied.

4. Materials and Methods

4.1. Participant Selection

Twenty-five healthy adults (15 females and 7 males, age 18–50 years) were recruited for the study (Figure 1) and provided written informed consent. Exclusion criteria were pregnancy, breastfeeding, use of medications, such as antibiotics, intestinal therapeutics, and immunosuppressants. Relevant violations of the dietary protocol did not occur. The study was conducted at the University of Hohenheim in 2020 according to the Declaration of Helsinki, has been approved by the local Ethical Committee (Ethik-Kommission der Landesärztekammer Baden-Württemberg), and was registered at ClinicalTrials.gov (NCT04288544). The study was planned as a proof-of-principle pilot study; therefore, no formal calculation of case numbers was performed. According to other studies in this area, our goal was to have at least 20 individuals available for analysis.

4.2. Study Design and Intervention

The study was designed as a randomized, single-blind, monocentric intervention trial in a crossover design (Figure 1) with 11 study visits. Participants were randomly assigned to group 1, starting with the microalgae PT (5.3 g per day) administered for two weeks (PTpost) and a FO capsule intake (one per day) for two subsequent weeks (FOpost), or to group 2 receiving the same intervention in the opposite order (FOpost and PTpost), with a two-week washout period before the first (pre-intervention 1) and the second phase (pre-intervention 2) of intervention. Due to restrictions related to the COVID-19 pandemic, all subjects underwent a five-week break after the first intervention phase (post-intervention 1) followed by the second washout phase (pre-intervention 2) before the second intervention.

All subjects who passed the crossover study with two interventions PT and FO were invited for a third intervention phase of two weeks without PT or FO, but fish as a positive control. These individuals (nine out of 22) were served four servings of salmon (125 g and 60 g in the first week, 60 g and 125 g in the second week) resulting in 185 g salmon per week (post-intervention fish; Fishpost). These chosen fish doses per week resulted in an EPA + DHA challenge similar to that achieved by PT or FO intervention in the previous study parts.

Fasting blood samples were collected at all-time points: at the baseline, after a two-week washout period (pre-intervention 1, 2, and 3), after one week of the taking study product, and two weeks of the taking study products (post-intervention 1, 2 and fish) at all three phases. Fecal samples were collected at pre-intervention 1, 2 (PTpre, FOpre) and post-intervention 1, 2 (PTpost, FOpost) and fish (Fishpost). During the whole study period, participants were asked to follow their habitual diet with some restrictions. They had to

avoid foods containing *n*-3 PUFA, such as fish, vegetable oils, vegetables, seeds, and nuts as well as *n*-3 PUFA-fortified foods. All participants underwent study-specific diet counseling and were instructed to take the study food in addition to their habitual diet.

4.3. Subject Characterization

Twenty-two out of 25 participants (10 men and 15 women) completed the study, namely the two major intervention phases PT and FO. Only three volunteers (three men) dropped out after four weeks for reasons not related to the study (COVID-19 pandemic and personal reasons). All 22 participants were invited to participate in a study extension with fish exposure instead of PT or FO. Nine of them agreed and completed the fish phase immediately after the previous study phase with a two-week washout in-between. Baseline characteristics of study participants are shown in Table 3. None of the participants showed any major changes in their health status during the study. Group 1 started with PT intake, group 2 started with FO intake and at the baseline, there was no difference between the study groups 1 and 2 and no changes were seen during the intervention (Table 3).

Table 3. Baseline characteristics of the study participants.

Parameter	All (*n* = 22)	Group 1 (*n* = 11)	Group 2 (*n* = 11)
Age [years]	25.7 ± 5.7	26.5 ± 7	24.8 ± 3.3
Female/male [n]	15 | 7	10 | 3	5 | 4
Body Weight [Kg]	65.9 ± 10.7	65.8 ± 12.8	68.2 ± 9
Height [m]	1.74 ± 1	1.72 ± 0.09	1.78 ± 0.09
BMI [kg/m^2]	21.5 ± 2.0	22.0 ± 2.6	21.5 ± 1.9
Vegetarian | Vegan |	2 | 2 |	2 | 0	0 | 2
Hemoglobin [g/dL]	14.4 ± 1.3	14.3 ± 1.1	14.4 ± 1.6
Hematocrit [%]	42.5 ± 2.9	42.2 ± 2.2	42.5 ± 3.8
Erythrocytes [cells/pL]	4.9 ± 0.3	4.9 ± 0.2	4.9 ± 0.4
Leucocyte [cells/nL]	5.6 ± 0.4	6.2 ± 1.2	5.3 ± 2.3
Platelet count [cells/nL]	240.8 ± 59.6	250.1 ± 54.5	245.5 ± 68
TSH [mU/L]	1.6 ± 0.6	1.8 ± 0.7	1.5 ± 0.4
Insulin [µE/mL]	7.1 ± 3.3	8.4 ± 3.6	6.0 ± 2.9
HOMA-IR	1.4 ± 0.7	1.7 ± 0.8	1.2 ± 0.7
Dietary intake (FFQ) [g/day]			
Fat	49.2 ± 23.7	44.8 ± 18.9	55.5 ± 29.4
Saturated fatty acids	22.23 ± 11.0	19.7 ± 7.9	25.9 ± 14.11
Unsaturated fatty acids	7.6 ± 4.1	7.0 ± 3.4	8.5 ± 5.0
Short chain fatty acids	1.2 ± 0.8	0.9 ± 0.4	1.4 ± 1.1
Long chain fatty acids	43.40 ± 20.9	39.6 ± 17.13	48.9 ± 25.5
Cholesterol	191.8 ± 109.2	177.2 ± 104.4	212.9 ± 118.7

Values are expressed as Mean ± standard deviation (SD) which is expressed in absolute numbers. Abbreviations: PT, *Phaeodactylum tricornutum*; FO, fish oil; BMI, body mass index; HOMA-IR, Homeostasis Model Assessment for Insulin Resistance; FFQ, Food Frequency Questionnaire. Group 1 started with PT intake; group 2 started with FO intake. Statistics: Comparison of group 1 and 2 revealed no difference for all parameters listed in the table ($p > 0.05$, paired *t*-test).

The compliance was checked every two weeks and revealed that all participants consumed the intervention products according to the protocol. All participants followed the instructions not to eat any *n*-3-rich foods during the washout and interventional phases. To record food intake in detail, a Food Frequency Questionnaire (FFQ) was requested during the whole study time, there was no difference between the study groups 1 and 2 (Table 3).

4.4. Study Products

PT SAG 1090-1b was cultivated in flat-panel airlift photobioreactors at Fraunhofer CBP as described previously [29]. After harvesting, the biomass was concentrated to 250 g/L using a centrifuge (Clara 20, Alfa Laval, Glinde, Germany) and stored at −20 °C

until further processing. After thawing, the biomass was diluted with deionized water to 100 g/L and subsequently, the cells were disrupted in a ball mill according to Derwenskus et al. [60]. To minimize oxidation during cell disruption, 0.2 g/L of all rac-α-tocopherol was added (Roth Inc., Karlsruhe, Germany). The biomass was freeze-dried for 36 h at −20 °C and 0.1 mbar (VaCo 5. Zirbus, Tiel, The Netherlands) and stored at −20 °C protected from light until further use.

The amount of PT and FO was chosen based on the national *n*-3 PUFA/EPA + DHA recommendation of 250 to 300 mg per day [17]. Accordingly, the subjects received 5.3 g PT daily (Table 4) divided into two servings, one at noon and one in the evening, delivering a total of 305 mg of *n*-3 PUFA. For comparison, we administered FO capsules resulting in an intake of a similar amount per day. Subjects took one commercially available capsule daily in the evening (softgel capsule "Essential Omega-3" containing 310 mg *n*-3 PUFA and antioxidants; Myprotein, Manchester, UK).

Table 4. Daily intake of omega-3 and omega-6 fatty acids by the study participants.

Fatty Acids [mg/day]	PT	FO	Fish
n-3 PUFA	305	310	554 *
EPA + DHA	286	300	299 *
20: 5 *n*-3 (EPA)	282	178	198 *
22:6 *n*-3 (DHA)	5	122	101 *
18:3 *n*-3 (ALA)	0.9	1.8	94.1 *
18:4 *n*-3	0.7	0.2	58.9 *
22:5 *n*-3	9.5	7	100 *
16:2 *n*-6	13.4	2.8	0.6 *
18:2 *n*-6	3.8	5.3	34.9 *
18:3 *n*-6	1.2	0.9	0.1 *
20:3 *n*-6	0.1	0.3	0.2 *
20:4 *n*-6	3.7	0	50.2 *

Abbreviations: PT, Intervention with the microalgae *Phaeodactylum tricornutum*; FO, Intervention with fish oil; Fish, Intervention with salmon served as two servings per week; *n*-3 PUFA, total amount of polyunsaturated omega-3 fatty acids; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid, ALA, alpha-linolenic acid * Average daily intake calculated based on weekly amount.

For the third intervention, we selected salmon (Salmo salar) from Norway from aquaculture, because this oily fish is rich in *n*-3 PUFA and a natural source for EPA and DHA. Since EPA and DHA are the essential *n*-3 PUFA, we adapted the number of salmon administered in the study to an EPA and DHA intake of about 300 mg per day. Since fish, in contrast to PT and FO, contains another *n*-3 PUFA in addition to EPA and DHA, the total *n*-3 PUFA intake during the fish intervention was higher than during the PT or FO intervention (Table 4). This approach resulted in the consumption of 185 g salmon per week, divided into 2 servings (125 g and 60 g). The frozen salmon of organic quality was purchased from a supermarket (Aldi Süd, Harsum, Germany). The detailed composition of the study products is shown in Supplementary Table S1.

4.5. Blood Plasma, Serum Measurements, and Fecal Samples

Blood samples were collected in two ethylenediaminetetraacetic acid (EDTA)-coated tubes and one serum tube. One plasma tube was used for blood count analysis (Sindelfingen laboratory GbR, Sindelfingen, Germany). The other one was centrifuged at 500 g for 7.5 min at 15 °C followed by separation of plasma, which was stored at −80 °C until analysis for fatty acids, carotenoids, retinol, tocopherols, and lipopolysaccharide-binding protein (LBP) quantification.

Serum tubes were centrifuged 15 min at 3000× *g*, and serum was used for quantification of γ-gamma-glutamyl transferase (γ-GT), aspartate aminotransferase (AST), alanine transaminase (ALT), c-reactive protein (CRP), plasma glucose, uric acid, triacylglycerols (TAG), cholesterol (Chol), High-density lipoprotein (HDL) and Low-density lipoprotein (LDL) (Sindelfingen laboratory GbR).

Stool samples were collected prior to the study date (max. 2 days before) in two tubes and stored at −20 °C at home or transported directly to the laboratory. In our laboratory, the two tubes were stored at −80 °C until further analysis. One tube was used to measure the gut barrier marker zonulin and SCFA, the other tube was used for DNA isolation for gut microbiome sequencing.

4.6. Quantification of Plasma Fatty Acids

In brief, 2 µL 10,11-dichloro-undecanoic acid (11:0) as internal standard and 2 mL methanol (Carl Roth GmbH, Karlsruhe, Germany) with 1% sulphuric acid for transesterification according to the method of Thurnhofer and Vetter was added to 100 µL plasma as described [61]. Next, 5 µL tetrade-canoic acid-EE (14:0-ethyl ester) was added before the samples were analyzed by gas chromatography with mass spectrometry on a 5890 series II/5972A system (Hewlett-Packard, Waldbronn, Germany) operated in selected ion monitoring mode according to Thurnhofer et al. [32,62].

4.7. Quantification of the Carotenoid FX and Its Metabolites FXOH and AxA in Plasma

To measure FX and their metabolites FXOH and AxA, 100 µL of human plasma was mixed with 200 µL of ethanol/butanol (50/50 (*v*/*v*)) containing 5 mg butylated hydroxytoluene. After vigorous mixing and centrifugation (17,000× *g* and 4 °C, 10 min) (Heraeus Fresco 17, Thermo Fischer Scientific, Waltham, MA, USA), 10 µL of clear supernatants were injected into a Shimadzu HPLC system (Mc Kinley Scientific, New York, NY, USA) with a F5 reversed-phase column (2.6 µm F5 100 Å 150 × 4.6 mm, Kinetex, Phenomenex Ltd., Aschaffenburg, Germany) maintained at 40 °C and an ultraviolet detector set to 450 nm. A mixture of methanol/water (85/15 (*v*/*v*)) with a flow rate of 1.0 mL/min for 15 min was used as mobile phase. The autosampler was kept at 15 °C and quantification was achieved using authentic commercial standards (FX purity ≥ 95%; FXO and AxA purity ≥ 97%, Merck Group, Darmstadt, Germany) diluted in ethanol. The method was tested for linearity, sensitivity, and selectivity before sample analysis.

4.8. Quantification of the Carotenoids Lutein/Zeaxanthin, Lycopenes, β-Cryptoxantins and α/β-Carotene, Retinol, and α/γ-Tocopherol in Plasma

The extraction of other carotenoids, retinol, and tocopherols from human plasma was performed as previously described [63] with some modifications. Forty microliters of plasma were mixed with 200 µL of ethanol/butanol (50/50 (*v*/*v*)) containing 12 µL beta-apo-8′-carotenal-methyloxime/100 mL (internal standard). After vigorous mixing and centrifugation (17,000× *g* and 4 °C. 10 min) (Thermo Fischer Scientific), 20 µL of clear supernatants were injected into a Shimadzu HPLC system (see above) with a ReproSil 80 ODS-2 column (3 µm, 250 × 4.6 mm) (Dr. A. Maisch GmbH, Ammerbuch-Entringen, Germany) maintained at 40 °C. A mixture of acetonitrile/1,4-dioxane/methanol (82/15/3; *v*/*v*) containing 100 mmol/L ammonium acetate and 0.1% trimethylamine, at a flow rate of 1.5 mL/min for 20 min was used as mobile phase and autosampler temperature was maintained at 5 °C. Detection of carotenoids was performed using an ultraviolet detector set to 450 nm while quantification of retinol and tocopherols used a fluorescence detector (Ex/Em at 325/470 nm for retinol for 0–5 min and Ex/Em at 296/325 nm for α/γ-tocopherol for 5–20 min). Quantification of all analytes was performed using authentic commercial standards corrected by the internal standard.

4.9. Quantification of Tocopherol, Tocotrienol, and Carotenoids in PT and FO by HPLC

In PT and FO, Vitamin E was determined by hexane-based extraction. PT determination was performed from freeze-dried material. For FO preparation ten capsules were chosen and mixed homogenously. Later triplicate samples of 100 mg PT or 25 mg FO were saponified with KOH, neutralized, extracted with hexane, and later vitamin E was resuspended in ethanol as previously described [64]. For measurement of vitamin E, 20 µL of the ethanolic suspension were injected into a Jasco HPLC system (JASCO Deutschland GmbH, Pfungstadt, Germany) with PFP reverse phase column (2.6 µm PFP 100 Å 100 × 4.6 mm,

Kinetex, Phenomenex Ltd., Aschaffenburg, Germany) maintained at 40 °C using a mobile phase methanol/water (85:15 (*v*/*v*)) with a flow rate of 1.2 mL/min for 30 min. Autosampler temperature was set to 5 °C and quantification was performed using a fluorescence detector with excitation/emission wavelengths of 296/325 nm against authentic standards for of RRR-α-tocopherol, RRR-β-tocopherol, RRR-δ-tocopherol, RRR-γ-tocopherol (purity ≥ 95 %, Merck Group), α-tocotrienol, β-tocotrienol, δ-tocotrienol, γ-tocotrienol (purity ≥ 97 %, Merck Group) diluted in ethanol. The method of quantification of FX and β-carotene in PT was previously described by Derwenskus et al. [60].

4.10. SCFA Analysis from Stool Samples

Raw fecal samples were homogenized, weighed (ca. 400 mg), diluted 1:4 with distilled water and 100 µL 50% phosphoric acid (Carl Roth GmbH, Karlsruhe, Germany) were added. Samples were homogenized with a whirlmix and centrifuged (20,000× *g* at 4 °C, 20 min) twice (5417R, Eppendorf, Hamburg, Germany). The supernatant was drawn up and filtered with a syringe filter with glas fiber (WIC 79545, Wicom, Heppenheim, Germany) to an autosampler glas (WIC 42100 with crimp caps, Wicom) with Micro Inserts (No 548-00060, VWR International GmbH, Darmstadt, Germany). With a capillary gas chromatograph (Clarus 690, Perkin-Elmer, Waltham, MA, USA), using a liquid autosampler with a capillary column (Cat. # N9316354, Perkin Elmer) with standards (Volatile Free fatty acid Mix CRM46975, Merck Schuchhardt OHG, Hohenbrunn, Germany), 1 µL filtrate was analyzed. For data integration, the software total-Chrome Version 6.3.4 (Perkin-Elmer, Waltham, MA, USA) was used. For fecal dry mass quantification, 200 mg fecal samples were weighed and dried for 12 h at 103 °C. SCFA data are expressed in relation to dry mass and identified by comparing the retention times of the respective peaks in the sample and standard chromatograms.

4.11. Analysis of Intestinal Permeability Markers Plasma Lipopolysaccharide-Binding Protein (LBP) and Fecal Zonulin

Both zonulin and LBP were measured using commercial enzyme-linked immunosorbent assay kits (K5600, KR6813, Immundiagnostik AG, Bensheim, Germany) following the manufacturer's protocols. The fecal samples were diluted to the working concentration in sample buffer using stool sample tubes (K6998SAS; Immundiagnostik AG, Bensheim, Germany) and for LBP analysis, 10 µL of blood plasma was used and processed as described [32].

4.12. Gut Microbiome Analysis

Bacterial DNA was extracted from fecal samples using the QIAamp Fast DNA Stool Mini Kit (Cat. No. 51604, Germantown, MD, USA) following the manufacturer's protocol. 16S Ribosomal RNA (rRNA) gene amplicons of 2 × 300 bp length were sequenced on the MiSeq platform at the University of Minnesota Genomics Center, targeting the V5–V6 regions using the primers V5F and V6R. For evaluation, the DADA2 and QIIME2 pipelines were used. First, paired-end reads were merged, demultiplexed, and quality control was conducted (length = 250 bp, mean sequence quality score ≥ 30). We refer to these adapted paired-end reads as amplicon sequences variants (ASVs). Samples with sequencing read below 8080 were excluded, resulting in 18 individuals within PT and FO and 9 individuals within fish intervention which were included in the microbiome analyses. After that, the data was converted into relative abundance and below 0.15% in all samples were removed. This resulted in 102 ASVs. Microbial alpha and beta-diversity were measured and taxonomic categories from the phylum to species (ASV) levels were categorized using a pre-trained Naive Bayes classifier 2 based on Silva 138 99% OTUs database [65].

4.13. Statistical Analyses

First, all parameters were tested for normal distribution using the Kolmogorov-Smirnov test. For parametric parameters a one-way ANOVA was conducted with the Geisser-Greenhouse correction and for multiple comparisons between visits (baseline, pre-

and post-intervention) within one intervention (PT, FO, fish) the Tukey test was used. Non-parametric parameters were tested with the Friedman test and, as a post hoc test, Dunn's multiple comparison test. To compare different interventions at given time points the two-tailed paired *t*-test was done for parametric variables and the paired Wilcoxon-matched-pairs-rank-test was used for non-parametric variables. Gut barrier marker, SCFA, and gut microbiome were measured at two time points and were also analyzed by the two-tailed paired t-test or the paired Wilcoxon-rank-test. For Fish pre phase, no stool samples were given, Fishpre represents the value at pre-intervention 2 before the fish phase- either FOpre (n = 5) or PTpre (n = 4) of the 9 participants. All data were analyzed per protocol with the exclusion of drop-outs after interventions 1 and 2. All statistical analyses were performed using GraphPad Prism version 9.0.1.

5. Conclusions

In conclusion, the study shows that PT is safe to consume for humans. EPA and FX are accessible from PT without processing the biomass, other than ball milling. In particular, the *n*-3 PUFA, and especially EPA, were absorbed in similar levels as those obtained from FO and fish. The carotenoids β-carotene and FX were also well absorbed, and the metabolites of FX were measured at higher concentrations in plasma than FX itself. This suggests that PT could serve as a novel food source in the future. Other potentially valuable components, such as proteins, need to be investigated in future studies. The taste and texture of the microalgae product need to be further improved for bringing this sustainable food source to the market.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/md19120700/s1, Table S1: Nutrient composition of PT, FO and fish (salmon) used in the study; Table S2: Plasma fatty acids concentrations during each of the experimental phases (in percent %); Table S3: Bacterial taxa in feces at pre- and post-intervention.

Author Contributions: Conceptualization, L.S. and S.C.B.; Methodology and Analysis, L.S., K.L., A.M.-A.; Formal Analysis, L.S.; Investigation, all; Resources, K.F., U.S.-S.; Writing—Original Draft Preparation, L.S.; Writing—Review & Editing, S.C.B.; Visualization, J.F., W.V., U.S.-S.; Project Administration, L.S.; Funding Acquisition, S.C.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry for Science, Research and Art within the Bioeconomy research Program of Baden-Württemberg, grant number BÖBW2-105A, FKZ-7533-10-5-185B.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the local Ethical Committee (Ethik-Kommission der Landesärztekammer Baden-Württemberg (F-2020-00; 28 February 2020) and was registered at ClinicalTrials.gov (NCT04288544).

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

Data Availability Statement: Data are available upon justified request to the corresponding author.

Acknowledgments: Stiefvatter L. and Frick K. are supported by the bioeconomy graduate program BBW ForWerts supported by the Ministry for Science, Research and Art, Baden-Württemberg.

Conflicts of Interest: The authors declare no conflict of interest related to the study.

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2.2 Überprüfung der sicheren Einnahme von zwei unterschiedlich kultivierten PT-Biomassen und Untersuchung von möglichen darmgesundheitlichen Vorteilen in Mäusen

Durch die Anpassung der Kultivierungsbedingungen der Mikroalge PT können unterschiedliche Nährstoffzusammensetzungen der Biomasse erreicht werden, die verschieden als Nahrungsquelle eingesetzt werden können. Wir haben zwei Varianten von Biomasse generiert, eine EPA/Fx-reiche und eine Chrl-reiche Biomasse. Die Einnahme der EPA/Fx-reichen Biomasse kann als eine zusätzliche EPA-Quelle verzehrt werden. Dies könnte gesundheitliche Vorteile haben, beispielsweise aufgrund der bekannten entzündungshemmenden und antioxidativen Wirkungen von EPA [127], womit die Darmgesundheit unterstützt werden kann [136,137]. Auch die Chrl-reiche Biomasse bietet aufgrund ihrer Nährstoffzusammensetzung großes Potenzial als zusätzliche Ballaststoffquelle genutzt zu werden. Zudem könnten durch immunmodulatorischer und antioxidativer Wirkungen Chrl als Gegenmaßnahme bei Darmentzündungen eingesetzt werden [112]. Beide Biomassen bieten daher ein großes therapeutisches Potenzial und könnten zusätzlich für die Deckung des Nährstoffbedarfs verwendet werden. Dieser Hypothese wurde im zweiten Promotionsprojekt nachgegangen. Das Ziel war es die beiden generierten PT-Biomassen an Mäusen in einem 14-tägigen Fütterungsversuch bezüglich der potenziellen darmgesundheitlichen Vorteile zu untersuchen. Zudem sollte die unbedenkliche Einnahme höherer Dosierungen beider kultivierten PT-Biomassen überprüft werden. Die Tiere erhielten eine Kontrolldiät oder eine der PT-Diäten. Die EPA/Fx-reiche Biomasse und die Chrl-reiche Biomasse wurde dem Futter der Mäuse in gefriergetrockneter Form zu 5, 15 bzw. 25 wt% zugesetzt. Es wurden histologische Untersuchungen durchgeführt, Leberfett und FS im Fettgewebe gemessen sowie Darmbarrieremarker, Entzündungsmarker, kurzkettige FS und das Darmmikrobiom sequenziert. Beide Mikroalgen-Diäten erhöhten die Produktion ausgewählter kurzkettiger FS und verringerten das Firmicutes/Bacteriodota-Verhältnis. Nach der Chrl-reichen Diät kam es zu einem Anstieg von *Akkermansia* in gesunden Mäusen. Algenmengen mit einem Gehalt von bis zu 4621 mg Chrl, 920 mg EPA und 231 mg Fx pro kg Körpergewicht täglich wurden ohne Nebenwirkungen vertragen. Diese vorklinische Studie zeigt, dass PT für die Fütterung von Nagern geeignet ist, mit positiven Auswirkungen auf die Zusammensetzung der Mikrobiota und die kurzkettige FS-Produktion, was auf eine präbiotische Wirkung durch den PT-Verzehr schließen lässt. Der Artikel wurde im Juni 2022 von der Fachzeitschrift Nutrients akzeptiert und publiziert [138].

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The Microalgae *Phaeodactylum tricornutum* Is Well Suited as a Food with Positive Effects on the Intestinal Microbiota and the Generation of SCFA: Results from a Pre-Clinical Study

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Abstract: Microalgae such as *Phaeodactylum tricornutum* (PT) are a sustainable source of nutrients, especially eicosapentaenoic acid (EPA), fucoxanthin (Fx), and chrysolaminarin (Chrl), the concentrations of which can vary depending on the culture conditions. We generated three types of diets containing either an EPA- and Fx-rich (EPA/Fx) or Chrl-rich microalgae (with 5, 15, or 25% added to the diet) or an isocaloric control diet (CD). These diets were evaluated over 14 days in young C57BL/6J mice for safety and bioavailability, short-chain fatty acid (SCFA) production, and microbiome analysis. Both microalgae diets increased body weight gain dose-dependently compared to the CD. Microalgae-derived EPA was well absorbed, resulting in increased liver and fat tissue levels and a decrease in the n-6:n-3 ratio in liver tissue. Both microalgae diets increased the production of selected SCFA and decreased the Firmicutes/Bacteriodota ratio, whereas the Chrl-rich diet led to an increase in *Akkermansia*. Doses of up to 4621 mg Chrl, 920 mg EPA, and 231 mg Fx per kg body weight daily were tolerated without adverse effects. This pre-clinical study shows that PT is suitable for mouse feed, with positive effects on microbiota composition and SCFA production, suggesting beneficial effects on gut health.

