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Oral intake of the microalgae *Nannochloropsis oceanica*, *Chlorella vulgaris*, or *Phaeodactylum tricornutum* improves metabolic conditions in hypercaloric-fed mice

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ABSTRACT

Diet-induced metabolic load is associated with excess body weight and liver steatosis. Here, selected microalgae, known to contain bioactive nutrients, were studied for beneficial metabolic effects in a mouse model of liver steatosis. Adult mice (8 per group) were fed either a Western-style diet (WSD) or a control diet +/− 15 % of the microalgae *Chlorella vulgaris* (CV), *Nannochloropsis oceanica* (NO), or *Phaeodactylum tricornutum* (PT) for 12 weeks. We evaluated liver fat content and liver damage, as well as fecal microbiota and lipopolysaccharide (LPS) translocation. NO supplementation to a WSD reduced the grade of liver steatosis (from 17 % to 4.7 %, $p < 0.002$), the liver damage score ($p < 0.001$), and LPS translocation ($p < 0.001$). PT had similar effects on liver damage score ($p < 0.001$) and LPS translocation ($p < 0.001$). CV supplementation reduced LPS translocation ($p < 0.001$). In conclusion, dietary supplementation of microalgae may be a novel sustainable approach to combat metabolic loads.

1. Introduction

In todaýs world, challenges arise from the excess energy intake of the Western-style diet (WSD), characterized by high-fat, high-carbohydrate content and high meat consumption (Odermatt, 2011). This diet is associated with chronic systemic inflammation and diseases such as diabetes, cardiovascular disease, and cancer (Clemente-Suárez et al., 2023). In addition, inflammatory responses and cytokine production are influenced negatively (Johnson et al., 2021). These changes also extend to the liver tissue, where genes associated with lipid metabolism and oxidative stress undergo altered expression (Roberts et al., 2015). Such conditions can also increase the development of metabolic-associated fatty liver disease (MAFLD) and thus contribute to obesity, dyslipidaemia, hypertension, and various types of cancer (Fabiani et al., 2016). Also, the gut microbiota composition and metabolite production can be affected negatively by the WSD (Martinez et al., 2017; Zinöcker & Lindseth, 2018). Combating liver steatosis is a major challenge as there is no specific treatment other than lifestyle intervention. Therefore, there is an urgent need for effective prevention strategies. Sustainable approaches like microalgae consumption are of particular interest.

Microalgae, which include both prokaryotic cyanobacteria and eukaryotic microalgae, represent a highly diverse group of microorganisms that can synthesize bioactive compounds such as polyphenols, fatty acids, proteins, and dietary fiber. Some of the best studied beneficial health effects of microalgae and their components are the improvement of insulin sensitivity and gut health, the reduction of inflammation and the possible prevention of the development of liver steatosis (Eilam et al., 2023; González-Arceo et al., 2021). Therefore, microalgae are promising implements in nutraceuticals and pharmaceuticals with proposed beneficial health effects (Kaur et al., 2023; Kumari et al., 2023; Tamel Selvan et al., 2023; Xia et al., 2021). In particular, the microalgae *Chlorella vulgaris* (CV), *Nannochloropsis oceanica* (NO), and *Phaeodactylum tricornutum* (PT) are promising health-promoting candidates (Gille et al., 2018). CV is particularly high in protein and contains all essential amino acids for humans (Bito et al., 2020). NO and PT are characterized by a high content of omega-3 fatty

Abbreviations: CV, *Chlorella vulgaris*; EPA, eicosapentaenoic acid; NO, *Nannochloropsis oceanica*; PCR, polymerase chain reaction; PERMANOVA, permutational multivariate analysis of variance; PT, *Phaeodactylum tricornutum*; WSD, Western-style diet.