Keywords: *Phaeodactylum tricornutum*; eicosapentaenoic acid; fucoxanthin; β-glucan; chrysolaminarin

Citation: Stiefvatter, L.; Neumann, U.; Rings, A.; Frick, K.; Schmid-Staiger, U.; Bischoff, S.C. The Microalgae *Phaeodactylum tricornutum* Is Well Suited as a Food with Positive Effects on the Intestinal Microbiota and the Generation of SCFA: Results from a Pre-Clinical Study. *Nutrients* **2022**, *14*, 2504. https://doi.org/10.3390/nu14122504

Academic Editor: Yoshitaka Hashimoto

Received: 25 May 2022
Accepted: 14 June 2022
Published: 16 June 2022

1. Introduction

Climate change will alter the quantity and quality of food. Consumption and production patterns need to be adapted in a sustainable way to achieve the 2030 sustainable development goals. One aim is the sustainable use of the oceans [1]; therefore, a change in fishing practice needs to be undertaken. Microalgae could be used as an alternative raw material; they can be grown in bioreactors without farmland and provide a nutrient-rich alternative to high-value compounds, such as proteins and fatty acids [2]. In particular, the diatom PT seems to be interesting for human nutrition. It is a unicellular microalga living in water and containing high amounts of protein, the carotenoid Fx, EPA, an omega-3 polyunsaturated fatty acid (n-3 PUFA), and Chrl, a β-glucan [3]. It can be cultivated at different salt concentrations (marine, brackish, and even lower (<10 gNaCl L^{-1}). Under nutrient-repleted growth conditions, PT generates large amounts of Fx and EPA.

Fx is synthesized by photosynthetic organisms for photoprotection and light harvesting and belongs to the xanthophylls. It is a significant carotenoid in brown (micro)algae and seaweeds [4,5]. Fx accumulation in the liver and adipose tissue [6] in mice was previously demonstrated, as well as metabolization and safe intake in humans [7]. Due to its

antioxidant and anti-inflammatory properties [8], as well as anti-obesity effects [9], Fx is already being investigated for health benefits [10]. Microalgae are generally the primary producers of EPA and docosahexaenoic acid (DHA). However, the microalga PT, which was used in this study, mainly produces EPA [11]. Its biomass composition varies according to species and environmental factors [12]. EPA and DHA are important for human nutrition because the essential fatty acid (FA) α-linolenic acid (ALA) is insufficiently converted into EPA and DHA [13]. The direct consumption of 250–300 mg EPA+DHA daily or consumption of fish one to two times per week is recommended [14], as the immune mediators produced from DHA and EPA shift the balance towards anti-inflammation [15]. Because fish only acquire n-3 PUFAs by eating microalgae, the direct consumption of microalgae is a sustainable alternative [16]. *Schizochytrium* sp. and *Ulkenia* sp. oils are already approved as DHA sources for human consumption. The microalga *Odontella aurita*, rich in EPA, is also approved as whole microalga but only in small amounts (0.5–1.5%) for flavoring [17]. For health benefits, more significant amounts of microalgal biomass in human nutrition need to be investigated. PT is not yet approved as a whole microalga, although its nutritional composition is similar to *Odontella aurita* [18]. In the USA, an EPA-rich oil from PT is already on the market; in Europe, the product is still in the approval process by the European Food Safety Authority (EFSA). Eating this microalga after cell disruption by a ball mill has already been shown to result in good bioavailability of EPA and accumulation in mouse liver tissue [19] and human plasma [7]. A further component of PT biomass is Chrl, a water-soluble β-(1,3)/β-(1,6 β−glucan (11:1) that is accumulated from PT under nutrient-depleted cultivation conditions [11]. It serves as primary energy and carbon storage and is dissolved in the cytosol [20]. Macroalgae, such as brown algae of the genera *Laminaria* and *Saccharina*, possess β-glucan laminarin, which is very similar to Chrl, with a β-(1,3)/β-(1,6) glucan structure (15:1) [21], and can be used in animal feed [22]. Other sources of β-glucans include grain, fungi, bacteria, and yeast [23]. They are already used in foods, medicine, and cosmetics [24] due to nutraceutical functions, such as antitumor, anti-inflammatory, antioxidant, anticoagulant, and immunostimulant effects [23,25]. Similar properties have already been reported for laminarin [26], Chrl [27,28], and Chrl from PT [29,30]. Due to the various ingredients of PT and their health-promoting properties, it is suspected to promote intestinal health. These include the formation of short-chain fatty acids (SCFA), the promotion of SCFA-producing bacteria, and strengthening of the intestinal barrier [31–33].

The current study evaluates the difference and safety of two different biomass samples of the microalga PT. An EPA- and Fx-rich (EPA/Fx) and Chrl-rich biomasses (Chrl-rich) were added at doses of 5%, 15%, and 25% to the feed of adult female C57BL/6 mice for 14 days and compared to a PT-free control diet (CD). Diets were evaluated for safety aspects, such as feed consumption, energy in feces, fatty acid content, and gut health benefits, such as gut intestinal permeability. Therefore, the relative expression of zonula occludens protein-1 (ZO1), which activates the tight junctions (TJ) and occludin, a tight junction protein of the intestinal barrels that keep the TJ closed, are measured in the ileum. In feces, SCFA and the gut microbiome were analyzed by 16S ribosomal RNA sequencing.

2. Materials and Methods

2.1. Phaeodactylum Tricornutum Culture and Experimental Diets

In this study, PT biomass was used from the strain PT SAG 1090-1b. This biomass was produced at the Fraunhofer CBP (Leuna, Saxony-Anhalt, Germany) using 180 L flat-panel airlift (Subitec GmbH, Stuttgart, Germany) reactors under outdoor conditions. Two batches of the biomass (Fx/EPA-rich and Chrl-rich) were produced under cultivation conditions. The EPA/Fx-rich biomass was produced under nutrient-repleted conditions. In contrast, the Chrl-rich biomass was cultivated under nitrogen-depleted conditions for several days before the harvest. After the harvest, both batches of biomass were treated the same. First, the biomass was concentrated to approximately 250 g L^{-1} via centrifugation (Clara 20, Alfa Laval, Glinde, Germany) and stored at −20 °C until further processing. After

thawing, the biomass was diluted with deionized water to 100 g L^{-1}. Subsequently, the cells were disrupted in a ball mill (PML-2, Bühler AG, Uzwil, Switzerland) according to Derwenskus et al. [34], freeze-dried, and pulverized as described previously [7]. The feed manufacturer, sSniff Spezialdiäten GmbH (ssniff Spezialdiäten GmbH, Soest, Germany) added both versions of PT at concentrations of 5%, 15%, and 2% (Table 1) to the diets, which were isocaloric and isoproteinogenic to the CD. The Fx content in chow was measured at the Fraunhofer IGB by high-performance liquid chromatography (HPLC) as described by Derwenskus et al. [34].

Table 1. Food composition of different diets.

Treatment	Suppl	ME	SFA	UFA	PUFA	MUFA	EPA	n-3:n-6	Fx	β-Carotin	Chrl
	[%]	[MJ/kg]	[g/kg]	[g/kg]	[g/kg]	[g/kg]	[g/kg]		[g/kg]	[g/kg]	[g/kg]
Control diet		15.6	9.33	11.18	3.60	7.58	0.00	0.08	0	0	0
PT_Chrl	5	15.6	16.98	30.83	51.45	21.59	0.00	0.10	0.14	0.02	10.92
	15	15.6	19.24	43.19	45.57	24.68	5.11	0.31	0.42	0.05	32.77
	25	15.6	19.11	49.58	27.50	24.41	8.32	0.90	0.71	0.09	54.62
PT_EPA/Fx	5	15.6	20.49	33.81	68.14	24.534	3.32	0.16	0.57	0.07	0.66
	15	15.6	22.44	52.96	72.56	26.68	11.14	0.30	1.71	0.22	1.98
	25	15.6	21.23	53.57	60.74	24.01	15.17	0.47	2.85	0.37	3.3

Measured fatty acids, carotenoids, and Chrl within the diet pellets and other parameters are available in [19]. Abbreviations: Suppl, supplementation; ME, metabolizable energy; SFA, saturated fatty acids; UFA, unsaturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; EPA, eicosapentaenoic acid; n-3:n-6, omega-3-to-omega-6 ratio; Fx, fucoxanthin; Chrl, chrysolaminarin belonging to the β-glycans; PT, microalgae *Phaeodactylum tricornutum*; PT_ Chrl, diet with Chrl-rich PT, PT_EPA/Fx, diet with EPA- and Fx-rich PT. Fatty acids were measured in chow by gas chromatography. Fx was measured by high-performance liquid chromatography.

2.2. Mouse Feeding Experiment

A total of 56 6- to 8-week-old female C57BL/6J mice were obtained from the animal care unit of the University of Hohenheim, Germany. The experiment was set up as described by Neumann et al. [19]. All experiments were approved by the local Institutional Animal Care and Use Committee (Regional Council Stuttgart, permit number: V326-15EM). The mice were divided into seven groups, with eight animals each supplemented with a CD diet; a Chrl-rich diet at concentrations of 5, 15, and 25%; and an EPA/Fx diet at concentrations of 5, 15, and 25%. Clinical health scores were assessed daily, and animals were weighed every three days. On days 9 and 14, the animals were housed solitary in metabolic cages (Tecniplast S.p.A, Buguggiate, Italy) to collect 24 h feces and determine food intake. Feces from day 9 were used for energy calculation, and feces from day 14 were used for SCFA measurement and 16-S gut microbiome sequencing. Mice were sacrificed, and organs (spleen, liver, and colon) were removed, weighed, and stored at −80 °C or in 4% PBS-buffered formalin solution (Carl Roth GmbH & Co., Karlsruhe, Germany).

2.3. Histological Analyses

Histological analysis was performed as described by Neumann et al. [19]. Formalin-fixed tissue samples of the 15% and 25% dosage diets, as well as the CD, were embedded in paraffin using hematoxylin-eosin (Sigma Aldrich, Schnelldorf, Germany) staining and analyzed. Samples of the ileum and colon were analyzed for cell infiltration (score 0–3) and tissue damage (score 0–3), and livers were scored from 0 to 3 for steatosis, infiltration, and tissue damage. The thickness of the muscularis externa was measured from captured images of the colon and ileum using the scaled ocular (20×) Axio Vision Rel. 4.8 software (magnification 200×, Zeiss, Oberkochen, Germany).

2.4. Measurement of Liver Fat Content

The fat content in the liver was measured by Folch extraction but with modifications, as described by Gille et al. [35].

2.5. Fatty Acid Analyses in Mouse Tissues and Chow

Liver and fat in white adipose tissue (WAT), especially inguinal (subcutaneous) WAT (iWAT) and epididymal (visceral) WAT (eWAT), were homogenized in 200 µL methanol with a TissueRuptor (Qiagen, Hilden, Germany). Furthermore, fatty acids were analyzed in the chow, the homogenized liver, and fat tissue as described in [36] using an Agilent 7890A (Agilent, Santa Clara, CA, USA) gas chromatograph with a Supelco SBP-PUFA 30 m × 0.32 mm × 0.2 µm column (Sigma-Aldrich, Schnelldorf, Germany) and an FID detector. Results were compared to a certified C4–C24 FAME mix (Supelco-18919-1AMP, Sigma-Aldrich, Schnelldorf, Germany). The analyses covered saturated fatty acids (C 14:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0, and C24:0) and unsaturated fatty acids (C 14:1, C15:1, C16:1n7, C17:1, 18:1n9c, -t, C18:2n6c, -t, 18:3n6, C18:3n3, C20:1n9, C20:2n6, C20:3n6, C20:5n3, C20:4n6, C22:1n9, C22:2n6, C22:3n6, and C24:1n9). For the measurement of the n-6:n-3 ratio, the totals of all n-6 PUFAs and n-3 PUFAs were calculated and divided by one another.

2.6. Real-Time Quantitative Reverse Transcription PCR

RNA was extracted from ileum tissue (50–100 mg) using peqGOLD TriFast (PEQLAB, Erlangen, Germany) according to the manufacturer's instructions, and 1 µg of RNA was transcribed into cDNA with SuperScript® IV Reverse-Transcriptase (Thermo FisherScientific, Darmstadt, Germany) after DNAse treatment (Promega, Madison, WI, USA). For the real-time PCR, Eva Green Universal PCR master mix (Bio-Rad Laboratories, Munich, Germany) was used to prepare the PCR mix as described in more detail by Zimmermann et al. [37]. The primers are shown in Table 2; the amplification programs for primers were as follows: Tumor necrosis factor-α (TNFα): 95 °C for 3 min, 39 cycles at 95 °C for 5 s, and 60 °C for 10 min; zonula occludens-1 (ZO1), occludin, interleukin (IL)-1β: 95 °C for 3 min, 40 cycles at 95 °C for 5 s, and 60 °C for 10 s. IL-6: 95 °C for 3 min, 45 cycles at 95 °C for 5 s, and 60 °C for 10 s. The comparative CT method was used to determine gene quantity as described in [37].

Table 2. Primer sequences used in quantitative real-time PCR.

Primer	Forward (5′-3′)	Reverse (5′-3′)
Occludin	ACTCCTCCAATGGACAAGTG	CCCCACCTGTCGTGTAGTCT
ZO1	CCACCTCTGTCCAGCTCTTC	CACCGGAGTGATGGTTTTCT
TNFα	ACCACCATCAAGGACTCA	AGGTCTGAAGGTAGGAAG
IL-1β	ACGGATTCCATGGTGAAGTC	GAGTGTGGATCCCAAGCAAT
IL-6	AGTCACAGAAGGAGTGGCTA	CTGACCACAGTGAGGAATGT

Abbreviations: ZO1, zonula occludens-1; TNFα, tumor necrosis factor-α; IL-1β, inflammatory-1β; IL-6, inflammatory-6.

2.7. Fecal Energy Loss and Fecal Energy Ratio

Energy was measured and energy loss was calculated with samples from day 9 as previously described [19]. The fecal energy ratio was calculated by dividing the intake (feed intake in calories) by the ingested energy at day 9.

2.8. SCFA Analysis

For SCFA analysis, a minimum of 200 mg of feces was used, following the method described in [7]. However, no dry mass of feces could be determined due to the low amount available. Thus, the SCFA concentrations are given as wet fecal mass. Due to the small masses of feces collected in the cages, some samples could not be measured as the biomass was used as well for microbiota analysis (CD n = 5; Chrl5 n = 7, Chrl15 n = 6, Chrl25 n = 3, EPA/Fx5 n = 7, EPA/Fx15 n = 2, EPA/Fx25 n = 6).

2.9. Gut Microbiota Analysis

For 16S ribosomal ribonucleic acid (rRNA) gene sequencing, feces samples from day 14 after the start of experimental feeding were used, bacterial DNA was extracted, and 16S Ribosomal RNA (rRNA) gene amplicons of 2 × 300 bp length were sequenced on the MiSeq platform at the University of Minnesota Genomics Center following a protocol similar to that described in [7]. Samples with sequencing reads below 10.000 were excluded, resulting in CD (n = 5), Chrl5 (n = 8), Chrl15 (n = 7), Chrl25 (n = 8), EPA/Fx5 (n = 4), EPA/Fx15 (n = 6), and EPA/Fx25 (n = 6), which were included in the microbiome analysis. Microbial alpha and beta diversity and taxonomies from phylum to species were assigned. Before analysis, data were converted into relative abundance, and values below 0.15% were in all samples. This resulted in 151 operational taxonomic units (OTUs).

2.10. Statistical Analyses

All parameters were tested for normal distribution using the Kolmogorov–Smirnov test; for normally distributed data, one-way ANOVA was used to compare statistically significant differences ($p < 0.05$) between microalga diet groups and the CD. Variances were tested with the Brown–Forsythe test. Tukey's multiple comparison post hoc test was used for equal variances, and for unequal variances, Dunnett's T3 multiple comparisons test was used. Correlation analyses were performed with two-tailed Spearman-rank correlation, with coefficients in the range of 0.0 to 0.3 (0.0 to −0.3) interpreted as a negligible correlation, whereas correlations in the range of 0.3 to 0.5 or −0.3 to −0.5 were regarded as positive or negative correlations, respectively. All statistical analyses were performed using GraphPad Prism version 9.3.1 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Microalgae Diet Acceptance and Effects on Body Weight (Bw) and Energy Uptake

Feed consumption varied between 2.5 and 2.6 g/day (Figure 1A), and the caloric intake (Figure 1B) was similar in all diets. Both PT diets increased the bw gain ($p < 0.001$), especially in higher doses compared to the PT-free CD (Figure 1C). Only the EPA/Fx5 diet, which did not induce weight gain, increased liver fat compared to the CD ($p = 0.001$; Figure 1D). Fecal energy loss measured on day 9 was lower in the 5 and 15% Chrl-rich diet groups and the 25% EPA/Fx diet group compared to the CD group ($p = 0.01$; Figure 1E). Figure 1F shows a higher energy ratio associated with the Chrl5-rich and −15 diets ($p = 0.001$) compared to the CD. Further analyses, including the weight of organs (not shown) and histological analyses, showed no differences in the CD-fed mice (see Table S1).

3.2. Short-Chain Fatty Acids, Markers of Intestinal Permeability, and Inflammation

Both microalgae diets caused increased production of SCFA in the feces of mice. The increase was less consistent in butyric acid in association with the Chrl-rich15 diet ($p < 0.001$) and occurred only in trend associated with the EPA/Fx25 ($p = 0.1$) diet compared to the CD (Figure 2A). Acetic acid increased the most in association with PT-rich diets compared to the CD ($p < 0.001$) (Figure 2B). Propionic acid increased only in association with the 25% EPA/Fx diet ($p = 0.04$) (Figure 2C), and valeric acid showed an increasing trend in association with the microalgae diets compared to the CD ($p = 0.01$) (Figure 2D). For intestinal permeability, the relative expressions of zonula occludens protein-1 (ZO1) and occludin were measured in the ileum, and no differences were observed. The proinflammatory tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-1β genes were measured in the ileum. Only TNF-α mRNA expression was higher ($p < 0.001$) in association with the lowest-dose Chrl-rich diet and the two highest-dose Chrl25-rich and EPA/Fx25 diets compared to the CD (Table 3).

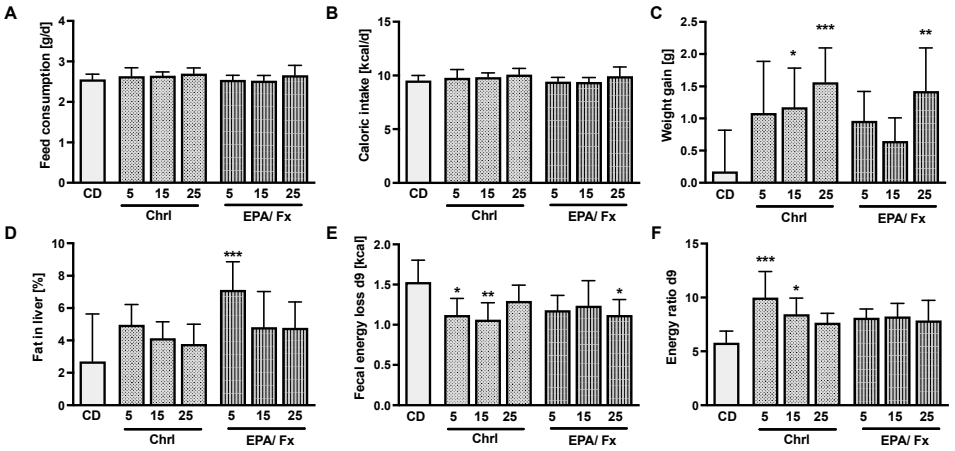

Figure 1. Daily feed (**A**) and caloric intake (**B**) of C57BL/6J mice, body weight gain within 14 days (**C**), and the percentage of liver fat content at day 14 (**D**). The fecal energy loss on day 9 was measured with a calorimeter (**E**), and the energy ratio of day 9 was calculated by dividing the feed intake on day 9 by the fecal energy loss on day 9 (**F**) of C57BL/6J mice. Concentration values are expressed as mean ± SD from $n = 8$ samples per group. Diets supplemented with concentrations of 5%, 15%, and 25%. Statistics: * indicates differences relative to CD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Abbreviations: CD, control diet; EPA, eicosapentaenoic acid; Fx, fucoxanthin; Chrl, diet with chrysolaminarin-rich PT; EPA/Fx, diet with EPA- and Fx-rich microalgae PT; d, day.

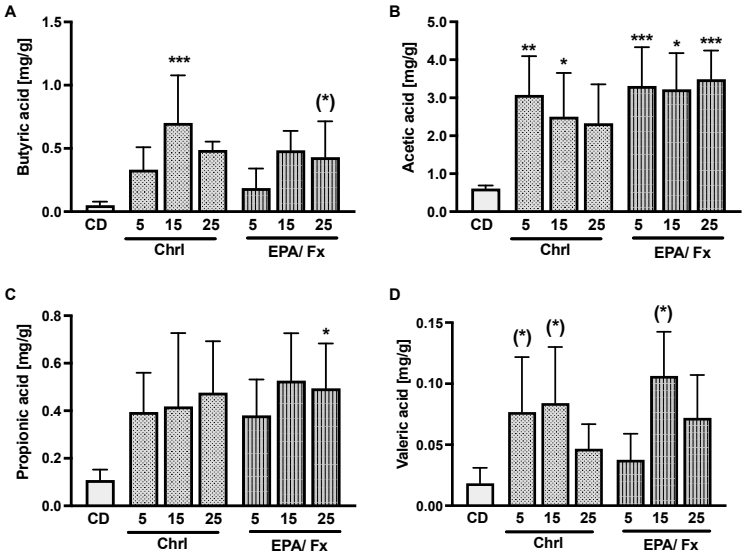

Figure 2. The microalgae diets increased the content of SCFAs, such as butyric acid (**A**), acetic acid (**B**), and propionic acid (**C**), with an increasing tendency of valeric acid (**D**), in feces after supplementation with both versions of the microalgae as measured on day 14 by gas chromatography. Data are expressed as mean ± SD (CD $n = 5$; Chrl5 $n = 7$, Chrl15 $n = 6$, Chrl25 $n = 3$, EPA/Fx5 $n = 7$, EPA/Fx15 $n = 2$, EPA/Fx25 $n = 6$). Statistics: * indicates differences from CD. (*) $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Abbreviations: see Figure 1.

Table 3. Intestinal and inflammatory markers measured in the ileum tissue.

Treatment	Suppl [%]	ZO1	Occludin	TNF-α	IL-6	IL-1β
CD		29.94 ± 15	39.17 ± 27	10.06 ± 8.6	49.75 ± 82	5.850 ± 2.8
Chrl	5	59.92 ± 30	46.56 ± 24	69.87 ± 28 **	148.8 ± 127	25.23 ± 15
	15	25.67 ± 21	28.50 ± 18	22.58 ± 17	113.2 ± 135	14.18 ± 14
	25	25.31 ± 26	48.57 ± 40	118.5 ± 94 **	8.526 ± 8	29.60 ± 49
EPA/Fx	5	24.15 ± 22	39.07 ± 26	32.88 ± 26	244.8± 344	18.73 ± 14
	15	16.90 ± 8	27.18± 15	27.57± 18	93.87 ± 129	7.355 ± 5.3
	25	96.19 ± 161	75.82 ± 105	62.58± 40 *	152.3 ± 99	32.19 ± 48

Increase in TNF-α associated with Chrl5/25 and EPA/Fx25 diets compared to CD analyzed by ANOVA. Diets supplemented with 5%, 15%, and 25%. Statistics: * indicates differences relative to CD. * $p < 0.05$, ** $p < 0.01$. Abbreviations: CD, control diet; Suppl, supplementation; ZO1, zonula occludens-1; TNF-α, tumor necrosis factor-α; IL-6, inflammatory-6; IL-1β, inflammatory-1β; for other abbreviations, see Table 1 and Figure 1.

3.3. Microbiome Analysis in Feces

Gut microbiome analysis showed no changes in β-diversity as measured by the Bray–Curtis distance and no effect on α-diversity compared to the CD (Figure 3A,B). Taxonomies changed between the microalgae diets and the CD from phylum to species. At the phylum level, Bacteriodota, Firmicutes, Desulfobacterota, Verrucomicrobiota, Cyanobacteria, and Proteobacteria were the most dominant bacteria and changed compared to the CD. The Firmicutes-to-Bacteroidota (F/B) ratio led to a significant reduction in association with all microalgae diets compared to the CD ($p < 0.001$; Figure 3C). In detail, the relative abundance of Bacteroidota was higher in association with all microalgae diets except for the Chrl25-rich diet ($p < 0.001$) (Figure 3D), and a lower abundance was demonstrated for the Firmicutes in association with all microalgae diets compared to the CD ($p < 0.001$). Further changes are shown in Figure 3D and Table S2. At the class level, the abundance of *Verrucomicrobiae* was higher following higher doses of Chrl15-rich and -25 ($p < 0.001$) diets compared to the CD (Figure 3E; other results see Table S2). The order level showed a reduction in *Lachnospirales* after higher doses of both PT biomass supplementations ($p < 0.01$; Table S2). At the genus level, results consistently showed a higher abundance of *Clostridia vadinBB60* in association with both highest-dosed Chrl25 and EPA/Fx15 and 25 diets ($p < 0.001$) compared to CD (Figure 3F). *Akkermansia* abundance was higher in association with both higher-dosed Chrl-rich diets ($p < 0.001$) compared to the CD (Figure 3G). The results were less consistent for *Alistipes*, which presented with higher abundance only in association with the lowest Chrl-rich supplementation ($p = 0.002$) and all EPA/Fx diets ($p < 0.04$) compared to the CD (Figure 3H). At the species level, the abundance of *Cyanobacteriia Chloroplast* was higher in association with all Chrl-rich diets compared to the CD and EPA/Fx diets ($p < 0.001$; Figure 3I). The abundance of *Muribaculaceae*;s_*unidentified* was increased in association with all Chrl-rich ($p = 0.003$; Figure 3J) diets. For more details, see Table S2.

3.4. Fatty Acids Measured in the Liver and the White Adipose Tissue (WAT)

Fatty acids were measured in the liver and WAT to compare their bioavailability and differences in absorption levels. Both microalgae diets showed a lower monounsaturated fatty acid (MUFA) concentration ($p < 0.001$) (Figure 4A) and a lower n-6: n-3 ratio in the liver at higher doses compared to the CD ($p < 0.001$; Figure 4B). The PUFA concentration did not show any differences in the liver (Figure 4C) and iWAT (Figure 4E) but higher concentrations in eWAT in association with the EPA/Fx diet compared to the CD ($p = 0.001$; Figure 4G). EPA concentrations increased dose-dependently in the liver ($p < 0.001$; Figure 4D) and fat tissue compared to the CD (Figure 4F,H). Following ingestion of the EPA/Fx diet with higher EPA doses, higher amounts were measured in the liver and eWAT compared to the Chrl diet (Figure 4D,H).

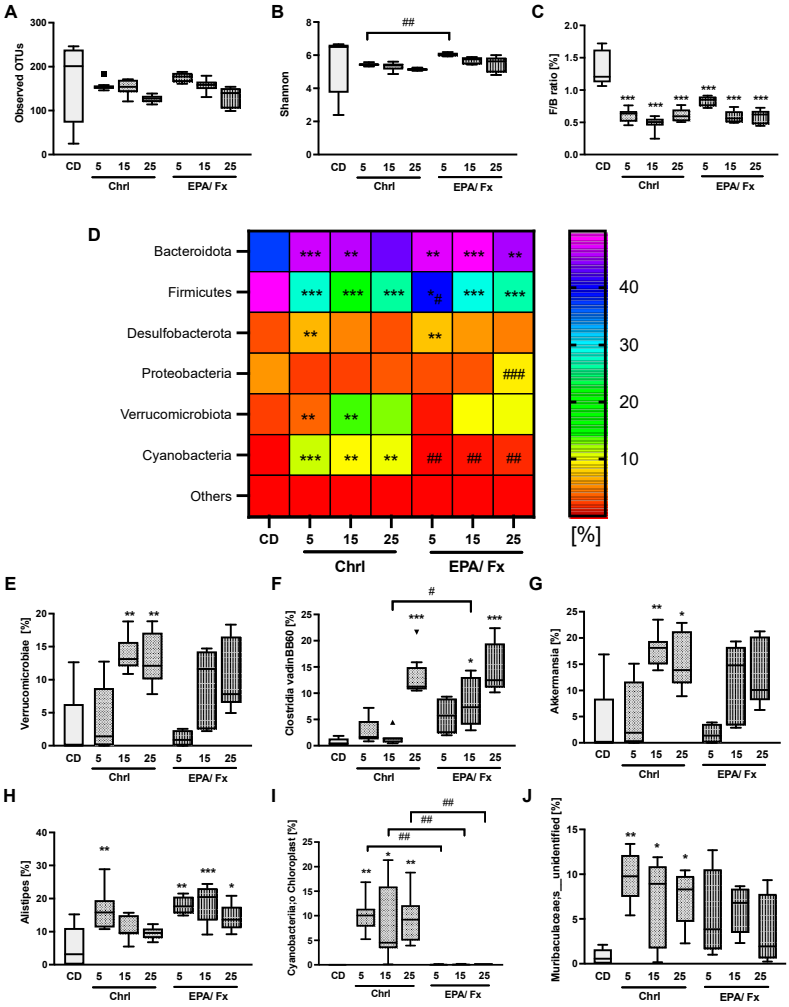

Figure 3. Both PT diets effected changes in the gut microbiome after 14 days of supplementation compared to the CD. The α-diversity, i.e., the species richness (observed OUTs, (**A**)), and the species diversity, i.e., the Shannon index (**B**) were not affected. At the phylum level, the F/B ratio was lower in association with PT diets (**C**), and the relative bacterial abundances [%] of Bacteroidota, Firmicutes, and others changed in association with PT diets compared to the CD (**D**). At the class level, the abundance of *Verrucomicrobiae* was higher in association with Chrl-rich diets (**E**). At the genus level, the abundance of *Clostridia_vadinBB60* (**F**), *Akkermansia* (**G**), and *Alistipes* changed compared to the CD (**H**). The abundances of Cyanobacteriia chloroplast (**I**) and Muribaculaceae at the species level were higher in association with Chrl-rich diets (**J**). Further results are presented in Supplementary Table S2. Data are shown as box plots with median, 25%, and 75% percentiles and a heat map of the phylum as relative bacterial abundances [%] (CD $n = 5$; Chrl5 $n = 8$; Chrl15 $n = 7$; Chrl25 $n = 8$; EPA/Fx5 $n = 4$; EPA/Fx15 $n = 6$; EPA/Fx25 $n = 6$). Statistics: * indicates differences relative to CD, and # indicates the difference between Chrl and EPA/Fx diets. $*/^{\#}$ $p < 0.05$, $**/^{\#\#}$ $p < 0.01$, $***/^{\#\#\#}$ $p < 0.001$. Abbreviations: OTUs, operational taxonomic units; F/B, Firmicutes/Bacteroidota ratio; for other abbreviations, see Table 1 and Figure 1.

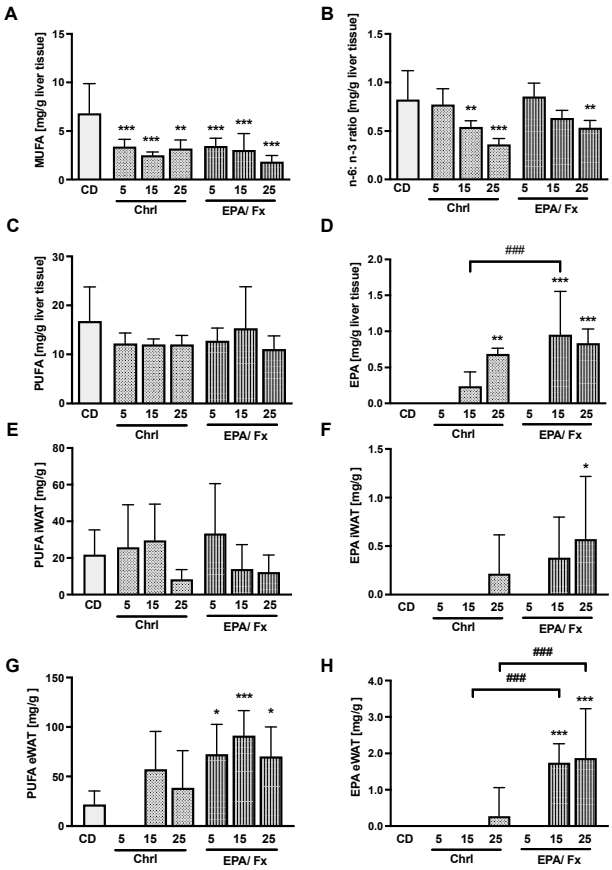

Figure 4. Fatty acids were measured by gas chromatography in the liver (**A**–**D**), subcutaneous WAT (**E**,**F**), and visceral WAT (**G**,**H**). MUFA amounts were lower in association with all microalgae diets (**A**), and the ratio of n-6-to-n-3 PUFA (**B**) decreased in association with higher supplementation of the two microalgae diets. PUFA content was increased in eWAT (**G**) in association with the EPA/Fx diets, and EPA content increased in all tissues with higher supplementation of the EPA/Fx diets and in the liver in association with the Chrl25 diet (**D**,**F**,**H**). Data are expressed as mean ± SD (n = 8 per group). Statistics: * indicates difference relative to CD, and $^{\#}$ indicates the difference between Chrl and EPA/Fx diets. * $p < 0.05$, ** $p < 0.01$, *** $/^{\#\#\#}$ $p < 0.001$. Abbreviations: WAT, white adipose tissue; iWAT, inguinal (subcutaneous) WAT; eWAT; epididymal (visceral) WAT; see Table 1 and Figure 1 for other abbreviations.

3.5. Correlations

For correlation analysis, the daily Chrl, Fx, and EPA intakes were calculated separately in mg/day and mg/g bw per day and correlated with fecal SCFA levels, the n-6:n-3 ratio in the liver, and the most important bacteria at different levels (Figure 5). The microalgae and EPA intake correlated with all SCFA amounts measured in feces. The daily Fx intake correlated with all SCFA levels except valeric acid, and the Chrl uptake expressed in mg/day correlated with SCFA levels except acetic acid. The Chrl intake expressed in mg/g bw correlated with butyric and valeric acid fecal concentrations. All intakes correlated negatively with the n-6:n-3 ratio in the liver. The intake of the whole microalgae and the components EPA, Fx, and Chrl measured daily was negatively correlated with the F/B ratio, Firmicutes, and Actinobacteria abundance at the phylum level and *Lachnospirales* abundance at the order level. A positive association was observed between the intake and the relative abundance of Verrucomicrobiae at the phylum level and *Akkermansia* at the genus level. *Clostridia vadinBB60* was positively associated with EPA and Fx but not with Chrl intake. Further results showed that a higher abundance of *Cyanobacteriia* at the class level and *Cyanobacteriia_Choroplast* at the genus level is only associated with Chrl intake.

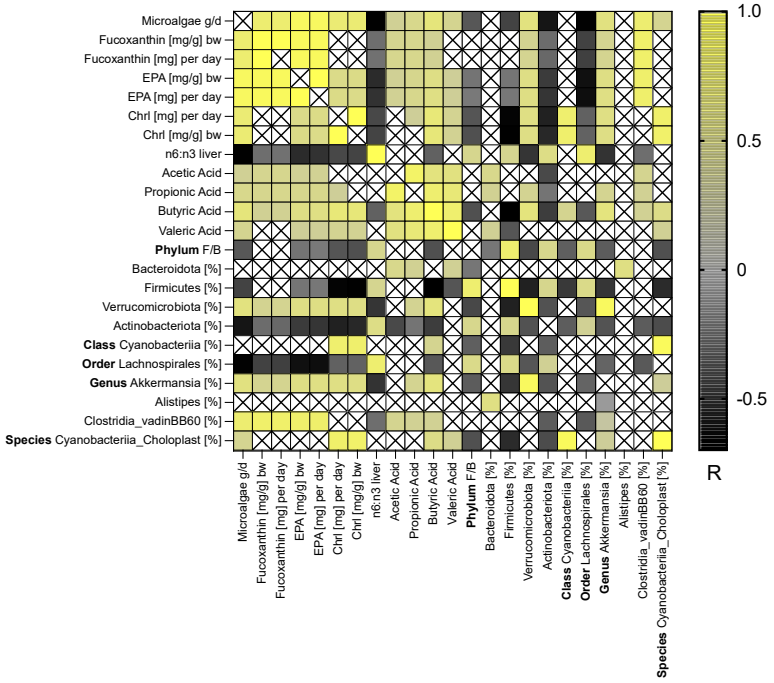

Figure 5. Heat map of Spearman's rank correlation measured by Spearman rho (R) comparing the microalgae intake/day, EPA, Fx, and Chrl intake mg/day and mg/g bw with the n:6-n:3 ratio in the liver, SCFA amounts (acetic, propionic, butyric, and valeric acid), and gut microbiome as the relative bacterial abundances [%] at the phylum, class, order, genus, and species levels. Abbreviations: X, no significance at *p*-values > 0.05; bw, body weight; EPA, eicosapentaenoic acid; Chrl, chrysolaminarin intake; F/B, Firmicutes/Bacteroidota ratio; for further abbreviations, see Table 1 and Figure 1.

The connection between the SCFA amounts and some bacteria was elucidated. A positive correlation was demonstrated for acetic acid between Bacteriodota at the phylum level and *Clostridia vadinBB60* at the genus level. The Bacteriodota Verrucomicrobiae at the phylum level and *Akkermansia* and *Clostridia vadinBB60* at the genus level are positively correlated with propionic acid. The propionic acid amount and the Actinobacteria abundance are negatively correlated at the phylum level. For butyric acid, a positive correlation was detected between the Verrucomicrobiae at the phylum level; *Cyanobacteriia* at the class level; and *Akkermansia*, *Clostridia vadinBB60*, and *Cyanobacteriia_Choroplast* at the genus level. A negative correlation was shown between the abundance of Firmicutes and Actinobacterota at the phylum level and *Lachnospirales* at the order level. For valeric acid, Bacteroidota and *Cyanobacteriia_Choroplast* were positively correlated at the phylum level, whereas Firmicutes correlated negatively at the phylum level.

4. Discussion

In this study, we investigated two biomass samples from the microalgae PT containing different amounts of EPA, Fx, and Chrl due to different cultivation conditions. The investigation in mice comprised acceptance and safety issues, as well as aspects of gut health, such as microbiota and fecal SCFA analyses. Such analyses are also needed to approve microalgae PT biomass as a novel food. This study provides information on the safe intake of microalgae biomass and demonstrates possible beneficial effects on gut health in a preclinical setting.

With respect to safety issues, we assessed the integrity of the intestinal barrier by measuring occludin and zonula occludens 1 in the intestinal mucosa. According to these measurements, the intestinal barrier was not impaired by the PT diets, suggesting the possibility of safe intake of PT. It has been reported that β-glucans can enhance the expression of TJ proteins in obese mice [38] and normal-weight pigs [39]. Although PT contains the β-glucan Chrl, we did not observe a beneficial effect of PT diets on the barrier, likely

because our mice were healthy and no severe barrier impairment was expected before the intervention. Other PT ingredients, such as EPA and Fx, might also play a protective role in the recovery of TJ proteins following inflammatory processes [40–42]. Whereas no benefit was observed in our healthy mice when fed PT diets, a beneficial effect can be anticipated if the recipients suffer from an impaired gut barrier.

Interestingly, the PT diets did not induce a clear inflammatory response, although we observed an increase in TNF-α mRNA levels in the ileum. However, other proinflammatory cytokine mRNA levels, such as IL-6 and IL-1β, were not affected. A negative correlation was measured between the n-3 PUFA in the liver, which was increased by the PT diets, and pro-inflammatory IL-1β, suggesting an anti-inflammatory effect of PT, which is further supported by the reducing effect of the PT diets on the n-6:n-3 ratio. The Chrl-rich extract of PT (0.06%) has immunomodulatory effects in sea bream [30], which might be related to induction of intestinal TNF-α levels. The increase in TNF-α levels observed in our studies requires further investigation, and measurement inaccuracies must be prevented. According to own previous data, PT intake has anti-inflammatory rather than proinflammatory effects [36].

Considering the medium dose of 15% microalgae content in the diet, we conclude that our data demonstrated a safe intake of Fx of up to 4.3 mg/day in mice with a bw of approx. 18 g (231 mg/kg bw per day). Such doses cannot be easily extrapolated to the human situation. Equivalent amounts in human diets are usually lower because of a slower metabolic turnover in humans compared to rodents; on the other hand, higher doses are often administered for medical use. In a recent pilot trial in humans, we demonstrated a safe intake of up to 30 mg/day of Fx [7], and the EFSA published a recommendation for 15 mg/day of pure Fx extracted from *Undaria pinnatifida thallus* [43]. Further human studies are needed to evaluate safe recommended dietary allowance.

With respect to EPA, this study confirms good bioavailability from both PT diets and a safe intake of EPA of up to 17 mg/day related in mice with a bw of approx. 18 g (920 mg/kg bw per day). As expected, higher EPA concentrations of the EPA/Fx diets resulted in higher absorption of EPA in the liver and fat tissue than the Chrl-rich diets. Regarding the dietary intake limits for Chrl, the present study showed no tissue changes or damage associated with intakes of up to 86.6 mg/day in mice with a bw of approx. 18 g (4621 mg/kg bw). EFSA has already been approved for the human diet with a dose of up to 600 mg/day of -(1,3)/(1,6)-glucan derived from the cell wall of baker's yeast *Saccharomyces cerevisiae* [44]. A toxicological study in rats demonstrated a safe intake of up to 100 mg/kg bw of this type of β-glucan [45]. Therefore, we assume that the Chrl type of β-(1,3)/(1,6)-glucan derived from PT should also be approved soon. Other microalgae containing β-(1,3)/(1,6)-glucan or β-(1,3)-glucans, such as *Odontalla auritia* and *Euglena gracilis*, are already authorized in the EU [17,46]. A drug derived from *Euglena gracilis* is already on the market for immune stimulation (BioGlenaTM; Algatechnologies Inc. Eilot, Israel). Thus, no safety issues should be expected because of the β-(1,3)/(1,6)-glucan contained in PT; instead, beneficial effects should be assumed, supporting the idea of using PT-based products for human nutrition.

Although a literature search did not reveal any studies reporting infection or intoxication by the diatom PT [47], some recent studies suggest that PT can produce β-N-methylamino-L-alanine (BMAA) under particular culture conditions [48,49]. BMAA is a neurotoxin produced by certain cyanobacteria, diatoms, and dinoflagellates, with adverse effects on humans [50]. BMAA is found in mussels and scallops and is thus transferred to the food chain [51,52]. However, our previous studies in cell culture, in mice, and humans, as well as the present study, did not reveal any signs of neurotoxicity, such as abnormal movement or behavior in the mice, which calls into question the relevance of BMAA in the PT biomass, possibly because of the different culture conditions we used in our studies [7,19,36]. On the other hand, BMAA in PT biomass should be quantified, and the amounts should be toxicologically evaluated in mice and humans to complete a

qualified presumption of safety (QPS) procedure used by EFSA Scientific Panels for risk assessment of biological agents [47].

Our preclinical data revealed that PT diets are safe and possibly also healthy, as they showed some beneficial effects on the intestinal microbiota and possibly also on the generation of SCFAs. SCFAs have been attributed to beneficial effects on gut health, nourishing the mucosa, preventing colon cancer, and protecting against leaky gut [53,54]. SCFAs are produced from dietary fibers, such as β−glucans fermented to SCFAs by commensal bacteria [55]. This concept was supported by studies showing an increase in acetic acid and butyric acid after supplementation with β-glucans from barley [56,57], oats [58], and laminarin from *Laminaria* spp. [59,60]. Interestingly, n-3 PUFA has also been found to promote SCFA production. Apart from their anti-inflammatory effects, n-3 PUFAs are considered prebiotics, as studies have provided evidence that n3-PUFAs promote SCFA production [61,62] and an increase in LPS-suppressing bacteria, such as *Bifidobacteria* [63]. The daily intake of Fx may also influence SCFA production, as demonstrated by Sun et al. [32].

Due to the similar structure of Chrl compared to other β-glucans, such as laminarin, and the high content of EPA and Fx, we assumed similar effects. We observed an increase in acetic acid and a higher amount of butyric acid after administration of the Chrl15-rich diet. Correlation analyses confirmed this assumption, and we demonstrated, for the first time, that SCFA levels correlated with Chrl uptake from diet. The current EPA/Fx diet increased acetic acid in association with all three diets. Additionally, correlation analysis confirmed that the highest dose of the diet also increased propionic acid levels. This increase suggests a possible benefit for gut health, as propionic acid is essential for mucosal healing and anti-inflammation [64]. A lower daily dose of 286 mg EPA+DHA did not change SCFA levels in a previous clinical trial conducted by our group after two weeks of PT intake [7]. The results suggest that Chrl, EPA, and Fx could promote SCFA production. Further evaluation is required, as the SCFA changes were minor and not always consistent for the different supplementation groups.

Due to the effects of PT diets on SCFA production in the gut, we also expected changes in the gut microbiome. As shown by other studies, the supplementation of n-3 PUFA, β-glucan [65,66], and Fx [32] increased bacterial diversity. However, in our study, we were not able to confirm these results. However, the PT diets modified the gut microbiome at the phylum level; they reduced the F/B ratio, confirming other studies supplementing with β-glucan from barley [66] and laminarin from macroalgae [67,68]. Correlation analyses revealed that the F/B ratio is negatively associated with microalgae intake, as well as EPA and Chrl intake. Furthermore, the Chrl-rich diets enhanced the abundance of the SCFA-producing *Akkermanisa* sp. [53]. Our data confirm an increase in *Akkermanisa* sp. after supplementation with the β-(1,3)-glucan paramylon, as reported in previous studies [69]. Additionally, n-3 PUFA supplementation is thought to increase *Akkermansia* sp. [70], especially *Akkermansia muciniphila* [71]. Therefore, *Akkermansia* sp. have been identified as "next-generation probiotics" [72]. Our murine data were less clear in this respect. However, a recent human study performed by our group demonstrated that consuming an EPA/Fx-rich PT diet increased *Akkermansia* after 14 days of intervention [7]. We conclude that Chrl-rich PT intake increases the abundance of *Akkermansia*.

The distinctive effect of the Chrl-rich diet could be due to the increase in the abundance of *Cyanobacteriia_chloroplasts*, possibly resulting from the formation of chloroplasts following cyanobacterial endosymbiosis [73]. Minor other changes included a lower abundance of *Lachnospirales* associated with PT diets, a butyric acid-producing bacteria [74]. Supplementation with n-3 PUFA was previously to shown to result in an increase in *Lachnospirales* due to conditional promotion by DHA [65]. The fact that the PT diet is mainly rich in EPA could be a possible explanation for the decline. Further changes were observed, including an increase in bacteria involved in mucosal sugar uptake [75], such as species *Muribaculaceae_unidentified* associated with the Chrl-rich diet and *Alistepes* in association with the EPA/Fx diet. The higher abundance of *Clostridia_vadinBB60* at the genus level is very poorly classified, and their role in the microbiota [76] requires further investigation.

5. Conclusions

Our preclinical study in healthy mice shows that the intake of two types of PT diets containing Chrl, EPA, and Fx as bioactive compounds is safe and well accepted by mice. With the same calorie intake and increased weight gain, we assume energy absorption. Due to the increase in TNF-α levels, further investigation is needed, and the mean dose of the two PT diets is described as the safest diet. Health benefits, such as the increase in SCFA, need to be further investigated, as the available results are inconsistent. The microbiota results could show positive effects, such as a decrease in the F/B ratio, and the Chrl-rich diet mainly led to an increase in the SCFA-producing bacteria *Akkermansia* sp. The data suggest that PT diets could be suitable for human nutrition, which must be confirmed in human trials.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu14122504/s1, Table S1: Histological scores of the liver, ileum, and colon after 14 days of diet supplementation.; Table S2: Bacterial taxa in feces after 14 days supplementation of the CD and Chrl-rich and EPA/Fx PT diets.

Author Contributions: Conceptualization, U.N., L.S. and S.C.B.; methodology and analysis, L.S., U.N. and A.R.; formal analysis, L.S.; investigation, all; resources, U.N., L.S., K.F. and U.S.-S.; writing—original draft preparation, L.S.; writing—review and editing, U.N. and S.C.B.; visualization., L.S.; project administration, L.S. and U.N.; funding acquisition, S.C.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry for Science, Research and Art within the Bioeconomy research Program of Baden-Württemberg, grant number BÖBW2-105A, FKZ-7533-10-5-185B.

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Review Board. All experiments were supported by the local Institutional Animal Care and Use Committee (Regional Council Stuttgart, permit number: V326-15EM).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available upon justified request to the corresponding author.

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

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2.3 Humane-Interventionsstudie zur Untersuchung von potenziellen gesundheitlichen Vorteilen im Alter nach der Einnahme der EPA/Fx-reichen Biomasse und des Chrl-reichen Überstandes von PT

Eine gesunde und altersgerechte Ernährung ist eine Grundvoraussetzung für den Erhalt von funktionellen Fähigkeiten bei älteren Menschen. Häufig treten mit zunehmendem Alter jedoch Störungen wie Sarkopenie und Mikronährstoffdefizite, Tumore und kardiometabolische Erkrankungen auf. Zugrunde liegen solchen chronischen Erkrankungen häufig eine subklinische Entzündung [139,140], Immundysfunktionen, Veränderungen der Darmepithelbarriere [141,142] oder oxidativer Stress [143]. Die Zufuhr von PT könnte nicht nur einen Mikronährstoffmangel ausgleichen, sondern auch durch die in PT enthaltenen bioaktiven Nährstoffe ein gesundes Altern fördern. Durch entzündungshemmende Wirkungen von EPA [127], antioxidative Wirkung von Carotinoiden [128] und immunmodulatorischen Eigenschaften von Chrl [112,144] sind positive Auswirkungen auf Biomarker im Zusammenhang mit gesundem Altern zu erwarten.

Dieser Hypothese wurde in der dritten Phase des Promotionsprojekts in einer Pilotstudie mit älteren Probanden nachgegangen. In dieser Studie wurde die Einnahme von PT mit verschiedenen Biomassen in vier Interventionsgruppen getestet. Diese Gruppen unterschieden sich hinsichtlich der 14-tägigen Intervention mit PT-Biomasse; Gruppe I war charakterisiert durch die Einnahme der EPA-/Fx-reichen Biomasse, Gruppe II durch die Einnahme des Chrl-reichen Überstandes, Gruppe III durch die Kombination aus I. und II., Gruppe IV. diente als Kontrollgruppe. Während der Studientermine wurden Sicherheitsparameter gemessen und Nebenwirkungen dokumentiert. Zudem wurden Marker für die Bewertung der Mobilität, inflammatorische Marker, oxidative Stressmarker, Darmbarrieremarker sowie kurzkettige FS in Stuhlproben gemessen und im Hinblick auf gesundes Altern bewertet. Des Weiteren wurden FS im Plasma und in der Erythrozytenmembran sowie Konzentrationen von Carotinoiden und Vitamin E für die Bewertung der Bioverfügbarkeit und Versorgung gemessen. Die primäre Ergebnisvariable Interleukin-6 (IL-6) im Plasma war nach der Behandlung in Gruppe III im Vergleich zur Kontrollgruppe reduziert. Die Mobilitätsparameter waren in Gruppe I und per Trend in Gruppe III verbessert im Vergleich zur Kontrollgruppe. Auch das n-6: n-3 Verhältnis wurde in Gruppe III verbessert. Darüber hinaus wurde in Gruppe II das fäkale Zonulin, ein Maker der Darmbarriere, signifikant verbessert. Die Daten zeigten eine entzündungshemmende und potenziell antioxidative Wirkung bestimmter PT-Präparate, was darauf hindeutet, dass sie zur Prävention altersabhängiger Erkrankungen geeignet sein könnten. Der Artikel wurde im November 2022 von der Fachzeitschrift *Marine Drugs* akzeptiert und publiziert [145].

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Article

Potentially Beneficial Effects on Healthy Aging by Supplementation of the EPA-Rich Microalgae *Phaeodactylum tricornutum* or Its Supernatant—A Randomized Controlled Pilot Trial in Elderly Individuals

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Abstract: Dietary supplements that promote healthy aging are mostly warranted in an aging society. Because of age-related risks, anti-inflammatory and anti-oxidative agents such as microalgae are potential candidates for intervention. In a randomized controlled trial, we tested *Phaeodactylum tricornutum* (PT), a microalgae rich in eicosapentaenoic acid (EPA), carotenoids, vitamins, and β-glucans, cultured in bioreactors. In this pilot trial, 19 healthy elderly received supplements for two weeks based on either the whole PT (*A*), the β-1,3-glucan-rich PT supernatant (*SupB*), the combination thereof (*A+SupB*), or a Comparator product (*Comp*). The primary outcome variable plasma interleukin-6 was reduced after treatment with *A+SupB* compared to the *Comp* group ($p = 0.04$). The mobility parameters 5 s sit-to-stand test ($p = 0.04$ in the *A* group) and by trend gait speed ($p = 0.08$ in the *A+SupB* diet) were improved compared to *Comp*. No treatment effects were observed for fatty acids, compared to *Comp* but omega-6 to -3 fatty acid ratio ($p = 0.006$) and arachidonic acid/EPA ratio ($p = 0.006$) were reduced within group *A+SupB*. Further, the *SupB* study product reduced faecal zonulin ($p = 0.03$) compared to the *Comp*. The data revealed an anti-inflammatory and potentially anti-oxidative effect of particular PT preparations, suggesting that they might be suitable for effects in healthy elderly.

Keywords: elderly; inflammageing; *Phaeodactylum tricornutum*; omega-3-fatty acids; eicosapentaenoic acid; fucoxanthin; β-glucan; chrysolaminarin

Citation: Stiefvatter, L.; Frick, K.; Lehnert, K.; Vetter, W.; Montoya-Arroyo, A.; Frank, J.; Schmid-Staiger, U.; Bischoff, S.C. Potentially Beneficial Effects on Healthy Aging by Supplementation of the EPA-Rich Microalgae *Phaeodactylum tricornutum* or Its Supernatant—A Randomized Controlled Pilot Trial in Elderly Individuals. *Mar. Drugs* **2022**, *20*, 716. https://doi.org/10.3390/md20110716

Academic Editors: Giuseppina Tommonaro and Annabella Tramice

Received: 29 September 2022
Accepted: 9 November 2022
Published: 15 November 2022

1. Introduction

The WHO declares the aging of the world population as the most important demographic problem worldwide. Therefore, healthy aging is becoming increasingly important to maintain functional abilities [1]. Aging is accompanied by immune system dysfunction, changes in intestinal epithelial barriers [2,3] chronic inflammation, and an increase in oxidative stress due to the imbalance between pro- and antioxidant species [4]. Due to these age-related changes, often named "inflammageing", microalgae have been proposed as an aid in the prevention of inflammation as their functional constituents exert multiple pharmaceutical and nutraceutical bioactivities. Microalgae contain long-chain omega-3 fatty acids (n−3 FA), especially EPA (20:5 n−3) and docosahexaenoic acid (DHA, 22:6 n−3), otherwise found in fish, but also proteins, phenols, carotenoids, vitamins and dietary fibres such as β-glucans, in particular chrysolaminarin [5–7]. The microalgae *Phaeodactylum tricornutum* (PT) is a microalgae of particular interest because it is a species highly effective in synthesizing EPA, proteins, fucoxanthin and chrysolaminarin, and therefore a candidate for promoting healthy

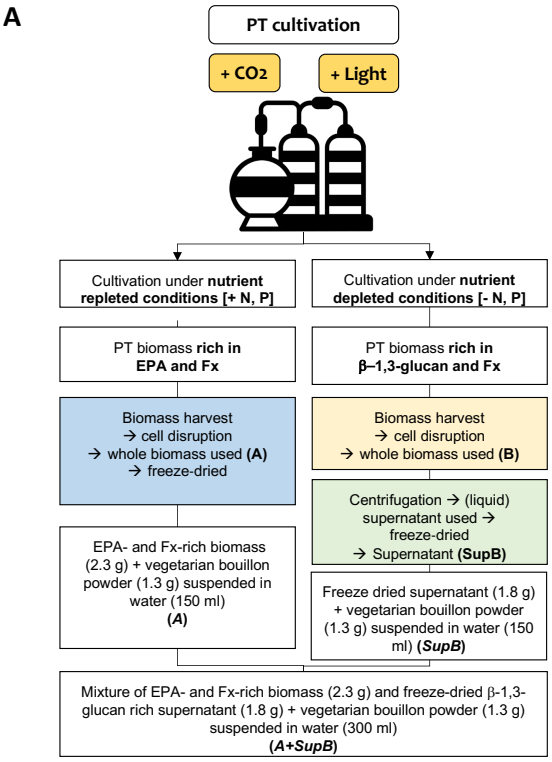
aging with preventive mechanisms. Compared to sea fish, the consumption of PT could be a sustainable nutritional alternative, as shown in previous studies from our and other groups [8,9]. By replacing sea fish consumption with microalgae such as PT the problem of overfishing of the oceans worldwide could be reduced [10]. Also, for vegans, PT could be of interest, because it offers a high content of EPA, which is available in plant food only through α-linolenic acid (ALA) known to be poorly converted into EPA in the human organism [11]. EPA plays a central role in the production of anti-inflammatory eicosanoids, cytokines and the reduction of reactive oxygen species [12]. This anti-inflammatory effect has already been demonstrated for PT [13] and could help tackle inflammageing in the elderly. Furthermore, adequate protein supply in old age is crucial due to muscle loss and the risk of sarcopenia [14]. PT contains up to 60% of protein and therefore can also serve as a valuable protein source for the elderly population. A preclinical study showed that 48% of protein could be replaced by PT without adverse effects on bioavailability [15]. Further functional compounds are phenols and carotenoids, which act as antioxidants by scavenging free radicals [16], and also the neuroprotective effects of carotenoids might be beneficial for healthy aging [17]. The most abundant carotenoid in PT is the xanthophyll fucoxanthin (Fx), a carotenoid without provitamin A activity, that is found in brown-coloured microalgae and macroalgae such as seaweeds [18,19]. Fx is converted into fucoxanthinol (FxOH) and amarouxiaxanthin A (A × A), which has already been demonstrated for PT in a clinical study [8]. Consumption of Fx may have health benefits for older people, such as neuroprotective effects [20], antioxidant and antiproliferative effects [21]. PT contains other carotenoids such as lycopene, which have antioxidant effects [22]. Also, the β-carotene with its pro-vitamin A activity is important for the visual process [23], and has antioxidant potential [24], and could provide further health benefits for the elderly. A further relevant nutrient is tocopherol, amounts of which depend on the growing conditions, and is a powerful antioxidant which could have a protective role in aging [25]. Another component of PT is the β-glucan (β-G) chrysolaminarin (Chrl), a water-soluble β-(1,3)/-(1,6)-glucan (11:1), which is produced in higher concentrations under nitrogen-limiting conditions [26]. The safe and potential gut health effect was shown in a preclinical study by the intake of the whole β-glucan- rich PT as well the EPA-rich biomass [27]. Further immunomodulatory properties and increased antioxidant status could already be shown in fish [28,29]. This regulation of antioxidant status and intestinal barrier function could benefit health, especially in older people.

Considering this background, we performed a randomized, controlled pilot trial in older individuals who received different preparations of the microalgae PT or a comparator product (*Comp*), which is considered a Placebo consisting of only vegetarian bouillon powder for two weeks to assess safety aspects, the bioavailability of selected ingredients and effects on biomarkers related to healthy aging.

2. Results

2.1. Analysis of the Phenolic Content and Oxidative Potential of the Intervention Products

The production of PT with different cultivation conditions resulted in changes in the composition of ingredients (Figure 1A,B). Under nutrient-repleted conditions, PT accumulated more EPA (Biomass A). Under nutrient-depleted conditions, Chrl-rich biomass (Biomass B) was produced. From biomass B, a supernatant was prepared (for details see 4.3) and analysed as well as biomass A and B for the total phenolic content (TPC) by measuring the gallic acid equivalents (GAE; Figure 1C). The TPC ranged from 8.31 ± 4.13 mg GAE per g dry weight in the SupB, to 14.07 ± 4.34 mg/g within biomass B and 16.82 ± 2.47 mg/g in biomass A. The whole biomass samples A and B had a higher TPC compared to SupB ($p < 0.001$ for A, $p = 0.009$ for B). To analyse the antioxidant potential, the ferric reducing ability of plasma (FRAP) assay was performed (Figure 1D). The highest FRAP value was measured for biomass B (2.65 ± 0.97 mmol FRAP per g dry weight), which was higher than for biomass A (1.97 ± 0.07 mmol/g; $p = 0.0480$) and the SupB (0.66 ± 0.27 mmol/g; $p < 0.001$). The FRAP value was also higher in the A biomass compared to the Sup ($p = 0.002$).



B

Nutrient	A	B	SupB
Protein [%]	34.9	29.9	2.4
EPA [%]	2.72	2.49	0.04
FA [%]	8.19	18.19	0.70
β-Glucan [%]	7.51	20.21	44.17
Fucoxanthin [%]	0.70	0.85	0.02

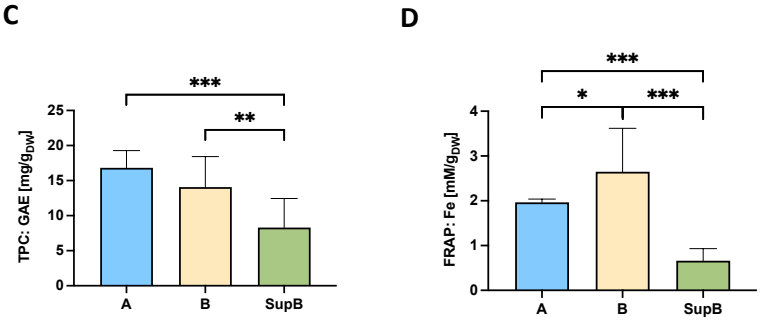


Figure 1. Different growing conditions were used for *Phaeodactylum tricornutum* (PT) production. Biomass A was grown under nutrient-repleted conditions leading to an EPA- and FX-rich biomass, whereas biomass B was grown under nutrient-depleted conditions (without nitrogen and phosphorous in the culture media) for several days before harvesting, leading to the accumulation of β-1,3-glucan. From biomass B, a supernatant was prepared (SupB). Based on biomass A and supernatant SupB, three supplements were prepared for the human trial (**panel A**). The nutrient composition of biomass A, biomass B and supernatant SupB differed (**panel B**). Total phenolics content (TPC) expressed as gallic acid equivalents (GAE) and ferric reducing antioxidant power (FRAP) were measured (**panels C**,**D**). Data are presented as mean ± SD (n = 3). Statistics: * indicates significant differences (ANOVA with Tukey post hoc test). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Further abbreviations: N, nitrogen; P, phosphor; EPA, eicosapentaenoic acid; Fx, fucoxanthin; FA, fatty acids; DW, dry weight.

2.2. Clinical Trial-Subjects' Anthropometric and Metabolic Characteristics at Baseline

Recruitment of study participants took place between June 2021 and August 2021. After the study entry and during the study, there were a total of three dropouts for personal reasons after screening (Figure 2). Thus 21 subjects were randomized into four study groups. Two additional subjects dropped out during the intervention phase due to lack of compliance, so 19 individuals could be finally analysed (12 females, 7 males). The mean (±SD) overall age of the subjects was 67.7 ± 6.5 years, with nine (37.5%) being ≥70 years old (Table 1). The participants were at the normal weight on average with a mean BMI of 24.5 ± 3.1 kg/m^2. Laboratory parameters did not show abnormal values except higher serum fat values than the reference values (cholesterol <200 mg/dl, LDL < 130 mg/dl, HOMA-Index < 1). There was no difference between study groups except the waist circumference was different within the groups ($p < 0.001$).

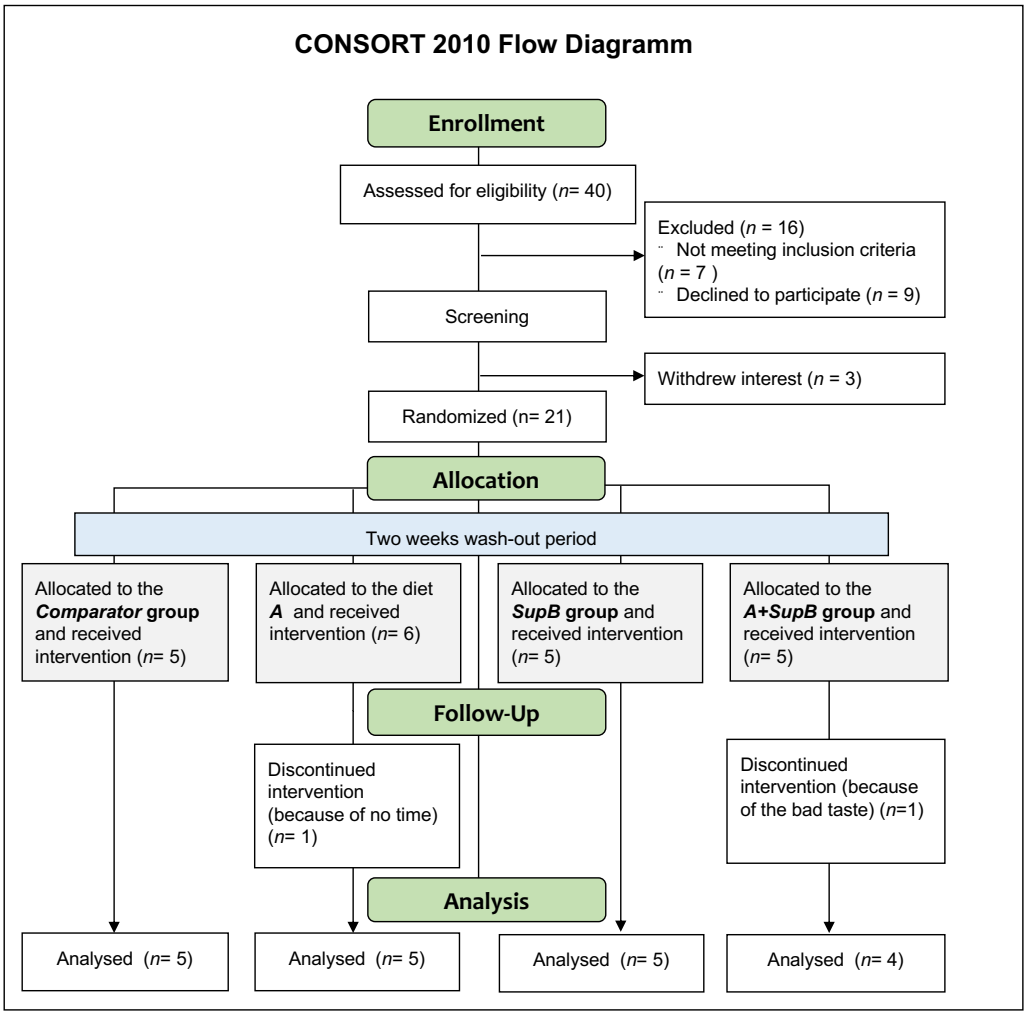


Figure 2. The study was designed as a pilot, randomized, single-blind, placebo (*Comp*)-controlled, parallel four-arm group study with a two-week wash-out and two-week intervention phase. After enrolment and screening (study start/time-point (0), a total of 21 participants were randomly assigned to four groups and underwent a two-week wash-out phase with dietary instructions. After these two weeks (time-point 2), they returned and received the study products assigned to their group. After one week of intervention (time-point 3), the third visit took place and two participants dropped out of the study. After two weeks of intervention (time-point 4), the fourth visit was conducted. The study was fully completed by 19 participants, which were analysed. Abbreviations: *Comparator*, is considered a Placebo with only vegetarian bouillon powder; *A*, diet with biomass A and vegetarian bouillon powder; *SupB*, diet with supernatant (*SupB*) of biomass B and vegetarian bouillon powder; *A+SupB*, Intervention with biomass A and supernatant of biomass B and vegetarian bouillon powder. For details of study products labelled with italic abbreviations (to separate it from biomass A and B in Figure 1) see chapter 4.3 and Table S7.

The study population was divided into four groups that received different supplements, (i) a Comparator (*Comp*) consisting of a vegetarian bouillon powder solved in 150 mL plain water (*Comp*), (ii) lyophilizate of PT biomass A mixed with bouillon powder dissolved in water (*A*), (iii) lyophilizate of supernatant of PT biomass B mixed with bouillon powder solved in water (*SupB*), (iv) a combination of (ii) and (iii) (*A+SupB*). For details of study products labelled with italic abbreviations (to separate it from biomass A and B in Figure 1) see chapter 4.3 and the nutrient composition in Table S7.

Table 1. Characteristics of the 19 study participants.

Female/Male [n]	***All*** **(*n* = 19)** **12/7**	***Comp*** **(*n* = 5)** **2/3**	***A*** **(*n* = 5)** **4/1**	***SupB*** **(*n* = 5)** **3/2**	***A+SupB*** **(*n* = 4)** **3/1**
Anthropometry					
Age [years]	67.7 ± 6.5	67.4 ± 7.9	65.4 ± 4.7	71.4 ± 5.7	67 ± 9.2
BMI [kg/m^2]	24.6 ± 3.1	26.9 ± 2.5	25.5 ± 1.8	22.3 ± 2.9	23.3 ± 3.7
Waist circumference [cm]	90.2 ± 11.6	100.5 ± 8.7	93.6 ± 4.5	81.2 ± 11.4	84.5 ± 11.2
Blood biomarkers					
Cholesterol (chol.) [mg/dl]	224.6 ± 50.0	248 ± 43.5	198 ± 44.7	219 ± 63.2	235 ± 46.5
Triglycerides [mg/dl]	99.9 ± 60.0	141 ± 102.1	83.2 ± 35.7	74 ± 26.7	101.8 ± 26.5
HDL-chol. [mg/dl]	71.1 ± 18.4	71.8 ± 24.2	64 ± 14	72.2 ± 12	77.8 ± 25.7
LDL-chol. [mg/dl]	126.4 ± 46.8	152.2 ± 24.1	120.2 ± 40.9	129.8 ± 51.2	129.7 ± 11.7
Insulin [µE/mL]	7.8 ± 4.1	10.8 ± 6.6	7.6 ± 2.8	5.9 ± 2.6	6.7 ± 1.5
HOMA-Index	1.9 ± 1.7	3.1 ± 3	1.7 ± 0.6	1.4 ± 0.6	1.5 ± 0.2

Values are expressed as mean ± standard deviation (SD). Abbreviations: diets see Figure 2; *Comp*, Comparator; BMI, body mass index; chol, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Statistics: Comparison of groups revealed no difference for all parameters listed in the table (p >0.05, ANOVA) except the waist circumference ($p < 0.001$).

2.3. Four-Week Food Diary and Food Frequency Questionnaire (FFQ)

Diet was assessed using an FFQ at the study start and a continuous food diary during the intervention period as described in Methods 4.10. The FFQ shows similar dietary intake between study groups (Table S1). The food diaries allowed for analysing the percentage compliance with recommended nutrient amounts for the corresponding age group, either 51–65 years or > 65 years. On average, the recommendations were exceeded for protein (124%), PUFA (151%), and vitamin A (257%) and were not fully met for energy (90%), carbohydrate (65%), fibre (73%) and vitamin D (9%) intakes.

2.4. Acceptance and Adverse Effects during the Intervention

All four study products were generally well tolerated. Adverse effects were monitored using a diary and at each study visit by the study staff (protocol). No serious adverse reactions were reported, but minimal and mild adverse effects occurred (Table 2). Most side effects occurred after taking *A+SupB*, e.g., belching, headache and increased urination. Gastrointestinal symptoms such as abdominal rumbling, flatulence and diarrhoea were described for all four treatments as well as for the *Comp*.

Table 2. Adverse effects during the two weeks intervention within the four study groups.

Side Effects	***Comp*** **(*n* = 5)** **Diary/Protocol**			***A*** **(*n* = 5)** **Diary/Protocol**			***SupB*** **(*n* = 5)** **Diary/Protocol**			***A+SupB*** **(*n* = 4)** **Diary/Protocol**		
	Min.	**Mild**	**Sev.**	**Min.**	**Mild**	**Sev.**	**Min.**	**Mild**	**Sev.**	**Min.**	**Mild**	**Sev.**
Abdominal rumbling	-	-	-	2|1$_1$	-	-	-	-	-	-	-	-
Flatulence	-	1|0$_2$	-	-	-	-	-	1|1$_3$	-	-	1|0	-
Stomach pain	-	-	-	-	-	-	-	0|1	-	-	-	-
Diarrhoea	-	1|1	-	-	-	-	-	1|0	-	-	1|0	-
Discoloration of the stool	-	-	-	-	-	-	-	-	-	1|0	-	-
Decreased frequency of bowel movements	-	-	-	-	-	-	-	-	-	1|0	-	-
Belching (at least 1×)	-	-	-	-	-	-	-	-	-	1|1	-	-
Headache	-	-	-	-	-	-	-	-	-	1|0	1|0	-
Increased blood pressure	-	1|0	-	-	-	-	-	-	-	-	-	-
Increased urge to urinate	-	-	-	-	-	-	-	-	-	1|0	-	-
Nausea	-	-	-	1|1	-	-	-	-	-	-	-	-

Adverse effects were documented in a diary by the participants and recorded upon questionnaire by the study personnel during the visits. Values from completers are expressed as absolute numbers. Minimal, transient symptoms with no impairment of the patient's daily activities; Mild, consistent symptoms with moderate impairment of the patient's daily activities; Severe, significant impairment of the patient's daily activities. Abbreviations: diets see Figure 2; *Comp*, Comparator; Min., minimal; Sev., severe; $_1$, the side effect was documented by two participants in a diary and one time during the protocol; $_2$, the side effect was documented one time in a diary but not during the protocol; $_3$, the side effect was documented one time in a diary and one time during the protocol.

2.5. Effect of Supplementation on Laboratory Parameters

Various laboratory parameters were determined at three time points at study start (0), after the wash-out phase (2) and after two weeks of intervention (4). Half of the subjects cholesterol levels were over the normal ratio. Within the *SupB* group, the cholesterol levels were lower by a trend at week four (206.2 ± 59.1 mg/dl) compared to before the intervention (209.8 ± 59.6 mg/dl; p= 0.06; Figure 3A). The LDL was higher within the *SupB* group at week 4 compared to week 2 (p= 0.04, Figure 3B). Triacylglycerols (TAG) were reduced at week 4 with 65.8 ± 17.1 mg/dl compared to week 2 (74.6 ± 22.2 mg/dl; p = 0.04, Figure 3C). Within the A group, the Homeostasis Model Assessment (HOMA) index for insulin resistance was higher at week two at 1.82 ± 0.3 compared to 1.28 ± 0.3 at week 4 (p= 0.03, Figure 3D). Further laboratory parameters are shown in Table S2. No difference was measured between groups and the change from week 4 to 2.

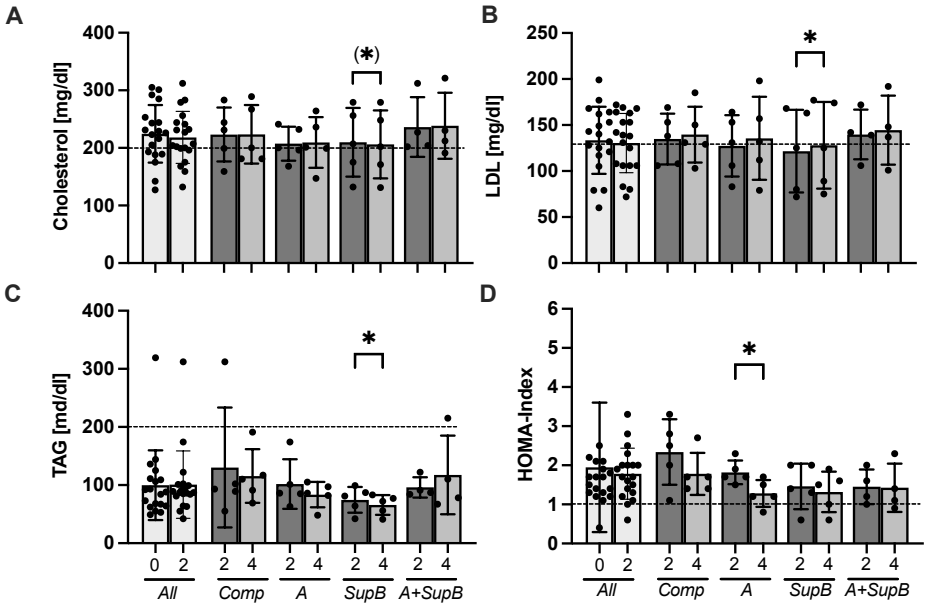


Figure 3. Effect of the study products on total serum cholesterol (**A**), LDL-cholesterol (**B**), triacylglycerols (**C**) and HOMA index (**D**). Parameters were measured at study start (0), after two-weeks wash-out (2), and after two weeks of intervention (4). Values are expressed in scatter plot with mean ± SD and individual values from 19 participants (*All* n = 19; *Comp*, *A*, *SupB* each n = 5; *A+SupB* n= 4). Below the dashed line is the normal reference range. Statistics: * indicates a difference within a group between week two (2) and week four (4) (t-test). No between-groups-differences were found at week 0, 2 and 4 (ANOVA). (*) $p < 0.1$, * $p < 0.05$. Abbreviations: diets see Figure 2; *Comp*, Comparator; LDL, Low-density lipoprotein; TAG, Triacylglycerols; HOMA index, Homeostasis Model Assessment for Insulin Resistance.

2.6. Mobility Markers and Body Composition before and after Intervention

The Western Ontario and McMaster Universities Arthritis Index (WOMAC) questionnaire was used to test the mobility of the knee and hip, including pain, stiffness, and physical functioning. There was no treatment effect measured (Table S7). The index was 9.2 ± 10.4 points at the study start and 11.1 ± 13.9 at study end. Within groups it varied at study end between 2.8 ± 3.0 points in the *A* group and 16.3± 18.9 in the *A+SupB* group. The time for the 5 s sit-to-stand test (5-STS) showed a reduction in the time from 11 ± 1.9 sec to 9.0 ± 1.2 sec within the *A* group from 0 to week 4 (p = 0.02; Figure 4A). The delta was lower by −0.7 sec within the *A* group which was significantly lower compared to the *Comp* group (p = 0.04; Figure 4B). Measurement of the gait speed test showed no changes within groups (Figure 4C), but there was a trend of a treatment effect by the delta reduction within the *A+SupB* group compared to the *Comp* group (p = 0.08, Figure 4D). The handgrip strength tended to increase

in the A group from 29.0 ± 5.2 kg at week 2 to 30.6 ± 6.3 kg at week 4 ($p = 0.09$, Figure 4E) but there was no delta change (Figure 4F). Body composition markers were not affected by the study interventions (Table S3) except the lean body mass was lowered after the *SupB* study product and by a trend of the others compared to the *Comp*.

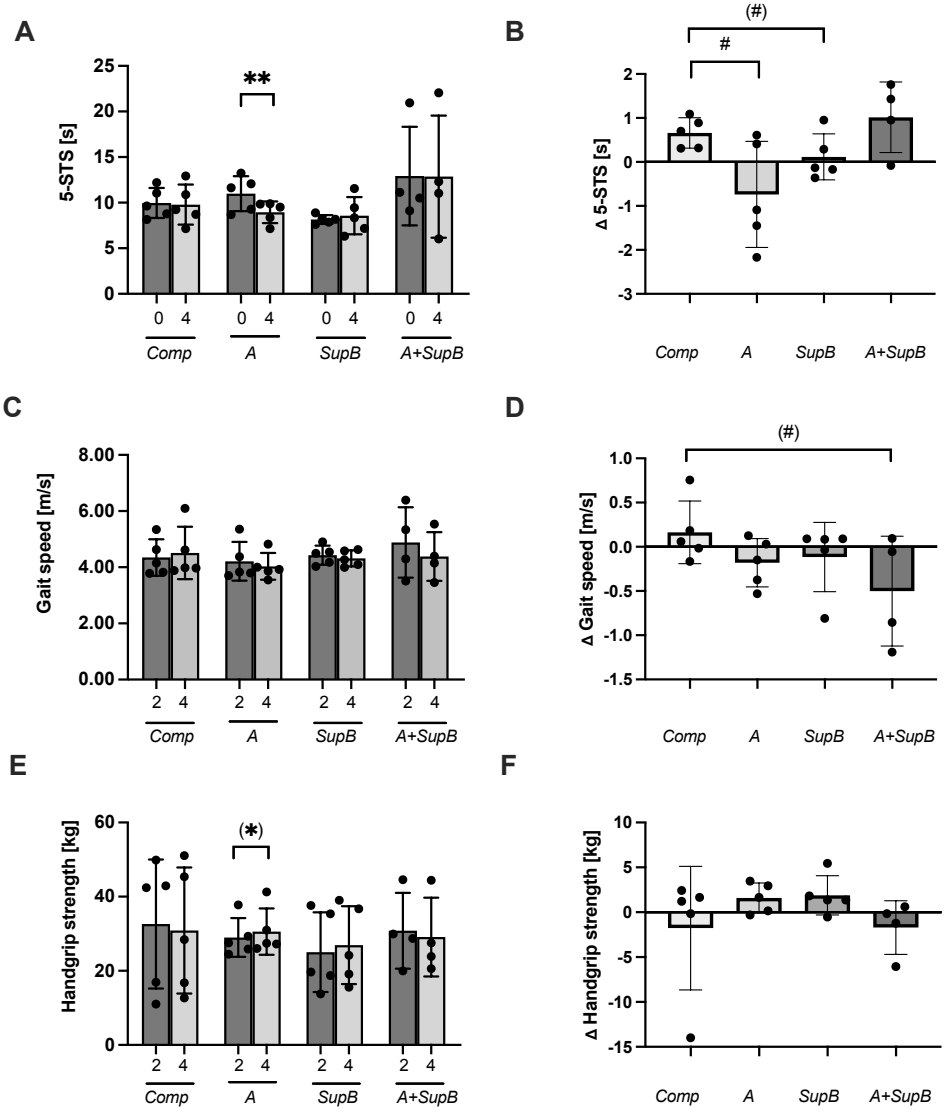


Figure 4. Effect of study products on the mobility markers gait speed (**panels A**,**B**), the 5 s sit-to-stand test (5-STS; **panels C**,**D**) and handgrip strength (**panels E**,**F**). Data at study start (0) and after (4) intervention (panel A), week 2 and 4 (**panels C**,**E**) and treatment effect expressed as differences between weeks 4 and 2 (Δ) (**panels B**,**D**,**F**) are shown. Values are expressed in scatter plots with mean ± *SD* and individual values from 19 participants (*All n* = 19; *Comp*, *A*, *SupB* each *n* = 5; *A+SupB n*= 4). Statistics: * indicate a difference within a group between study start (0) and week four (4) (t-test); # indicate a difference between groups (ANOVA). (*/#) $p < 0.1$, # $p < 0.05$, ** $p < 0.01$. Abbreviations: diets see Figure 2 *Comp*, Comparator.

2.7. Fatty acid Changes in Plasma and Erythrocyte Membrane/Red Blood Cells (RBC)

The plasma FA levels were determined at study start (0), after the wash-out phase (2) and after two weeks of intervention (4) (Figure 5 and Table S4). Comparing all plasma data at the study start (0) with after the washout period (2), when no fish was consumed, plasma levels of EPA ($p = 0.005$; Figure 5A), n−3 FA ($p = 0.02$; Figure 5B), DHA ($p = 0.008$), EPA + DHA ($p = 0.005$) decreased and others by a trend (Table S4). Further, the AA/EPA ratio increased ($p < 0.001$; Figure 5C) and n−6:n−3 ratio ($p = 0.01$; Figure 5D) after the washout period compared to the study start (Table S4).

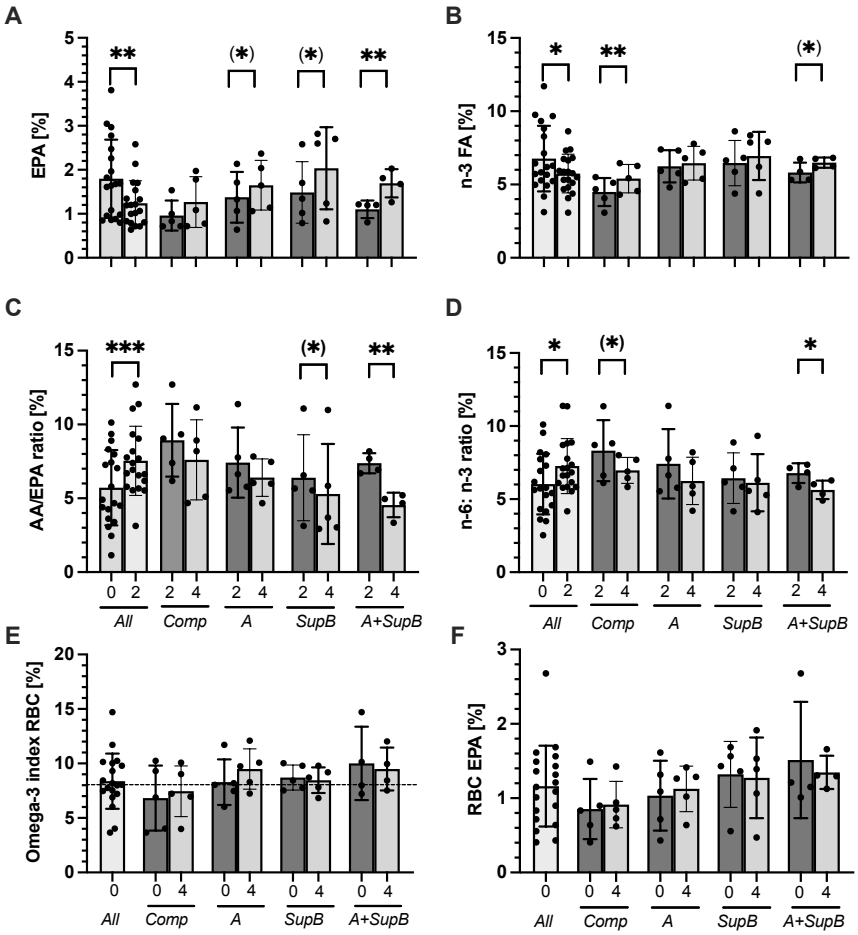


Figure 5. Effect of study products on the plasma fatty acid (FA) levels eicosapentaenoic acid (EPA; **panel A**), omega-3 fatty acids (n−3 FA; **panel B**), arachidonic acid (AA)/EPA ratio (**panel C**) and n−6 FA to n−3 FA ratio (**panel D**). Parameters were measured at study start (0), week two (2) and week four after the intervention (4). Further, red blood cell (RBC) FA were measured, namely the omega-3 index (**panel E**; marked threshold value at 8%) and the RBC EPA content (**panel F**). Values are expressed in scatter plots with mean, *SD* and individual values from 19 participants (*All n* = 19; *Comp*, *A*, *SupB* each *n* = 5; *A+SupB n* = 4). Statistics: * indicate a difference within a group between week two (2) and week four (4) (t-test). No between-groups-differences were found at week 0, 2 and 4 (ANOVA). (*) $p < 0.1$, * $p < 0.05$, ** $p < 0.01$., *** $p < 0.001$. Abbreviations: diets see Figure 2; *Comp*, Comparator.

Regarding within-group differences from week 2 to week 4, the EPA plasma concentrations tended to increase by 0.3% within the *A* group and 0.55% within the *SupB* group ($p = 0.1$; Figure 5A). Only within the *A+SupB* group, the EPA level increased significantly by 0.6% ($p = 0.006$). The DHA level did not change due to the intervention (Table S4). The EPA + DHA level did not change except for an increase within the *Comp* group (Table S4). Total n−3 FA plasma concentration increased within all interventions but only significantly within the *Comp* group by 0.9% ($p = 0.005$) and by a trend within the *A+SupB* group by 0.7% ($p = 0.05$) (Figure 5B). The AA/EPA ratio decreased from week 2 to week 4 after all supplementations but only significantly within the *A+SupB* group from 7.4 ± 0.7% to 4.5 ± 0.8% ($p = 0.006$; Figure 5C) and by a trend within the *SupB* group ($p = 0.09$). Furthermore, the n−6:n−3 ratio was lower within the *A+SupB* group at week four with 5.6 ± 0.6% compared to week two with 6.8 ± 0.1% ($p = 0.006$, Figure 5D). A reducing trend was further measured within the *Comp* group ($p < 0.01$). Minor other changes in plasma FAs were measured as shown in the Supplementary Materials in Table S4. Regarding treatment changes, which were calculated as the difference between week 4 to 2, the 18:3n−3 FA level was lowered after the *A* diet compared to the *Comp* group ($p = 0.008$). The 22:6n−3

level in the *SupB* group ($p = 0.02$) and the 20:3n−6 level within the *A* group were reduced compared to the *Comp* group ($p = 0.05$; Table S4).

The RBC FA concentration was measured at the study start (0) and after 4 weeks. The mean total Omega-3 index at the beginning was 8.4 ± 2.5% (Figure 5E), consisting of levels of EPA (1.16 ± 0.5%, Figure 5F) and DHA (7.21 ± 2.1%). Within the *A* group, the index changed from the study start from 8.29 ± 2.1% to 9.49 ± 1.9%, but not significantly, and no changes were measured comparing both time points.

2.8. Carotenoids, Vitamin E Changes in Plasma

Due to the carotenoid and tocopherol concentrations in PT, the amounts in the blood plasma were measured at four different time points and shown in Figure 6A and C at weeks 2, 3 and 4 and the delta between weeks 2 and 4. The *A* and *A+SupB* diets contained Fx in the diet and the participants took 21.4 mg or 21.6 mg daily. After one week of intake at week 3, no Fx could be measured in the blood plasma of the subjects. FxOH was detected after one week (3) (0.03 ± 0.03 µM) and two weeks (4) of *A* diet intake (0.02 ± 0.001 µM) and the level significantly increased from week 2 to 4($p = 0.007$, Figure 6A). Within *A+SupB* group, FxOH was detected after one week (3) (0.05 ± 0.05 µM) and two weeks (4) (0.08 ± 0.08) in the plasma. The change between weeks 4 to 2 (Δ) for FxOH, was higher in group *A* ($p = 0.001$; Figure 6B) and *A+SupB* group by a trend compared to the *Comp* group. A × A, to which FxOH is converted in the liver [30], could be measured in plasma at 0.003 ± 0.006 µM in only one participant after the study product *A+SupB*. Through the *A* and *A+SupB* diets, subjects consumed 0.3g of β- carotene daily. After two weeks of supplementation, plasma β-carotene increased from 0.5 to 0.7 µM. ($p = 0.02$, Figure 6C) within the *A+SupB* group. No changes between week 4 to 2 were measured (Figure 6D, Table S5). Further, carotenoids such as retinol did not change in any group, nor did the lycopene plasma levels. Furthermore, no increase in γ-tocopherol and α-tocopherol were measured (Table S5).

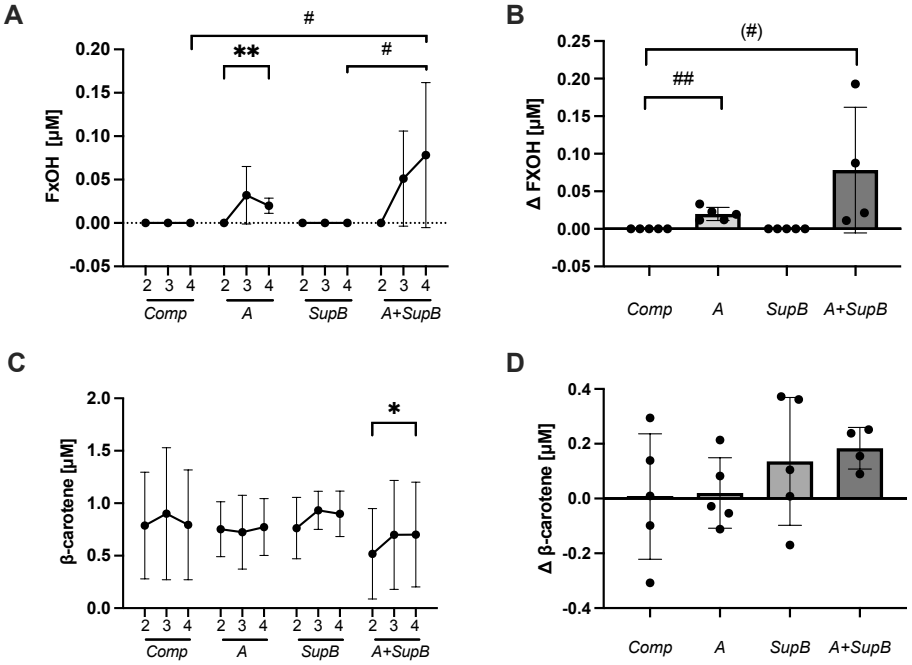


Figure 6. Plasma carotenoids changes following interventions. Fucoxanthinol (FxOH, **panels A**,**B**) and β-carotene (**panels C**,**D**) were shown before the intervention (2), after week three and one week of supplementation (3) and week 4 after two weeks of supplementation (4) (**panels A**,**C**). The treatment effect expressed as differences between weeks 4 and 2 (Δ; **panels B** and **D**) with *Comp, A, SupB,* and *A+SupB*. Values are expressed as mean and SD error bars. Statistics: *indicate a difference within a group between week two (2) and week four (4) (t-test); # indicate a difference between groups (ANOVA). (#) $p < 0.1$, */# $p < 0.05$, **/## $p < 0.01$. Abbreviations: diets see Figure 2; *Comp*, Ccomparator.

2.9. Inflammatory Parameters and the XOR as an Oxidative Stress Marker

A two-week intervention with *A* and *SupB* had no negative effect on inflammatory markers such as high-sensitivity C-reactive protein (hs-CRP), interleukin (IL)-10, IL-6 and tumour necrosis factor (TNF)-α. Comparing before (2) and after the intervention (4) within the group of *A+SupB* a trend was shown to decrease the pro-inflammatory marker IL-6 level from 5.3 ± 1.6 pg/mL at week two to 3.3 ± 1.9 pg/mL at week four (p = 0.5; Figure 7A). The treatment effect measured as the change of weeks four to two, showed a significant difference between the *A+SupB* group (−2.0 ± 1.3 pg/mL) and the *Comp* group (+1.5 ± 2.5 pg/mL; p = 0.042; Figure 7B). The hs-CRP, IL-10 and TNF-α did not change according to the diet or differ between the diets. As an oxidative stress parameter, the xanthine oxidoreductase (XOR) was measured. The XOR is the final enzyme in purine catabolism and catalyses hydroxylation to xanthine and then to uric acid. The results showed a tendency to decrease levels within the *A* and *A+SupB* groups compared to the levels before the intervention (2) and after intervention (4) ($p < 0.1$). The XOR tended to increase within the *SupB* group (Figure 7C). The treatment effect showed no effect compared to the *Comp* group only between *A* and *SupB* (Figure 7D).

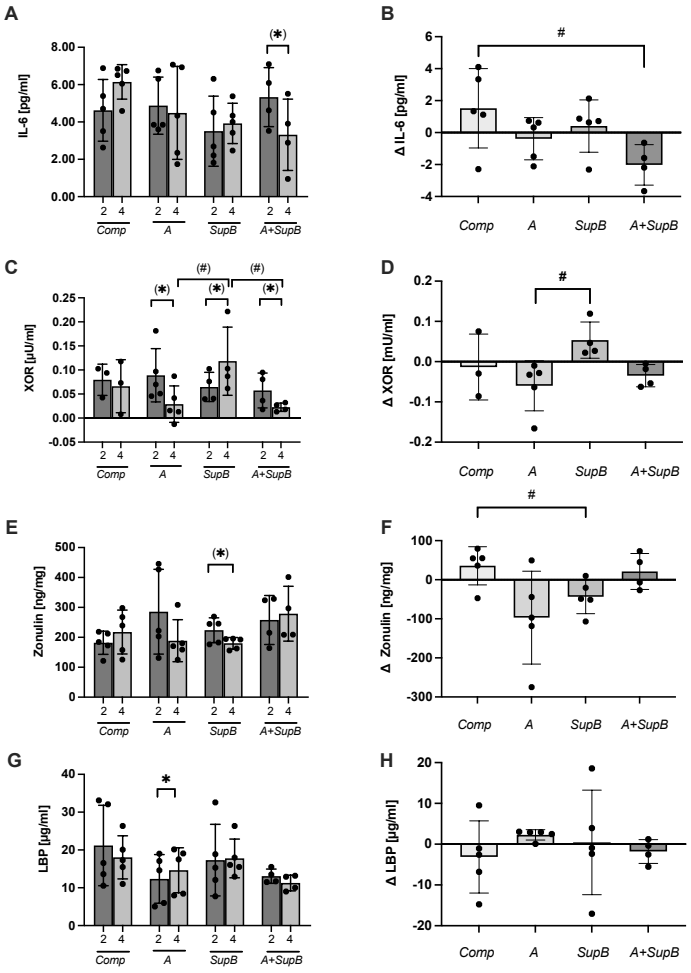


Figure 7. Effect of the study products on plasma interleukin-(IL)-6 (**panels A**,**B**), plasma xanthin oxidoreductase (XOR; **panels C**,**D**), and the gut barrier markers faecal zonulin (**panels E**,**F**) and plasma lipopolysaccharide-binding protein (LBP) in plasma (**panels G**,**H**). Data before (2) and after (4) intervention (**panels A**,**C**,**E**,**G**) and treatment effect expressed as differences between weeks 4 and 2 (Δ) (**panels B**,**D**,**F**,**H**) are shown. Values are expressed in a scatter plot with mean and *SD*-individual values from 19 participants (*Comp* , *A*, *SupB* each n = 5; *A+SupB* n = 4). Statistics: *indicates a difference within a group between week two (2) and week four (4) (t-test); $^{\#}$ indicates a difference between groups (ANOVA). (*/$^{\#}$) $p < 0.1$, */$^{\#}$ $p < 0.05$. Abbreviations: diets see Figure 2; *Comp*, Comparator.

2.10. Gut Barrier Markers and Short-Chain Fatty Acids

The gut barrier marker lipopolysaccharide-binding protein (LBP), an acute-phase protein that binds to bacterial lipopolysaccharides derived in part from translocation from the intestine and zonulin, which is an acute-phase response protein and controls intestinal permeability by reducing the stability of tight junctions (TJ), revealed small changes within the different groups. The faecal zonulin showed a trend of reduction within the *SupB* group comparing the level of 223.8 ± 41.5 ng/mg at week 2 to 180.3 ± 19.3 ng/mg at week 4 ($p = 0.088$; Figure 7E) and a treatment effect compared to the *Comp* group ($p = 0.03$; Figure 7F). The LBP was higher at week 4 with 14.6 ± 5.9 µg/mL within the *A* group compared to 12.4 ± 6.4 µg/mL at week 2 ($p = 0.045$; Figure 7G) but no treatment effect was measured compared to the *Comp* group (Figure 7H). SCFA concentrations in faeces showed no significant changes between groups or due to the interventions (Table S6).

2.11. Correlations

Spearman correlation showed a positive association between different FAs, especially EPA and the age of the participants (Figure 8A). A positive correlation was measured between the AA/EPA plasma ratio with TNF-α and IL-6 (Figure 8B,C) and the n−6: n−3 ratio with IL-6 (Figure 8D). A negative association was found between the WOMAC score and the carotenoid intake measured by the FFQ (Figure 8E). Regarding the gut barrier marker LBP, a negative association with the omega-3 Index was measured (Figure 8F).

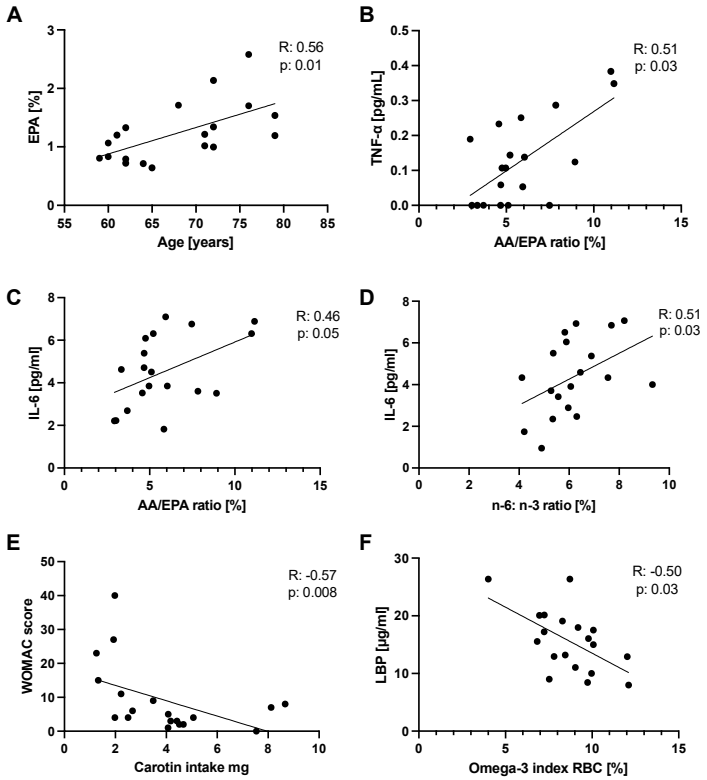


Figure 8. Positive correlations were shown by Spearman (R) correlation as the EPA plasma concentration (week 2) to the age (**panel A**), AA/EPA plasma ratio to the TNF-α (week 4) (**panel B**), Il-6 to the AA/EPA ratio (week 4) (**panel C**), and the n−6: n−3 ratio (week 4) (**panel D**). A negative association was found between the WOMAC score and the carotenoid intake measured by the FFQ (**panel E**) and the LBP level and the omega-3 index (week 4, **panel F**). Statistics: R, Spearman rho; *p*-values > 0.05. Abbreviations: EPA, eicosapentaenoic acid; AA/EPA, arachidonic- and eicosapentaenoic acid ratio; n−6: n−3, n−6 FA to n−3 FA ratio; TNF-a; tumour necrosis factor; IL-6, interleukin-6; WOMAC, Western Ontario and McMaster Osteoarthritis Index; LBP, lipopolysaccharide-binding protein; RBC, red blood cells (erythrocyte).

3. Discussion

The present study investigated the anti-aging effect of different preparations of the microalgae PT and Comparator in a randomized controlled manner. So far, PT has not been approved as a novel food; therefore, the safe intake, as well as possible health promotion effects, were investigated.

3.1. Safety and Bioavailability

Microalgae are not yet widespread on the food market and are mostly sold in capsules, tablets, or dried powder as dietary supplements. A new approach is to use the whole microalgae and convert it into novel and tasty food products with potential health-beneficial effects. The incorporation of microalgae biomass into foods has encountered some difficulties mainly due to the colour and fishy taste [31]. PT with its unique content of functional nutrients is particularly difficult to process due to its intense brown/green colouration and the oxidation of FA and carotenoids, which favours the fishy taste [32]. However, due to the combination with the vegetarian bouillon powder, the ingestion became acceptable by most study participants, although other studies rated the taste fishy and unpleasant [33]. The general intake of the β-G-rich supernatant (*SupB*) was very well accepted as it was almost tasteless when dissolved in water. Previously, it was shown that β-Gs contained in beverages and liquid test meals turned out to be the best carriers [34], as it was used in the present study.

Our study provides further evidence for safe intake by evaluating several laboratory parameters. Except for the *SupB* group, which showed an increase in LDL-cholesterol, but still within the normal reference range, no negative effects on laboratory parameters were observed. As we could not measure treatment effects compared to the *Comp*, the results still suggest health-promoting effects within the groups, such as the reduction in HOMA-index within the *A* group after the whole PT supplementation. n−3 FA could generally improve insulin sensitivity, although these results are mostly based on animal models [35]. Further n−3 FAs have been reported to lower TAG, which may lead to a reduction of the risk of cardiovascular diseases (CVD) [36]. In our study, such results could not confirm after PT supplementations (*A* or *A*+*SupB* diets), possibly because it was underpowered for such an effect. However, a pre-clinical study in Wistar rats fed a high-fat (HF) diet supplemented with 12% PT already shows a TAG and HOMA-index lowering effect compared to the HF diet without PT supplementation [37].

A reduction of cholesterol by a trend and TAG levels was measured within the *SupB* group, after supplementation with the Chrl-rich supernatant. Although our data could not confirm previous pre-clinical studies with zebrafish showing cholesterol-lowering and LDL-lowering effects following PT supernatant supplementation similar to *SupB* [38], they did suggest a possible trend. Especially in older people, in whom lipid metabolism is frequently altered [39], LDL cholesterol-lowering could be a valuable goal, since LDL cholesterol is a modifiable cardiovascular risk factor for prevention [40]. Meta-analyses have already demonstrated the β-G cholesterol-reducing and LDL-lowering effect of taking a dose >3 g of β-G per day by consuming oats and barley for at least three weeks in individuals with mild hypercholesterolemia [34]. In our study, we administered only about 0.5 g of β-G per day, which might be suboptimal. A higher supplementation of the Chrl-rich supernatant (*SupB*) could produce greater effects, which needs to be confirmed in future trials. In a pre-clinical study, the safe intake of up to 4621 mg/kg body weight of the β-G Chrl in mice has been shown [27]. For human nutrition, β-Gs are an important dietary fibre supporting preventive effects [41], and microalgae containing β-Gs like *Odontalla auritia* and *Euglena gracilis*, have already been approved by the European Food Safety Authority (EFSA). Since the recommended daily fibre levels were not reached by the participants, additional supplementation with PT Chrl-rich supernatant would be conceivable for older people and others.

In terms of FA bioavailability, the wash-out phase was effective due to the plasma FA reduction. Further, an increase of EPA was expected after the PT diets *A* and *A+SupB*, comparable to the previous study with younger participants [8]. However, no treatment effect was observed. Our study could not confirm such findings, possibly because it was underpowered. Age differences might be related to altered FA metabolism in older people, such as slower plasma clearance and lower incorporation into cell membranes [42], as well as changes related to FA release and/or β-oxidation in older people [39]. However, our present data showed that plasma n−3 FA levels were already higher in the older participants before the study than in the younger study participants [8]. This is consistent with other studies [43,44] and with our correlation analyses, which showed higher plasma EPA levels with increasing age. In addition, nutritional data also show a higher EPA+DHA intake in older women and men (65–79 years) at 232.1/277 mg/day (women/men) compared to 18–24-year-olds at 199.9/232.1 mg/day (women/men) [45]. The general recommended intake of EPA+DHA is 250–500 mg daily and might be even higher for some prevention goals [46], suggesting that no age group is meeting the recommendations. Therefore, PT could be an additional dietary source of EPA-FA. Measurement of the Omega-3 index in erythrocytes was performed to verify adequate supply. This varied between 6.8% and 10%, confirming a good supply and moderate to low risk for CVD in our study population [47]. As the Omega-3 index measured in RBC is considered a marker for long-term FA changes, no changes were expected and observed in our study after two weeks of PT supplementations. Our present study results indeed suggest a possible altered FA metabolism in older adults. Therefore, the recommended intake of EPA and DHA should be reconsidered and possibly adjusted in future for the elderly population.

Regarding the uptake of carotenoids, the present study confirms the metabolization of Fx to FxOH, as previously shown [8]. Further metabolization to A × A could only be measured in one subject. This seems to be dependent on the ingested amount of Fx, since a previously conducted study with supplementation of about 30mg Fx per day led to a significant increase of both metabolites [8]. So far, the EFSA recommends an amount of 15 mg of pure Fx per day, e.g., derived from *Undaria pinnatifida thallus* extract (wakame) [48]. Despite several possible health benefits, no health claims have been awarded so far for Fx-containing microalgae [49,50]. Future studies not only on health benefits but also regarding dose-finding and toxicity levels are needed to promote the acceptance of Fx as a valuable food supplement in future.

For tocopherol and β-carotene, PT might not be the best selection, because their content is rather low and therefore would require an intake of higher amounts of the microalgae than those chosen in our study. This might explain why we found no plasma increase of tocopherol and only some increase of β-carotene in the *A+SupB* group. This group tended to have the lowest baseline levels, suggesting that PT supplementation can increase plasma β-carotene levels as recently demonstrated [8].

In terms of gut health, minimal and mild side effects were noted after ingestion of the study products, as reported earlier [8]. Because loss of gut barrier function [2] and changes in the gut microbiome [51,52] may occur with aging, we were interested in whether PT supplementation could prevent such alterations in the study population. The gut barrier markers plasma LBP and faecal zonulin were measured to assess intestinal barrier function. The results yielded a treatment effect of zonulin, which decreases within the *SupB* group compared to the *Comp* group. Since PT contains high amounts of n−3 PUFAs, which are thought to promote the intestinal barrier [53], we expected a treatment effect as well within the *A* and *A+SupB* groups. We found no such effect, but a negative correlation between the Omega-3 index in erythrocytes and plasma LBP levels. The reduction of faecal zonulin levels within the *SupB* group could also have implications beyond the gut since zonulin is positively correlated with the concentration of pro-inflammatory cytokines (TNF-α and IL-6) and negatively correlated with muscle strength and usual physical activity [54]. In a pre-clinical study supplementing the whole EPA-rich PT biomass and β-G-rich PT biomass, we found an increase in SCFA and SCFA-producing bacteria, suggesting possible healthy

gut promoting effects [27]. However, in the present human trial, we could not confirm such findings, possibly because of smaller amounts of PT biomass administered based on a bodyweight-related dosage.

3.2. Potential Antioxidative and Anti-Inflammatory Effects

In terms of antioxidant potential in the PT biomass, the highest phenolic content was measured for both whole biomasses rich in EPA (A) and those rich in β-G Chrl (B) and half of the potential in the supernatant (Sup). The results show that for a higher phenolic content, the use of whole biomass is necessary. Previously, it was shown that PT contains fourteen phenolic compounds [55], which could be useful for human nutrition. Regarding the antioxidant potential measured by the FRAP assay, the highest value was measured for the β-G-rich PT biomass B. This biomass contained the highest Fx amount, which confirms that carotenoids and xanthophylls could have a high antioxidant potential [56]. The antioxidant effect of Fx extracts from PT has been reported previously [21]. However, the EPA-rich biomass A also exhibited antioxidant potential, as FAs may also have antioxidant activity [57].

Our human study provides the first evidence of a potential anti-oxidative effect of the EPA-rich PT biomass, as plasma XOR levels tend to be lower in group *A* and group *A+SupB*, which ingest the whole PT. In the *SupB* group, which ingested the Chrl-rich supernatant, this trend toward lowering was not observed. XOR levels are often elevated in inflammatory bowel diseases [58] and age is positively correlated with xanthine oxidase activity [59]. Therefore, the trend of reducing the XOR levels is a possible positive indication. Other studies with PT have already shown that the microalgae reduce the activity of nuclear factor kappa B (NF-κB) in mouse macrophages [13], which is activated by the production of reactive oxygen species (ROS). Moreover, it has been shown that the free radical scavenging activity is presumably related to the high Fx content of PT [30,60]. These findings are consistent with our results, as diets *A* and *A+SupB*, which have the highest Fx content, showed a trend for some XOR reduction within the groups. Not only Fx, but also n−3 FAs might have a stimulatory effect on mitochondrial function and fusion processes by reducing ROS production [61]. Higher doses and more participants are required to prove statistically significant effects.

To tackle inflammageing in elderly the current study shows potential anti-inflammatory treatment effects of the *A+SupB* group, by the reduction of the pro-inflammatory cytokine IL-6 compared to the *Comp* group. Other inflammatory parameters such as TNF-α and C-reactive protein remained unchanged. For PT, an anti-inflammatory effect has already been shown in vitro [13] and in vivo [37]. The effect could be addressed by the high Fx content, which regulates the NF-κB and NLRP3 inflammasome activation [50]. Other PT compounds such as polysaccharide and EPA could be further involved in the reduction of pro-inflammatory cytokines such as IL-6 [62,63]. A further anti-inflammatory indicator could be the reduced AA/EPA ratio and the n−6:n−3 ratio in plasma within the *A+SupB* group. Both are reliable indicators of nutritional status, and a higher n−6:n−3 ratio can lead to the development of various metabolic disorders [64]. Indeed, our correlation analyses confirm this hypothesis, as the AA/EPA ratio was positively associated with IL-6 and TNF-α levels and the n−6:n−3 ratio with IL-6 level.

Considering age-related muscle wasting, supplementation of PT could prevent such deficits and promote functional ability. If higher amounts of PT are administered, it could even be an additional source of protein. n−3 FAs are thought to have an anabolic effect [65] and indeed cause improvements in muscle strength and protein synthesis [65,66], however, other studies did not find such effects [67,68]. The current study showed some modest treatment effects after the PT supplementation in terms of improvement in mobility markers such as the 5-STSs in the *A* group and gait speed within the *A+SupB* group compared to the *Comp* group. Regarding the effects of carotenoids, higher intake is associated with better grip strength [69], and reduction of hip fractures [70], therefore PT in higher doses may be a valuable nutrient for older adults. Higher dietary intakes of α-tocopherol and lycopene are negatively associated with the WOMAC score (a higher score means more severe pain

and functional limitations) [71]. The present data suggest this association, as a negative correlation was found between the WOMAC score and carotenoid intake. In addition, supplementation with n−3 FAs, carotenoids, and vitamin E has been found to improve working memory in older adults [72], which closely matches the constituent nutrients of PT and suggests a potential benefit in the elderly.

3.3. Limitations

The study shows limitations, as some effects such as cholesterol- and TAG-lowering effects are demonstrated only in the *SupB* group, but not in the *A+SupB* group, which consumed the same amount. It has been expected that the effects would occur in both groups. The findings from this pilot trial need confirmation from larger confirmatory trials.

4. Materials and Methods

4.1. Participant Selection

Both females and males, aged between 60 to 90 years were screened with a BMI between 18.5 to 30 kg/m^2 and a weight of more than 50 kg. Participants had to be willing to adhere to certain dietary rules (no significant changes in diet, no fish or seafood and no probiotics) and the willingness to follow the prescribed diet for the duration of the study (14 days). Physical activity should not be changed throughout the study. Exclusion criteria were the intake of intestinal therapeutics, antibiotics, immunosuppressants, cholesterol-lowering drugs or similar, relevant violations of the dietary protocol, occurrence of relevant diseases—diabetes mellitus, lipid metabolic disorders, severe acute COVID disease within the last six weeks according to a case-by-case decision, and acute COVID disease. Inclusion and exclusion criteria remained unchanged throughout the study. The study was conducted according to the Declaration of Helsinki at the Center for Clinical Nutrition Stuttgart at the University of Hohenheim in 2021 and has been approved by the local Ethical Committee (Ethik-Kommission der Landesärztekammer Baden-Württemberg, F-2021-061), and was registered at ClinicalTrials.gov (NCT05120791).

4.2. Study Design and Outcome Parameters

The study was designed as a randomized, single-blind, 1:3 Comparator controlled (considered a Placebo with only vegetarian bouillon powder) parallel group with four visits (Figure 2). An intervention period of two weeks was chosen due to the increase in FA of the previous human study [8] and to estimate a safe intake of the β-G supernatant (*SupB*). When participants fulfilled all inclusion and no exclusion criteria at the study start, visit parameters were assessed. They were requested to eat no fish and seafood for all four weeks. The first two weeks were planned as a wash-out period for the n−3 FAs and the second visit was after two weeks (2). The third visit was after three weeks (3) and the fourth visit was after four weeks (4). The study was planned as a proof-of-principle pilot study; therefore, no formal calculation of case numbers was performed.

The primary outcome parameter was the effect on inflammation markers (hs-CRP, IL-6, 10). Secondary outcome parameters were laboratory parameters, n−3 FA in plasma, erythrocyte/ RBC, the improvement of the n−6: n−3, and AA/EPA ratio, carotenoids, body weight, waist circumference, handgrip strength, BIA assessing, gut barrier marker (LBP, zonulin) and SCFAs. For muscle function 5-STS, gait speed, handgrip, and the WOMAC questionnaire were used. Diet was evaluated using a food diary and an FFQ. At the study entry, the subject got verbal and written informed consent and the inclusion and exclusion criteria were obtained. The demographic data were collected, the medical history and the collection of former and current medication. The bioelectrical impedance analysis and the 5-STS were done and the fasting venous blood collection for blood markers and FAs (plasma and RBC), as well as anthropometric measurements and hand strength were done at the institute. The nutrition diary, FFQ and the instructions for the faecal sample collection were handed out.

At the second visit (after week 2) the products were distributed, and fasting blood samples were taken to analyse blood markers and FAs (plasma). Faecal samples were collected and used for SCFA measurement and barrier permeability. Further, the gait speed and the 5-STS were completed. Participants started to take the capsules after the second visit. In the third visit after three weeks (3), fasting blood samples were obtained for blood markers and FAs (plasma) and the instructions for the faecal sample collection were handed out. The fourth visit after four weeks (4) was the same as the study entry and on the second visit and participants returned their investigational products.

4.3. Study Products, Randomization and Blinding

The EPA and Fx-rich PT biomass (biomass A) was produced under nutrient-repleted conditions in flat panel airlift reactors. The biomass was harvested and concentrated via centrifugation to 250–270 g/L (Clara 20, Alfa Laval, Glinde, Germany) as described before [8]; see also Figure 1. The cells were disrupted using a bead mill (PML 20, Bühler, Uzwil, Switzerland), and freeze-dried (VaCo 5, Zirbus). For the generation of supernatant, PT biomass B was produced in flat panel airlift reactors under nutrient-depleted conditions (without nitrogen or phosphorous source in the culture media) for several days before harvesting. Harvesting and concentrating were performed by centrifugation to 250–270 g/L (Clara 20, Alfa Laval) as described before [8]. After cell disruption via bead milling (PML 20, Bühler), the biomass was centrifuged again, and the liquid supernatant was separated from the biomass pellet to obtain the supernatant (SupB). Afterwards, the supernatant was freeze-dried (Avanti J-26 XP, Beckman Coulter, Brea, USA). The detailed nutrient composition of the study products is shown in Table S7.

For a better taste of PT and for the blinding, 1.3 g vegetarian bouillon powder (Gemüse Bouillion, Knorr, Hamburg, Germany) was added to the lyophilised biomass/supernatant. The study population was divided into four groups receiving different study products. The first study product was the *Comp*, which consisted of daily 1.3 g of vegetarian bouillon powder solved in 150 mL plain water. The second study product *A* consisted of 2.3 g of lyophilised biomass A containing 312.1 mg n−3 FA (293.5 mg EPA+DHA) per day and additionally 1.3 g of vegetarian bouillon powder suspended in water (Table 3). The amount of PT was chosen based on the national n−3 PUFA/EPA + DHA recommendation of 250 to 300 mg per day. The third study product was based on β-G recommendations of yeast β-G by the EFSA, which is 600 mg per day [73]. Because Chrl is not yet a novel food, a total concentration of 500 mg Chrl was taken, which was 1.8 g daily of lyophilised Sup B biomass and 1.3 g vegetarian bouillon powder suspended in water (*SupB*). The fourth study product *A+SupB* consisted of 2.3 g of biomass A, 1.8g of SupB and 1.3 g of vegetarian bouillon powder suspended in water (Table 3). The study groups are named as the study products *Comp*, *A*, *SupB* and *A+SupB*.

All participants were given a diary to document their intake of the study products. The participants were instructed to suspend the study products freshly in a glass of water (around 150 mL) before usage and to take these products at breakfast time. To meet the compliance criteria, participants had to achieve 100% compliance, if the intake was forgotten for one day, the intake was extended by one day. The study was single-blinded; study participants were blinded for the study products. The randomization was generated using the Randlist software (datinf GmbH, Tübingen, Germany, available at randomisation.eu).

Table 3. Daily nutrient intake of the participants through the study products.

Daily Nutrient Intake	*Comp*	*A*	*SupB*	*A+SupB*
Protein g/day	0.007	0.81	0.05	0.85
β-1,3-glucan g/day	0	0.17	0.54	0.71
FAs [mg/day]	13.02	678.3	22.9	688.2
n−3 FA	0.34	312.51	1.79	313.97
n−6 FA	5.19	63.56	5.57	63.94
SFA	3.69	90.85	7.35	94.51
MUFA	3.80	104.38	7.70	108.28
PUFA	5.53	483.07	7.85	485.38
n−6: n−3 ratio	20.00	20.43	20.47	20.90
AA/EPA ratio	0.00	0.22	0.00	0.22
EPA+DHA	0.03	293.65	1.20	294.82
EPA	0.02	288.52	1.44	289.94
DHA	0.01	5.13	0.06	5.19
Carotenoids [mg/day]				
Fucoxanthin	0.00	21.39	0.22	21.61
β-carotene	0.01	0.29	0.01	0.30
α-carotene	0.00	0.00	0.00	0.00
Lycopene	0.00	0.21	0.01	0.21
Vitamine E [mg/day]				
α-Tocopherol	0.00	0.10	0.03	0.13
β-Tocopherol	0.00	0.00	0.00	0.00
γ-Tocopherol	0.00	0.01	0.00	0.01

Abbreviations: diets see Figure 2; FA, fatty acids; n−3 FA, omega-3 fatty acids; n−6 FA, omega-6 fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n−6: n−3, n−6 FA to n−3 FA ratio; AA/EPA, arachidonic- and eicosapentaenoic acid ratio; EPA, eicosapentaenoic acid; DHA, docosahexaeonic acid.

4.4. Blood Plasma, Serum and Faecal Measurements

Blood samples were collected in two ethylenediaminetetraacetic acids (EDTA)-coated tubes, and one serum tube. One EDTA- tube was used for blood count analysis (Sindelfingen laboratory GbR, Sindelfingen, Germany). The other one was centrifuged at 500 g for 7.5 min at 15 °C, followed by plasma separation. Plasma was stored at −80 °C until further analysis (FA, carotenoid, inflammatory markers, LBP). The RBC (underneath the centrifuged plasma) were washed with NaCl and centrifuged three times and stored at −80 °C. The serum tube was centrifuged for 15 min at 3000× g at 15 °C. 1 mL Serum was stored at −80 °C and the rest was used for quantification of gamma-glutamyl transferase (γ-GT), aspartate aminotransferase (AST), alanine transaminase (ALT), prealbumin, albumin, triacylglycerols (TAG), total cholesterol (Chol), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), haemoglobin, haematocrit, erythrocytes, leucocytes, platelet count, haemoglobin beta-N-1-deoxy fructosyl component of haemoglobin (HbA1c), insulin, HOMA-index, plasma glucose, thyroid-stimulating hormone = thyrotropin (TSH), C-reactive protein (CRP), uric acid, 25-hydroxy vitamin D (25(OH)D) (measured at Laborärzte Sindelfingen GbR, Sindelfingen, Germany).

Stool samples were collected before the study visit (max. two days before) in two tubes and stored at −20 °C at home or transported directly to the laboratory. In our laboratory, the two tubes were stored at −80 °C until further analysis. One tube was used to measure the gut barrier marker zonulin and the other for SCFA analysis.

4.5. Antioxidant Assays

The total phenolics content (TPC) was measured as was measured using Folin–Ciocalteu method as described previously for microalgae (compounds) by Neumann et al. [21]. It is expressed as gallic acid equivalents (GAE). The unit GAE (mg/g dry weight (DW)) describes how many milligrams of gallic acid are needed to achieve the same antioxidant

effect as one gram of (biomass) sample. For conducting the TPC assay a sample solution with a defined biomass concentration was prepared. 20 mg freeze-dried biomass sample was suspended in 5 mL of dimethyl sulfoxide. 150 µL Folin–Ciocalteu reagent (diluted 1:10 in ddH_2O) and 120 µL sodium carbonate solution (75 g/L) were mixed with 30 µL sample in a 96-well plate. The samples were then incubated and protected from light for 120 min at room temperature. Afterwards, the absorbance was measured at 765 nm using a plate reader (infinite M200 PRO, Tecan Group, Männedorf, Switzerland). Each sample was analysed in triplicate. As a blank, each sample was mixed with sodium carbonate solution and ddH_2O, to obtain an individual blank for each sample. Using a calibration curve, made with gallic acid (10–50 mg/L), the total phenolic content of the defined sample solution was calculated as gallic acid equivalents (GAE in mg/L). Via the biomass concentration of the defined sample solution (see above), the total phenolic content per (biomass) dry weight (DW) was calculated (GAE in mg/g_{DW}). As part of the antioxidative effect, the ability of substances to reduce substances by absorbing electrons was quantified by the ferric reducing antioxidant power assay (FRAP) and expressed in the form of FRAP values (ferric-reducing-antioxidant-power-values). The unit FRAP (mM/g_{DW}) describes how many µM of iron (II) sulphate are needed to produce the same antioxidant effect as one gram of (biomass) sample. The FRAP assay was performed based on the method of Benzie and Strain [74] as described by Neumann et al. [21]. For conducting the assay a solution with a defined biomass concentration was prepared as already described for the TPC assay. FRAP reagent was prepared with 10 mL sodium acetate buffer (300 mM, pH 3.6), 1 mL TPTZ solution (10 mM in 40 mM HCl) and 1 mL of iron (III) chloride solution (20 mM) immediately before the experiment and stored at 37 °C until use. In a 96-well plate, 220 µL FRAP reagent was mixed with 10 µL of sample solution before incubation at 37 °C for 90 min. Absorbance was measured at 593 nm using a plate reader (infinite M200 PRO, Tecan Group). Each sample was analysed in triplicate. Individual blanks for each sample were prepared by mixing 10 µL of sample with 220 µL of ddH_2O. To establish a calibration curve iron (II) sulphate was used (100–1000 µM) and the results are presented as FRAP values (iron (II) sulphate equivalents.

4.6. Quantification of Plasma and Erythrocyte Fatty Acids, Carotenoids and Tocopherols

The same protocol was used for plasma and erythrocyte fatty acids quantification by gas chromatography with mass spectrometry (GC/MS) as described before [8]. The transesterification was performed as previously reported with slight modification [75]. In short, 2 µL 10,11-dichloro-undecanoic acid (DC-11:0) as internal standard and 2 mL methanol (Carl Roth, Karlsruhe, Germany) with 1% sulphuric acid for transesterification were added to 100 µL sample. During incubation at 80 °C for 1 h, the samples were sonicated three times for 5 min. Thereafter, samples were cooled down on ice, mixed with 0.5 mL demineralised water and 0.35 saturated NaCl solution and extracted with 2 mL n-hexane. Prior to measurement, 5 µL tetradecanoic acid- ethyl ester (14:0) was added as the second internal standard. Fatty acid methyl esters (FAMEs) were analysed by GC/MS on a 5890 series II/5972A system (Hewlett-Packard, Waldbronn, Germany). A commercial standard (Supelco, Taufkirchen, Germany) was used for identification based on mass spectra and retention times in full scan mode, quantification was carried out in selected ion monitoring mode [76]. Five saturated fatty acids (14:0, 16:0, 18:0, 20:0, 22:0) were measured by GC/MS. Unsaturated fatty acids were measured in form of the monounsaturated fatty acids (MUFAs) 14:1 n−5 (myristoleic acid), 16:1, 16:1 n−7 (palmitoleic acid), 17:1, 18:1 n−9 (oleic acid), 18:1 (isomer of oleic acid), 20:1 n−9 (gondoic acid) and the polyunsaturated fatty acids (PUFAs) 18:2 n−6 (LA), 20:2 n−6 (eicosadienoic acid), 18:3 n−6 (γ-linolenic acid), 18:3 n−3 (ALA), 20:3 n−6 (dihomogammalinolenic acid), 20:4 n−6 (AA), 20:5 n−3 (EPA), 22:5 n−3 (DPA), 22:6 n−3 (DHA). The n−6:n−3 ratio in plasma levels was calculated from the total area of the n−6 FA (18:2 n−6, 20:2 n−6, 18:3 n−6, 20:3 n−6, 20:4 n−6) divided by the total area of the n−3 FA (18:3 n−3, 20:5 n−3, 22:5 n−3, 22:6 n−3). For the AA/EPA ratio, percentual contributions of both fatty acids were determined and divided through each

other. According to its definition, the Omega-3 index was determined by the percentual share of EPA and DHA in erythrocyte membranes relative to the sum of 26 fatty acids like in the method of Omegametrix (HS-Omega-3-Index®) [77].

Carotenoids and tocopherols were measured in plasma as described in detail previously. For FX, FXOH and A × A analysis, 100 µL of human plasma were mixed with 200 µL of ethanol/butanol (50/50 (*v*/*v*)) containing 5 mg butylated hydroxytoluene (BHT). After vigorous mixing and centrifugation (17,000× *g* and 4 °C, 10 min, Heraeus Fresco 17, Thermo Fischer Scientific, Waltham MA, USA) clear supernatants were injected into a Shimadzu HPLC system (Mc Kinley Scientific, New York, USA) equipped with an autosampler (15 °C), an UV detector (450 nm) and a C18 reversed-phase column (2.6 µm F5 100Å 150 × 4.6 mm, Kinetex, Phenomenex Ltd., Aschaffenburg, Germany) at 40 °C. A mixture of methanol/water (85/15 (*v*/*v*)) at 1.0 mL/min for 15 min was used as mobile phase [8].

For determination α/γ-tocopherol, lutein/zeaxanthin, lycopene, β-cryptoxanthin, α/β-carotene, and retinol, 40 µL of plasma were mixed with 200 µL of ethanol/butanol (50/50 (*v*/*v*)) containing 12 µL beta-apo-8′-carotenal-methyloxime/100 mL as internal standard. After vigorous mixing and centrifugation (17,000× *g* and 4 °C. 10 min; Heraeus Fresco 17, Thermo Fischer Scientific, Waltham, MA, USA), clear supernatants were injected into a Shimadzu HPLC system (Mc Kinley Scientific, New York, NY, USA) equipped with an autosampler (5 °C), and a ReproSil 80 ODS-2 column (3 µm, 250 × 4.6 mm) (Dr. A. Maisch GmbH, Ammerbuch-Entringen, Germany) at 40 °C. A mixture of acetonitrile/1.4-dioxane/methanol (82/15/3; *v*/*v*) containing 100 mmol/L ammonium acetate and 0.1% trimethylamine, at 1.5 mL/min for 20 min was used as mobile phase. Carotenoids were detected using an UV detector (450 nm). Retinol and tocopherols were detected using a fluorescence detector (Ex/Em at 325/ 470 nm for retinol and Ex/Em at 296/325nm for α/γ-tocopherol). carotenoids and tocopherols were quantified using authentic commercial standards and respective standard curves [78]. Quantification of FX [8], other carotenoids and tocopherols [79] in study products were performed as described before.

4.7. Inflammatory Markers and Anti-Oxidative Stress Parameter

The inflammatory markers were performed using the Human IL-10 ELISA Kit (RAB0244), the Human IL-6 ELISA Kit (RAB0306; Millipore Sigma Aldrich, Saint Louis, USA) and Human TNF-α Immunoassay (HSTA00E; R&D Systems, Inc., Minneapolis, USA) according to the manufacturer's protocol. For IL-10 and IL-6 plasma was used and TNF-α serum was used. For an oxidative stress marker, the xanthin oxidase fluorometric assay kit was performed following the manufacturer's protocol (Item No. 10010895; Cayman).

4.8. Analysis of the Intestinal Permeability Marker Plasma LBP, Faecal Zonulin, and Faecal SCFA

The measurement of the faecal samples for zonulin measurement were diluted to the working concentration in sample buffer using stool sample tubes (K6998SAS; Immundiagnostik AG, Bensheim, Germany) and for LBP analysis, 10 µL of blood plasma was used and processed. Zonulin and LBP were measured using the enzyme-linked immunosorbent assay kit (K5600, KR6813, Immundiagnostik AG, Bensheim, Germany) following the manufacturer's protocols. For SCFA analysis the same method was used as described before [8] by gas chromatography (Clarus 690, Perkin-Elmer, Waltham, MA, USA).

4.9. BIA, Muscle Function Test (5 STS, WOMAC, Gait Speed, Hand Grip Strength)

The BIA was done as a multi-frequency BIA according to Kyle et al. [80] (Data Input, Pöcking, Germany). The individual ASM and ASMI were calculated with the dietic pocket guide (www.dieteticpocketguide.com (accessed on 21 September 2022)) with the individual weight, gender, age, body height, BIA resistance (50 kH) and reactance (50 kH).

For muscle function, the 5-STS was conducted as previously described by Jones et al. [81]. Using the WOMAC questionnaire, the activity levels of the study participants were assessed at weeks zero and eight. WOMAC was developed for patients with osteoarthritis and registers signs of physical disability and relevant changes in health status because of

treatment intervention [82]. This questionnaire consists of three scales: pain, stiffness, and function. The higher the WOMAC value, the higher the pain, stiffness, and functional limitations. The maximum score which can be achieved is 96 (maximum score for pain is 20, 8 for stiffness and 68 for functional limitations). Gait speed in elderly was assessed in meters per second measured as a four-meter usual walking speed test two times. The hand grip strength was measured on each side two times (Hydraulic hand dynamometer, Jamar).

4.10. Diet Evaluation

Food diary of four weeks was evaluated with EPISpro 2016 (Software, Willstätt-Legelshurst, Germany). The dietary pattern was documented by the validated German Food Frequency Questionnaire (FFQ) [83].

4.11. Statistical Analyses

All parameters were tested for normal distribution using the Kolmogorov–Smirnov test. Normally distributed data, one-way ANOVA was used to compare statistically significant differences ($p < 0.05$) between microalga diet groups and the *Comp*. Variances were tested with the Brown–Forsythe test. Tukey's multiple comparison post hoc test was used for equal variances, and Dunnett's T3 multiple comparisons test was used for unequal variances. Within one group a t-test was performed between weeks two and four or study entry and week four. The study product treatment effect was measured as differences between the values of weeks 4 and 2 (Delta, Δ). This change was calculated for each subject. The four groups were compared with each other with a one-way ANOVA and a t-test respectively to the *Comp* group. For the mortality and body composition data, the value after intervention (4) minus the study start value (0) was calculated. Correlation analyses were performed with two-tailed Spearman-rank correlation. All statistical analyses were performed using GraphPad Prism version 9.4.1 (GraphPad Software, San Diego, CA, USA) and IMB SPSS statistics 25 (IBM Corp., Armonk, NY, USA).

5. Conclusions

The study demonstrates anti-inflammatory effects and potential antioxidant effects, as well as possible preservation of functional ability after ingestion of the whole microalgae and the β-G rich supernatant in the elderly. In addition, the present results provide the first evidence of the safe use of the β-G-rich microalgae supernatant over a two-week period and reconfirm the safe ingestion of whole PT in humans. Supplementation with PT could be a source of functional compounds that contribute to healthy aging from a nutritional perspective and, could be a candidate for anti-aging effects as a supplement. Higher doses of PT with higher amounts of functional compounds are warranted for future interventions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/md20110716/s1, Table S1: Food Frequency Questionnaire at the study start; Table S2: Laboratory parameter and inflammatory markers; Table S3: Mobility markers and body composition at baseline and the study change; Table S4: Plasma fatty acid blood composition at different time-points; Table S5: Carotenoid plasma levels at the study start, after two-weeks and four weeks of intervention; Table S6: The concentration of short-chain fatty acids (SCFA) per dry mass in faecal samples at week 2 and week 4 after the intervention within the four study groups; Table S7: Nutrient composition of *Comp, A, SupB* and *A+SupB* diets used in the study.

Author Contributions: Conceptualization, L.S. and S.C.B.; methodology, L.S., K.L. and A.M.-A.; formal analysis, L.S.; investigation, L.S., K.L. and A.M.-A.; resources, K.F., U.S.-S., W.V. and J.F.; data curation, L.S.; writing—original draft preparation, L.S.; writing—review and editing, S.C.B.; visualization, L.S.; supervision, L.S.; project administration, S.C.B.; funding acquisition, L.S.; S.C.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Gesellschaft für Angewandte Vitaminforschung (GVF) and the Society of Nutrition and Food Science (SNFS), to the topic "Healthy aging-The contribution of

adequate nutritional status with essential nutrients (Gesundes Altern—Der Beitrag eines adäquaten Ernährungsstatus mit essenziellen Nährstoffen)" as a master degree project by Annika Köhler.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the local Ethical Committee (Ethik-Kommission der Landesärztekammer Baden-Württemberg (F-2021-061; 27 April 2021) and was registered at ClinicalTrials.gov (NCT05120791).

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

Data Availability Statement: Not applicable.

Acknowledgments: Many thanks to the participants and students who made the project possible, as well as the measurements of the SCFAs by Andreas Rings.

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

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3. Diskussion

Der Trend zu einer ressourcenschonenden Ernährung wird in den nächsten Jahren durch den Anstieg der Weltbevölkerung und globalen Anforderungen verstärkt werden. Neue alternative Ressourcen wie Insekten, *In-vitro*-Fleisch und Algen werden in den Vordergrund rücken [5]. Mikroalgen haben aufgrund ihrer vielfältigen bioaktiven Nährstoffe ein großes ernährungsphysiologisches Potenzial für die menschliche Ernährung. Die Algenforschung steht aufgrund von hohen Produktionskosten und einer fehlenden Einarbeitung des Algenmaterials in schmackhafte Lebensmittelzubereitungen allerdings noch an ihren Anfängen [9,17]. Mikroalgen sind jedoch vielversprechende Organismen für die nachhaltige Produktion von Lebens- und Futtermitteln sowie für die Verwendung als funktionelles Lebensmittel [10,50,146]. Es gibt nur wenige bereits zugelassene Mikroalgen und die meisten werden in Form von Nahrungsergänzungsmittel verkauft. Die Mikroalge PT hat aufgrund ihrer Nährstoffzusammensetzung ein großes Potenzial für die humane Ernährung eingesetzt zu werden. Ein neuartiger Ansatz ist die Verarbeitung der ganzen Mikroalge, ohne eine Extraktion von Nährstoffen. Da die Mikroalge PT unter das Novel-Food-Gesetz fällt und nicht als Lebensmittel zugelassen ist, muss eine sichere Einnahme, der Nachweis der Bioverfügbarkeit der Nährstoffe sowie der Nachweis von potenziellen gesundheitlichen Vorteilen für die humane Ernährung erfolgen. Hier setzen unsere Untersuchungen an.

Drei zentrale Fragestellungen wurden durch die drei Arbeiten, die der Promotionsarbeit zugrunde liegen, adressiert:

1. Bioverfügbarkeit und Sicherheit von Mikroalgennährstoffen bei gesunden Probanden
2. Bioverfügbarkeit und biologische Wirkungen von Mikroalgennährstoffen aus zwei Mikroalgenpräparationen in Mäusen
3. Biologische Wirkungen von Mikroalgennährstoffen aus zwei Mikroalgenpräparationen auf Alterungsprozessen bei älteren Menschen

Es konnte gezeigt werden, dass die untersuchten Mikroalgenpräparationen aus PT sicher *in vivo* verabreicht werden können, sowohl bei Mäusen als auch beim Menschen. Zudem werden wichtige Mikroalgennährstoffe wie EPA, FX und Chrl ausreichend intestinal aufgenommen, um biologische Wirkungen zu erzielen und es gibt erste Hinweise dafür, dass der Verzehr von Mikroalgen gesundheitliche Vorteile bewirkt.

Die Ergebnisse dieser drei Arbeiten werden im Folgenden detailliert diskutiert.

Humane Interventionsstudie zur Untersuchung der sicheren Einnahme und Bioverfügbarkeit von Inhaltsstoffen der Mikroalge PT

Da die Mikroalge PT bislang nicht für die humane Ernährung getestet wurde, war das Ziel des Projektes die Durchführung einer Humanstudie. Für die Studie wurde die EPA- und Fx-reiche Biomasse als Interventionsprodukt verwendet. Diese wurde in einem Photobioreaktor am Fraunhofer-Institut kultiviert. Für die Verbesserung der Bioverfügbarkeit wurde die Zellwand mechanisch mit einer Laborkugelmühle aufgeschlossen [46], anschließend gefriergetrocknet und bei –20°C gelagert. Eine der größten Herausforderungen bei der Entwicklung eines schmackhaften Interventionsproduktes war die bräunliche Farbe und der fischige Geschmack der Mikroalge, der die Verarbeitung erschwerte. Eine Kombination mit einer vegetarischen Gemüsebrühe und dem anschließenden Lösen in Wasser wurde von den meisten Studienteilnehmern akzeptiert, obwohl bereits andere Studien den Geschmack von PT als fischig und unangenehm bewerten [147]. In einer randomisierten Pilotstudie mit 22 gesunden Probanden im Alter von 18 bis 50 Jahren wurde in einem Cross-over-Verfahren über zwei Wochen täglich die EPA/Fx-reiche Biomasse von PT (5,3 g/Tag) oder eine Fischölkapsel verabreicht. Die Einnahme der Fischölkapsel diente der Vergleichbarkeit des möglichen FS-Anstiegs und dem Vergleich mit einer herkömmlichen n-3-FS-Quelle. Die gewählte Dosis von ca. 300 mg EPA und DHA täglich wurde in Anlehnung an die Empfehlung der Deutschen Gesellschaft für Ernährung gewählt [148]. In einem weiteren Versuch wurden die Auswirkungen der Einnahme von gekochtem Lachs bei neun Probanden, die eine Menge von 185 g pro Woche verabreicht bekamen, zusätzlich untersucht, was im Vergleich zu PT und Fischöl zu einer ähnlichen EPA- und DHA-Aufnahme führte. Für die Bewertung der sicheren Einnahme im Rahmen der menschlichen Ernährung wurden verschiedene Laborparameter gemessen und Nebenwirkungen bei den Teilnehmern dokumentiert.

Die Ergebnisse der vorliegenden Studie zeigen, dass nach der Einnahme von PT keine Laborparameter verändert waren und die Mikroalgen im Allgemeinen gut vertragen wurden. Einige Probanden berichteten über leichte Bauchbeschwerden wie Blähungen, Magenschmerzen, Aufstoßen, Verstopfung oder Durchfall. Diese Darmbeschwerden wurden nicht nur nach dem Verzehr von PT, sondern auch nach der Einnahme von Fischöl berichtet. Aufgrund der Darmbeschwerden wurden zwei Darmbarrieremarker in den Stuhlproben und Plasma der Probanden gemessen, die jedoch keine negativen Veränderungen nach der PT-Einnahme aufwiesen. Auch zeigte die Konzentrationsmessung der kurzkettigen FS sowie das Darmmikrobiom gemessen in den Stuhlproben keine Veränderungen nach der PT-Einnahme. Anzeichen für einen möglichen gesundheitlichen Vorteil war die Zunahme des Darmbakteriums *Akkermansia* nach zweiwöchigem Fischverzehr und als Trend nach der PT-Einnahme. Die Messung dieser Parameter und Dokumentierung der Nebenwirkungen lassen

insgesamt auf eine sichere Einnahme der Mikroalge PT für die gewählte Dosierung für den Menschen schließen. Bisher kann eine tägliche Einnahme von PT von bis zu 14 Tagen als sicher angesehen werden, da sowohl in der früheren Mausstudie als auch in der hier durchgeführte Humanstudie die Mikroalge täglich über diesen Zeitraum verabreicht wurde [46]. Daher müssen Langzeitstudien diese Annahme bestätigen.

Bezüglich der Bioverfügbarkeit der FS zeigen die Ergebnisse erstmalig nach einer 14-tägigen PT-Einnahme den Anstieg von n-3-FS- als auch von EPA-Konzentration im Blutplasma. Dies lässt auf die Bioverfügbarkeit der FS für den Menschen schließen. Daher bestätigt die Studie die Bioverfügbarkeit von EPA und n-3 FS aus PT nach dem alleinigen Aufschließen der Zellmembran und anschließendem Verzehr der getrockneten Biomasse für den Menschen. Auch zeigt die Reduzierung des n-6: n-3 Verhältnisses im Plasma der Probanden eine verbesserte n-3-Versorgung nach der zweiwöchigen PT-Einnahme. Unerwartet war, dass der EPA-Anstieg mit 0,9 % nach einer zweiwöchigen Einnahme von PT vergleichbar mit dem nach dem Fischkonsum war. Durch den Verzehr von Lachs nahmen die Probanden eine deutlich geringere EPA-Menge von 198 mg täglich zu sich als durch die tägliche PT-Einnahme mit 282 mg. Der vergleichbare EPA-Anstieg ist vermutlich auf den höheren DHA-Gehalt im Fisch (101 mg DHA/Tag gegenüber 5 mg DHA/Tag durch PT) zurückzuführen, da bereits gezeigt wurde, dass höhere DHA-Dosierungen zu einem EPA-Anstieg führen [149]. Dieser Umstand könnte, wie früher vermutet, an einer Rückumwandlung von DHA zu EPA liegen [150]. Neuere Studien zeigen, dass bei DHA-Einnahme ein verlangsamter Stoffwechsel von Plasma-EPA und eine damit verbundene Akkumulation stattfindet. Auch können zusätzliche EPA Mengen nachweislich in DHA umgewandelt werden [149]. Daher ist der direkte Verzehr von EPA notwendig und PT könnte eine alternative Nährstoffquelle zum Fisch essen darstellen. Durch den direkten Verzehr von PT, welche in einem kontrollierten Photobioreaktor kultiviert wird, könnten weitere Nachteile, wie die Belastung mit Schwermetallen und Mikroplastik wegfallen, die durch den Verzehr von Fisch entstehen [151].

Des Weiteren liefert die durchgeführte Humanstudie durch den Konzentrationsanstieg von Fx im Plasma der Probanden nach einer zweiwöchigen Einnahme von PT weitere Hinweise auf die Bioverfügbarkeit des Carotinoids für den Menschen. Nicht nur Fx, sondern zugleich höhere Konzentrationen der Metaboliten FxOH, und AxA wurden im Blutplasma nachgewiesen, was auf eine Absorption im Darm und in der Leber hindeutet. Auch unterstützt die Annahme der Bioverfügbarkeit von Fx für den Menschen, dass vor der PT-Einnahme kein Fx gemessen werden konnte. *In vitro* konnte bereits eine Bioverfügbarkeit von Fx aus PT von 20,4 ± 5 % nachgewiesen werden. Zusätzlich zeigte eine präklinische Studie mit Mäusen eine Anreicherung von Carotinoiden in Leber und Fettgewebe, was auf eine Bioverfügbarkeit schließen lässt. Jedoch werden auch erhöhte Mengen von Fx ausgeschieden [84]. Daher sind

weiterführende pharmakokinetischen Studien beim Menschen und die Messung von Plasmakonzentrationen in kürzeren Zeitabständen nötig, um die Absorption und den Metabolismus von Fx aus PT zu bestimmen. Da die EFSA eine tägliche Menge von bis zu 15 mg reinem Fx pro Tag empfiehlt [152], sind weitere Toxizitätsstudien und klinische Studien hinsichtlich des gesundheitlichen Nutzens erforderlich. Mausstudien zeigen bereits eine sichere Einnahme höherer Dosierungen, jedoch wurde auch ein Anstieg des Gesamtcholesterin und eine Organfärbung der Fäzes aufgezeigt [153]. Die gesammelten Stuhlproben der Probanden der vorliegenden Studie wiesen dagegen eher leichte Grünfärbung auf, was auf den hohen Chlorophyllgehalt von PT zurückzuführen ist und in Zukunft weiter untersucht werden muss [154].

Weiterführend zeigen die Ergebnisse der Humanstudie nach einer zweiwöchigen Einnahme von PT die Bioverfügbarkeit des β-Carotin aufgrund eines Konzentrationsanstiegs im Plasma. Zuvor konnte bereits *In-vitro* eine Bioverfügbarkeit von 19 % gemessen werden [84]. Für andere Carotinoide wie Lutein und Zeaxanthin, aber auch für Tocopherole, wurden nach der PT-Einnahme keine Veränderungen im Blutplasma gemessen. Auch konnte kein Anstieg des Retinols im Blutplasma gemessen werden, was aufgrund der Pro-Vitamin-A-Aktivität und dem Anstieg von β-Carotin zu erwarten war. Die zugrundeliegenden Mechanismen für keinen Anstieg der Carotinoide und Retinol sind nicht bekannt, jedoch könnte eine Wechselwirkung mit anderen Carotinoiden wie Zeaxanthin, die auch in PT enthalten sind [155], oder ein Vorhandensein von Retinol in der Nahrung mögliche Gründe sein [156]. Dies schließt die Bioverfügbarkeit dieser Stoffe nicht aus, es sind allerdings weitere genauere pharmakokinetische Studien erforderlich, um die Bioverfügbarkeit insoweit zu beurteilen. Bei der Untersuchung der Bioverfügbarkeit von Carotinoiden und Vitaminen aus PT ist daher zukünftig auf weitere endogene und exogene Faktoren zu achten. Die enthaltenen Ballaststoffe in PT, wie Chrl, könnten die Bioverfügbarkeit von Carotinoiden reduzieren. Andererseits könnte durch die Einnahme von PT mit einer Fettquelle die Bioverfügbarkeit der Nährstoffe gesteigert werden [76]. Zudem muss ein weiterer Aspekt wie die Stabilität der Nährstoffkonzentration bei der Lagerung von Mikroalgen untersucht werden [157]. Zusammenfassend zeigen die Ergebnisse der humanen Pilotstudie eine sichere Einnahme der Mikroalge PT und die Bioverfügbarkeit vereinzelter Nährstoffe. Es konnten keine relevanten negativen Veränderungen von Laborparametern oder Darmbarrieremarkern gemessen werden, daher kann PT als Lebensmittel für den Menschen weiterhin in Betracht gezogen werden.

Überprüfung der sicheren Einnahme von zwei unterschiedlich kultivierten PT-Biomassen und Untersuchung von möglichen darmgesundheitlichen Vorteilen in Mäusen

Da bislang nur wenig über eine sichere Einnahme höherer Dosierungen, sowie darmgesundheitliche Auswirkungen nach der Einnahme verschieden kultivierter PT-Biomassen bekannt ist wurde eine *In-vivo*-Studie mit weiblichen C57BL/6J-Mäusen durchgeführt. Die EPA-/Fx-reiche und Chrl-reiche PT-Biomassen wurden durch die Anpassung von Kultivierungsbedingungen im Fraunhofer-Institut generiert und in 5, 15 bzw. 25 wt% dem Futter zugesetzt. Die Kontrolldiät und Mikroalgendiäten waren beides isokalorisch und isoproteinogen. Die klinischen Parameter wurden täglich ermittelt und die Tiere alle drei Tage gewogen. Zur Bewertung einer sicheren Einnahme wurden Darmbarriere- und inflammatorische Marker gemessen.

Die Ergebnisse der Studie zeigen, dass nach der 14-tägigen Supplementierung keine Gewebeveränderungen festgestellt werden konnten und keine histologischen Untersuchungen der Leber und des Magen-Darm-Trakts. Nach der Einnahme der Mikroalgendiäten waren die gemessenen Darmbarrieremarker unverändert, jedoch wurde ein Anstieg der TNF-α-mRNA-Expression im Ileum nach den höchsten Dosierungen beider PT-Biomassen, sowie der niedrigsten der Chrl-reichen Biomasse gemessen. Dieser Effekt ist negativ zu bewerten, jedoch waren andere gemessene Darmbarriere- und inflammatorische Marker im Vergleich zur Kontrollgruppe unverändert. Da Chrl aus PT [112] und aus anderen Mikroalgen bereits immunmodulatorische und antioxidativen Wirkungen zugesprochen werden [144,158], könnte ein TNF-α-Anstieg im Darm mit dieser Wirkungsweise zusammenhängen. Die Ergebnisse waren unerwartet, da bei einer Einnahme von PT von entzündungshemmenden und nicht entzündungsfördernden Auswirkungen auszugehen ist [127], was durch die Verbesserung des Verhältnisses von n-6-: n-3-FS im Lebergewebe unterstützt wird. Ein geringeres Verhältnis beider FS weist gesundheitliche Vorteile auf, wie die Unterdrückung von Entzündungserkrankungen [66]. Daher müssen die zugrundeliegenden Mechanismen des Anstiegs der TNF- α-mRNA-Expression im Ileum weiter untersucht werden. Da jedoch keine anderen Sicherheitsparameter verändert wurden ist von einer sicheren Fütterung von Nagern von einer PT-Konzentration von mindestens 15 % im Futter auszugehen.

Für die Bewertung der Bioverfügbarkeit der Nährstoffe aus den PT-Biomassen wurden das Gewicht und die Futteraufnahme bestimmt, sowie der Energiegehalt in den Fäzesproben mittels Bombenkalometrie gemessen. Dabei war festzustellen, dass trotz vergleichbarer Kalorienaufnahme gegenüber den Mäusen der Kontrolldiät insbesondere die Tiere mit den höchsten PT-Diäten eine Gewichtszunahme aufwiesen. Auch hatten einige PT-Diäten einen statistisch geringeren Energieverlust im Vergleich zur Kontrolldiät. Sowohl die

Gewichtszunahme als auch ein reduzierter Energieverlust in den Fäzes deuten auf eine Energieabsorption durch die PT-Einnahme beider Biomassen hin. Die vorangegangene Mausstudie nach Supplementierung der 25-%-EPA/Fx-haltigen PT-Diät wies einen erhöhten fäkalen Energieverlust auf [46], wohingegen die vorliegende Studie gegenteilige Ergebnisse hervorbrachte. Diese Effekte müssen in weiteren Langzeitstudien untersucht werden, jedoch deuten die Ergebnisse auf eine Energieaufnahme durch beide PT-Diäten hin.

Zur Bewertung der Verfügbarkeit von n-3-FS und EPA wurden die Konzentrationen in Leber und Fettgewebe gemessen. Ein Anstieg von EPA im Lebergewebe war nach den PT-Diäten zu verzeichnen. Wie erwartet führte die EPA-/Fx-reiche-Diät im Vergleich zur Chrl-reichen Diät aufgrund ihrer höheren EPA-Konzentration zu einer größeren Absorption von EPA in der Leber und im Fettgewebe. Die Ergebnisse bestätigten somit erneut die Bioverfügbarkeit von FS in Mäusen, wie in der vorangegangenen Studie [46]. Berechnungen ergaben, dass Algenmengen mit einem Gehalt von bis zu 4621 mg Chrl, 920 mg EPA und 231 mg Fx pro kg Körpergewicht täglich ohne Nebenwirkungen vertragen werden. Solche Dosen lassen sich nicht ohne weiteres auf die Situation beim Menschen extrapolieren. Die entsprechenden Mengen sind in der menschlichen Ernährung in der Regel niedriger, jedoch werden für medizinische Zwecke häufig höhere Dosen verabreicht.

Neben der Bestätigung der unbedenklichen Einnahme höherer Dosierungen beider kultivierten PT-Biomassen, wurde die Einnahme von PT bezüglich darmgesundheitlicher Vorteile untersucht. Durch die verschiedenen Nährstoffe von PT, denen bereits gesundheitsfördernde Eigenschaften, wie die Bildung von kurzkettigen FS, die Förderung von kurzkettigen FS-produzierenden Bakterien und die Stärkung der Darmbarriere zugesprochen werden, ist auch nach einer Einnahme von PT von einer möglichen darmgesundheitlichen Verbesserung auszugehen [137,159,160]. Die Ergebnisse der vorliegenden Studie geben erste Hinweise darauf, wie die Erhöhung der Konzentrationen von Essigsäure und Buttersäure in den Fäzes nach der Chrl-reichen Diät, sowie Essigsäure und Propionsäure nach der EPA-/Fx-reichen PT-Diät. Diese gemessenen Effekte waren nicht wie erwartet dosisabhängig. Durchgeführte Korrelationsanalysen bestätigten jedoch einen Zusammenhang zwischen einer höheren EPA-, Fx- und Chrl-Aufnahme und höheren Konzentrationen kurzkettiger FS in Fäzes. Daher geben die Studienergebnisse erste Hinweise auf eine mögliche Förderungen von kurzkettigen FS, die bei der Aufrechterhaltung der Darm- und Immunhomöostase beteiligt sind [161]. Im nächsten Schritt wurde das Darmmikrobiom sequenziert und aufgrund der erhöhten Bildung von kurzkettigen FS nach den PT-Diäten war eine Veränderung zu vermuten. Die bakterielle Vielfalt veränderte sich nicht, jedoch wurden Veränderungen auf verschiedenen Bakterienebenen gemessen. Zum einen zeigte sich eine Reduzierung des Verhältnisses der Bakterien *Firmicutes* zu *Bacteriodes* nach allen PT-Diäten auf Stammebene im Vergleich zur

Kontrolldiät. Ein senkender Effekt oder eine Wiederherstellung eines Ungleichgewichtes dieses Verhältnisses kann grundsätzlich als positiv angesehen werden, da höhere Verhältnisse mit Fettleibigkeit und geringgradigen Entzündungen in Verbindung stehen [162]. Da die Mäuse jedoch gesund waren, ist von keinem Ungleichgewicht auszugehen und muss weiter untersucht werden. Andere Studien zeigen jedoch auch ähnliche reduzierende Effekte nach der Einnahme von β-Glucan aus Gerste [163], Laminarin aus Makroalgen [164,165] und n-3-FS [136]. Fernerhin zeigte die Sequenzierung eine Erhöhung der FS-produzierenden *Akkermansia* nach der Chrl-reichen Diät auf Gattungsebene. Die genauen Veränderungen auf Spezienebene waren jedoch nicht eindeutig und müssen zukünftig weiter untersucht werden. Der Anstieg von *Akkermansia* zeigen auch andere Studien nach β-1,3-Glucan [166], und n-3-FS Supplementierung vor allem von *Akkermansia muciniphila* [136], dem eine probiotische Funktion zugeschrieben wird [167]. Diese Effekte sind auch durch die PT-Einnahme denkbar, da in der vorangegangenen Humanstudie des ersten Promotionsprojektes der Verzehr von Fisch und die Einnahme der EPA-/Fx-reichen PT-Biomasse zu einem Anstieg von *Akkermansia* führten. Die Betrachtung und Untersuchung dieses Bakteriums auf Spezienebene sowie weitere Darmbakterienveränderungen müssen in weiteren Studien untersucht werden. Zusammenfassend zeigen die Ergebnisse der vorklinischen Studie, dass die gewählten PT-Dosierungen beider Biomassen bei Mäusen nach der Einnahme zu einem gesundheitsfördernden Anstieg von kurzkettigen FS und vereinzelten Veränderungen des Mikrobioms führen. Diese präbiotische Wirkung wurde erstmalig für die Chrl- und EPA/Fx-reiche PT-Biomasse nachgewiesen, die auch zukünftig für die humane Ernährung von Relevanz ist.

Humane Interventionsstudie zur Untersuchung von potenziellen gesundheitlichen Vorteilen für ein gesundes Alter nach der Einnahme der EPA/Fx-reichen Biomasse und des Chrl- reichen Überstandes von PT

Ziel des dritten Promotionsprojekts war es, mögliche gesundheitsfördernde Wirkungen z. B. entzündungshemmende Eigenschaften oder einen präventiven Nutzen nach der Einnahme der EPA/Fx-reichen PT-Biomasse, des Chrl-reichen Überstandes und der Kombination aus beidem bei älteren Menschen zu untersuchen. Zudem wurden weitere Sicherheitsaspekte der Einnahme sowie die Bioverfügbarkeit untersucht. Dafür wurde eine randomisierte, verblindete, Kontrollgruppe-kontrollierte, vierarmige Studie mit einer zweiwöchigen Wash-out-Phase und einer zweiwöchigen Interventionsphase durchgeführt. Die Probanden waren im Alter von 60 bis 90 Jahren, mit einem BMI zwischen 18,5 und 30 kg/m^2. Auch in diesem Projekt wurde die PT-Mikroalge auf zwei Arten kultiviert. Die erste Studiengruppe erhielt die gefriergetrocknete EPA-/Fx-reiche ganze PT-Biomasse als Interventionsprodukt. Die zweite Gruppe erhielt den Chrl-reichen Überstand, der aus der Chrl-reichen Biomasse gewonnen wurde. Die dritte

Gruppe erhielt eine Kombination (Kombi) der Präparate der Gruppen 1 und 2. Die vierte Gruppe galt schließlich als Kontrollgruppe und erhielt Gemüsebrühe, die auch in allen anderen Studienprodukten enthalten war. Die Menge der eingenommenen EPA-/Fx-reichen PT-Biomasse wurde auf Grundlage der nationalen Empfehlung für EPA + DHA von 250 bis 300 mg pro Tag gewählt, wie bereits in der ersten Humanstudie [148]. Die Einnahmemenge des Chrl-reichen Überstandes, basierte auf den β-Glucan-Empfehlungen der EFSA von 600 mg pro Tag [114]. Da Chrl aus PT bis zum derzeitigen Zeitpunkt nicht zugelassen ist, wurde eine Gesamtkonzentration von 500 mg Chrl gewählt.

Die Ergebnisse der Studie bezüglich der Sicherheitsparametern nach der Einnahme der PT-Diäten zeigten ähnliche Nebenwirkungen wie in der vorangegangenen Humanstudie des ersten Projektes. Dazu zählten unter anderem Blähungen, abdominales Grummeln, Magenschmerzen und Stuhlverfärbungen. Nach der Einnahme der gesamten EPA-/Fx-reichen Biomasse wurden keine negativen Veränderungen und nach der Chrl-reichen Diät nur eine Erhöhung des Low-Density-Lipoprotein (LDL)-Cholesterins festgestellt, das jedoch weiterhin im Normalbereich lag. Die Ergebnisse deuten daher auf eine sichere Einnahme der EPA/Fx-reichen als auch des Chrl-reichen Überstandes von PT bei älteren Menschen hin.

Des Weiteren wurde die Eignung von PT zur Prävention altersabhängiger Erkrankungen untersucht und es gibt erste Hinweise, dass der Verzehr von PT gesundheitliche Vorteile bewirkt. Die Untersuchung von Mobilitätsmarkern der älteren Menschen zeigten einen Behandlungseffekt durch eine Verbesserung des „Fünf-Sekunden-Sitz-Steh-Test“ und der Ganggeschwindigkeit. Da die Aufnahme von Carotinoiden mit einer Steigerung der Griffkraft [168] und einer Verringerung von Hüftfrakturen [169] zusammenhängt, ist eine ausreichende Zufuhr von Carotinoiden für ältere Menschen von hoher Bedeutung. Da die Ergebnisse der Studie zeigten, dass in der Kombi-Gruppe, in der ein niedrigerer Plasmawert zu verzeichnen war, einen signifikanten Anstieg des β-Carotin-Plasmaspiegels nach der Supplementierung zu messen war, könnte PT als eine zusätzliche Quelle eingesetzt werden. Die eher geringen Verbesserungen könnten auf die niedrige Dosierung der PT-Diäten und Nährstoffe zurückzuführen sein.

Des Weiteren sanken die „Homeostasis Model Assessment (HOMA)“-Indizes der Probanden, ein Marker für die Insulinresistenz, die eine EPA-/Fx-reiche Biomasse zu sich nahmen. Erhöhte Serumcholesterinwerte stehen mit einer verminderten Insulinsekretion und damit einer höheren Insulinresistenz in Verbindung [170]. Da die Insulinresistenz im Alter zunimmt und somit ein höheres Risiko besteht, an Typ-2-Diabetes zu erkranken [171], könnte die Senkung der Insulinresistenz bei älteren Menschen ein Hinweis auf einen potenziellen positiven Effekt nach der PT Einnahme sein. Diese cholesterinsenkende Wirkung konnte bereits *in vivo* an Ratten [172] und Mäusen nach der Einnahme von PT nachgewiesen werden

[131]. Einen weiteren Hinweis auf eine mögliche Veränderung des Lipidstoffwechsels durch die PT-Einnahme ist die Reduzierung der Triglyceride der Probanden infolge der Einnahme des Chrl-reichen Überstandes. Da insbesondere ältere Menschen häufig einen veränderten Lipidstoffwechsel aufweisen [173], könnte eine lipidsenkende Wirkung gesundheitliche Vorteile haben [174]. Eine Veränderung des Lipidstoffwechsels konnte bereits *In vivo* an Zebrafischen durch die Supplementierung des Chrl-reichen PT-Überstandes erzielt werden [126]. Die Ergebnisse geben daher Hinweise auf einen möglichen präventiven Vorteil nach einer PT-Einnahme, der jedoch in weiteren Studien und höheren Dosierungen bestätig werden muss.

Ein weiterer Aspekt der Studie war die Untersuchung der Darmgesundheit bei älteren Menschen, da diese häufig einen Verlust der Darmbarrierefunktion aufweisen [141]. Die Hypothese war, dass die Darmbarriere verbessert und kurzkettige FS durch eine Supplementierung von PT ansteigen, aufgrund der Ergebnisse der vorangegangenen Mausstudie. Die Daten der Teilnehmer zeigten eine Reduzierung des Darmbarrieremarkers Zonulin der Probanden nach der Einnahme des Chrl-reichen Überstandes im Vergleich zur Kontrollgruppe. Dies lässt auf eine Verbesserung der Darmbarriere schließen. Innerhalb der Gruppe der EPA-/Fx-reichen Diät wurde dagegen ein gegenteiliger Effekt und Anstieg für den Darmbarrieremarker Lipopolysaccharid-bindendes Protein (LBP) festgestellt. Bezogen auf die Messung der kurzkettigen FS in den Stuhlproben konnten keine Konzentrationsveränderungen gemessen werden. Da die vorangegangene Mausstudie im zweiten Promotionsprojekt einen Anstieg von kurzkettigen FS zeigte, ist von einer zu gering gewählten Dosierung der Mikroalge PT und des Chrl-reichen Überstandes (500 mg/ Tag) in beiden Humanstudien auszugehen. Da ältere Menschen insofern geringe Mengen von kurzkettigen FS aufweisen, wäre eine Förderung ein möglicher Therapieansatz für eine gestörte Darmbarriere [175].

Weitere präventive Wirkungen wurden untersucht. Durch die Reduzierung des proinflammatorische Zytokin IL-6 nach der PT-Kombi Diät und im Vergleich zur Kontrolldiät ist von einer entzündungshemmenden Wirkung von PT auszugehen. Ein weiterer Indikator dafür war die Reduzierung des AA/EPA- und des n-6: n-3-Verhältnisses im Plasma der Gruppe mit der PT-Kombi Diät. Höhere Verhältnisse neigen dazu, Entzündungen zu fördern [176] und ein unausgewogenes Verhältnis wird mit der Entwicklung von Herz-Kreislauf-Erkrankungen in Verbindung gebracht [66,177]. Da Entzündungen im Alter häufig auftreten und als „Entzündungs-Altern“ bezeichnet werden [178], könnte die Zufuhr von PT weitere positive Auswirkungen auf proinflammatorische Zytokine haben und eine mögliche Nahrungsergänzung für ältere Menschen darstellen.

Des Weiteren wurde die antioxidativen Wirkung von PT untersucht. Mit steigendem Alter nimmt die antioxidative Kapazität ab [178,179]. Da die Inhaltsstoffe von PT Radikalfängeraktivitäten aufweisen, wie Fx [128] und n-3-FS [180], könnte eine zusätzliche Einnahme antioxidative Wirkungen erzielen. Dieser Hypothese wurde nachgegangen. Die Xanthinoxidoreduktase (XOR), ein Marker für die Messung von oxidativem Stress war als Trend nach der EPA-/Fx-reichen und PT-Kombi Diät reduziert. XOR katalysiert die Oxidation von Hypoxanthin zu Xanthin und von Xanthin zu Harnsäure [181]. Der Effekt nach der PT-Einnahme ist somit als positiv zu bewerten, da Korrelationsanalysen zeigen, dass ein steigendes Alter positiv mit der XOR-Aktivität korreliert [182]. Jedoch sind die Regulierungen durch XOR weiter zu untersuchen, da sowohl physiologische als auch pathologischer Zustände durch XOR reguliert werden [181]. Weitere Messungen von Markern zur Untersuchung des oxidativen Stresses sind notwendig. Neben der Messung des oxidativen Stresses bei den Probanden wurde auch die antioxidative Kapazität der verschiedenen PT-Biomassen mittels Eisen (III)-Reduktionsvermögen (FRAP-Assay) untersucht. Das höchste Potenzial wies die Chrl-reiche PT-Biomasse auf. Daher wäre auch die Einnahme der Chrl-reichen Biomasse und nicht nur der Chrl-reiche Überstand für die menschliche Ernährung denkbar für das Erreichen einer stärkeren antioxidativen Wirkung. Die Einnahme der Chrl-reichen Biomasse konnte im zweiten Promotionsprojekt mit Mäusen bereits als sicher eingestuft werden. Weitere Untersuchungen im klinischen Zusammenhang sind nötig, um allgemeine antioxidativen Wirkung von PT zu bestätigen. Insgesamt zeigt die klinische Studie die mögliche Eignung von PT zur Prävention altersabhängiger Erkrankungen, jedoch müssen diese Wirkungen durch höhere Dosierungen verstärkt und in weiteren klinischen Untersuchungen bestätigt werden. Neuste Studien zeigen das Interesse von Mikroalgen als funktionelles Lebensmittel für die Förderung des gesunden Alterns [183].

Zusammenfassung

Zusammenfassend konnte im Rahmen des Promotionsprojektes erstmals die sichere Einnahme zweier verschiedener PT-Biomassen und des Chrl-reichen Überstandes *in vivo* an Mäusen und in zwei Humanstudien gezeigt werden (Abbildung 4). Es wurden neue Erkenntnisse über ernährungsphysiologisch relevante Bestandteile wie EPA, Fx und β-Carotin gewonnen. Zudem konnte die Bioverfügbarkeit nachgewiesen werden, ohne dass die Biomasse, abgesehen von der Kugelmahlung, weiter aufgearbeitet werden muss. Insbesondere die n-3-FS und vor allem EPA wurden in vergleichbaren Mengen wie aus Fischölkapseln im Blutplasma gemessen und in Mäusen in Leber und Fettgewebe angereichert, was auf eine gute Bioverfügbarkeit schließen lässt. Daher kann PT als eine alternative EPA-Quelle für die humane Ernährung zukünftig erwogen werden. Zudem zeigen die Ergebnisse eine Absorbierung und Metabolisierung von Fx zu FxOH und AxA, jedoch sind zukünftige Toxizitätsstudien erforderlich. Auch geben die Ergebnisse Hinweise auf eine

Bioverfügbarkeit des β-Carotin aus PT, wobei die tägliche Empfehlung von 5 bis 15 mg pro Tag nicht überschritten werden sollte [184]. Inwieweit PT eine Proteinquelle darstellt, muss in weiteren Studien untersucht werden. Eine Einnahme von PT zeigte potenzielle gesundheitliche Vorteile auf, wie nach der Chrl-reichen Diät vor allem bei Mäusen in Form von darmgesundheitlichen Effekten und bei älteren Menschen durch eine Verbesserung des Zonulin. Weitere Veränderungen wie die Reduzierung des IL-6, die Reduzierung von XOR, die Verbesserung von Mobilitätsparametern bei älteren Menschen, sowie eine Reduzierung des n-6: n-3- und AA/EPA-Verhältnisses innerhalb beider Humanstudien deuten auf entzündungshemmende sowie antioxidative Wirkungen hin. Diese potenziellen gesundheitlichen Vorteile nach einer PT-Einnahme müssen durch weitere klinische Studien bestätigt werden. Insgesamt zeigen die Studien die grundsätzliche Eignung der Mikroalge PT für die menschliche Ernährung und bestätigen die sichere Einnahme, die Bioverfügbarkeit ausgewählter Nährstoffe und potenzielle gesundheitliche Vorteile, was die Zielsetzung dieser Arbeit war. Die Mikroalge PT könnte in Zukunft als neues Lebensmittel in Betracht gezogen werden (Abbildung 4).

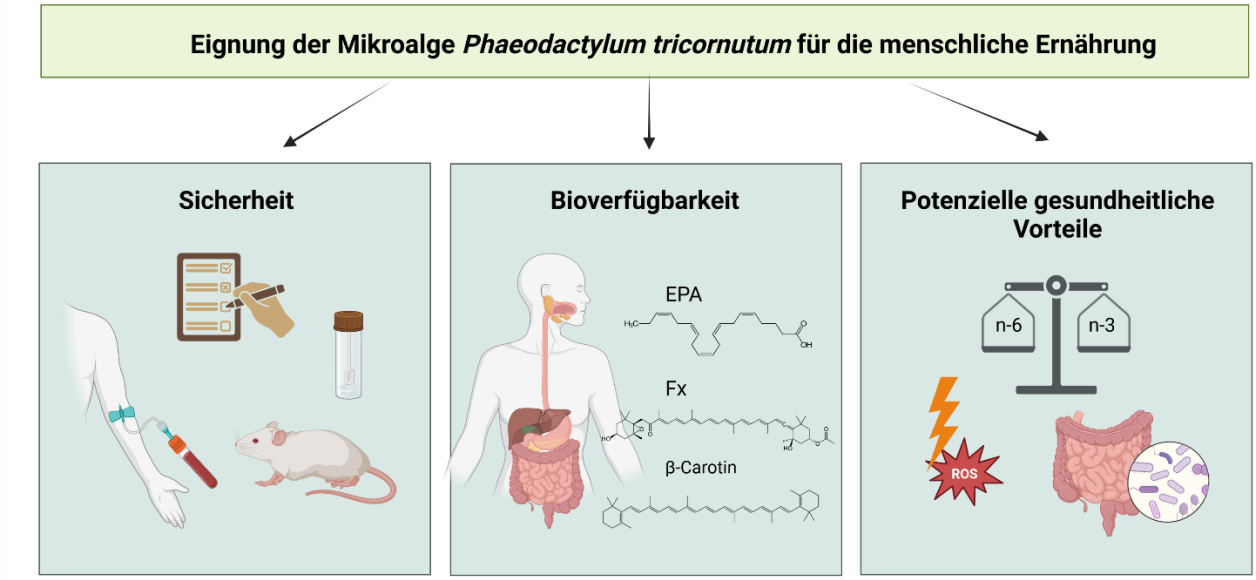

Abbildung 4. Zusammenfassung der erzielten Ergebnisse im Zuge der Promotion. Es wurden Sicherheitsparameter, Bioverfügbarkeit und potenzielle gesundheitliche Vorteile der PT-Einnahme in Maus- und Humanstudien untersucht. Das Bild wurde mit BioRender.com erstellt.

Ausblick

Die Untersuchungen bezüglich der Eignung der Mikroalge PT für die humane Ernährung werden aufgrund der vielversprechenden Ergebnisse der Promotionsarbeit fortgesetzt. Zukünftig werden weitere Untersuchungen in Richtung Toxikologie und Allergologie

durchgeführt, um die Sicherheitsbedenken der qualifizierten Sicherheitsvermutung (QPS) zu widerlegen [120]. Damit PT jedoch als Lebensmittel verwendet werden kann, müssen nicht nur die gesetzlichen Bestimmungen erfüllt werden, sondern auch der Geschmack und die Beschaffenheit des Mikroalgenprodukts weiter verbessert werden, um dieses nachhaltige Lebensmittel marktfähig zu machen. Erste Prototypen konnten bereits entwickelt und in einer Verkostungsstudie an der Universität Hohenheim getestet werden. Zudem gibt es bereits andere sensorische Überlegungen zu möglichen Darreichungsformen, wie PT in Cookies zu integrieren [147]. Jedoch muss vor allem der Geschmack für ein Lebensmittelprodukt verbessert werden, was zukünftig eine der größten Herausforderungen sein wird.

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Danksagung

An dieser Stelle möchte ich meinen herzlichen Dank an alle Personen aussprechen, die mich während meiner Dissertation tatkräftig unterstützt haben. Besonders möchte ich meinem Doktorvater Prof. Dr. Stephan C. Bischoff, für seine motivierende und engagierte Betreuung danken, die maßgeblich zum Erfolg meiner Projekte beigetragen hat.

Ebenso möchte ich meine aufrichtige Wertschätzung meinen Mentoren und Gutachtern, Prof. Dr. Jan Frank und Prof. Dr. Walter Vetter, aussprechen, die mir wertvolle Ratschläge während meiner Promotion gegeben haben.

Ein besonderer Dank gebührt auch Frau Dr. Ulrike Schmid-Staiger und Konstantin Frick vom Fraunhofer-Institut für Grenzflächen- und Bioverfahrenstechnik IGB für die hervorragende Zusammenarbeit und Unterstützung bei der Produktion der Mikroalgen. Weiterhin möchte ich mich bei Katja Lehnert für ihre wertvolle Hilfe bei der Fettsäuremessung und bei Dr. Alexander Montoya-Arroyo für die Messung der Carotinoide und Vitamine bedanken. Ein weiteres Dankeschön gilt Andreas Rings für die Unterstützung bei analytischen Fragen und Anna Schweinlin für die tatkräftige Hilfe bei der Durchführung der Humanstudien.

Ebenfalls möchte ich meinen aufrichtigen Dank an meine geschätzten Kolleginnen und Kollegen richten, allen voran Prof. Dr. Axel Lorenz, Dr. Sabrina Bilotta, Dr. Julia Zimmermann, Dr. Benjamin Seethaler, Louisa Filipe-Rosa, Dr. Nils Noelle, Patricia Peterson, Eva Haasis, Yvonne Soltow, Nina Constroffer und Dagmar Schuhmacher für den regen Austausch und die gegenseitige Unterstützung. Ein besonderer Dank geht auch an meine Projektkolleg:innen aus Schweden Dr. Lina Tingö, Dr. Ashley Hutchinson und Prof. Dr. Robert Brummer, sowie an Dr. Morten Jensen von GSK für die angenehme Zusammenarbeit. Meinen Dank möchte ich auch Dr. Jens Pfannstiel und Dr. Klaus Schwadorf von der Core Facility der Universität Hohenheim aussprechen, die verschiedene Inhaltsstoffe der Mikroalgen gemessen haben.

Ein herzliches Dankeschön gebührt auch den zahlreichen Bachelor- und Masterstudenten, die mit ihrer Arbeit und Mithilfe zum Erfolg dieser Dissertation beigetragen haben.

Abschließend möchte ich meiner Familie und meinen Freunden meinen tiefsten Dank aussprechen. Ihre stetige Ermutigung und Motivation haben mich in dieser Zeit begleitet und unterstützt. Ein besonderes Dankeschön gilt meinen Eltern, Tina und Peter Stiefvatter, meiner Schwester Anna Stiefvatter und meinem Ehemann Phil Kopp, für ihre Geduld und Unterstützung während meiner gesamten Doktorarbeit. Eure Hilfe war von unschätzbarem Wert, und dafür bin ich euch zutiefst dankbar.

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Die Mikroalge *Phaeodactylum tricornutum* – Bioverfügbarkeit, Sicherheit und potenzieller gesundheitlicher Nutzen für die humane Ernährung

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