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acids, especially eicosapentaenoic acid (EPA) as well as the carotenoid β-carotene (Celi et al., 2022; Zanella & Vianello, 2020). PT also contains the carotene fucoxanthin (Celi et al., 2022), which was shown to exhibit anti-obesity and anti-inflammatory effects (Ha et al., 2013; Maeda et al., 2005). Recently, a study by Lü et al. showed that marine algae oils derived from PT and *Laminaria japonica* alleviate obesity, insulin resistance, and gut microbiota dysbiosis in high-fat diet-fed mice (Lü et al., 2024). In the present study, we aimed to investigate the effect of the consumption of the whole microalgae CV, NO, and PT as dietary supplements in high-fat diet-fed mice and investigated the effects on metabolic parameters, as well as on liver and gut health markers.

2. Materials and methods

2.1. Microalgae and diets

The microalgae were purchased from Algomed (Klötze, Germany; for CV and PT [phototrophic]) and Allma (Lisbon, Portugal; for NO [mixotrophic]) with food-grade quality. The microalgae were cultivated in closed photobioreactors and were harvested by centrifugation. For the mixotrophic cultures of NO, organic carbon was added, while the phototrophic CV and PT cultures used only CO_2 as a carbon source. CV and PT were spray-dried, while NO was obtained as frozen moist biomass (10 % w/v) and subsequently freeze-dried (WKF *L*10, WKF, Darmstadt, Germany). Afterwards, the microalgae were stored at −20 °C until they were ball milled. Here, the microalgae were resuspended in water (10 % w/v) and ground using a laboratory ball mill (PML 2, Bühler, Germany) with a pump rate of 70 L/h and a total disintegration time of 2.5 h. The crushed biomass was then freeze-dried for 36 h at −20 °C and 0.04 mbar (WKF *L*10, WKF, Darmstadt, Germany). The biomass was vacuum-packed and stored at −20 °C until it was added to the feed by the feed manufacturer sSniff Spezialdiäten GmbH (Soest, Germany). The composition of the main micro- and macronutrients of the microalgae are shown in Table 1.

The basic diet was either a normocaloric control diet (CD, ssniff S0514-E730; 15.3 MJ per kg feed) or a hypercaloric WSD (ssniff S0514-E735, 18.6 MJ per kg feed) without microalgae. For both diets the microalgae CV (CD-CV ssniff S0514-E73; WSD-CV ssniff S0514-E736), NO (CD-NO ssniff S0514-E733; WSD-NO ssniff S0514-E738), or PT (CD-PT ssniff S0514-E732; WSD-PT ssniff S0514-E737) were added as dried biomass by 15 % to the CD or the WSD by the feed manufacturer. The amount of 15 % which was supplemented was found to be the optimum amount of microalgae for bioavailability of the nutrients in preliminary tests (Neumann, Derwenskus, et al., 2018; Stiefvatter et al., 2022). The composition of the feed is shown in Table 2.

2.2. Animals

All experiments were carried out from the end of 2017 to the beginning of 2018. A total of 64 male, 6- to 8-week-old C57BL/6J mice were obtained from the animal care unit (ZVH) of the University of Hohenheim, Germany in the year 2017. Only male mice were included because they show less variability in the biological outcomes tested compared to female mice, due to the estrous cycle of females. Animals were housed in groups of two to four in a specific pathogen-free barrier facility with controlled temperature, a 12:12 inverted light–dark cycle and ad libitum access to food and water. All experiments were approved by the local Institutional Animal Care and Use Committee (Regional Council Stuttgart, permit number V330/17 EM). Animals were divided into 8 groups receiving different diets, with 8 animals in each group. The experimental animals were fed ad libitum for 12 weeks (84 days). On days 0 and 69, fecal samples were collected and on day 70 Urine samples were collected over 24 h housed singly in metabolic cages (TECNIPLAST S.p.A, Buguggiate, Italy). At the end of the experiment, after 12 h fasting the animals were anesthetized (ketamine/xylazine-100/16 mg/kg body weight). The abdomen was then opened, and the blood was removed from the portal vein. The animals are then sacrificed by terminal cervical dislocation, mice were 4–5 months old. Blood samples was centrifuged for 10 min at 1000 *g* with 4 °C and the obtained serum was stored at −80 °C. Organs were taken, weighed and stored at −80 °C. The intestine was divided into duodenum, jejunum, ileum, colon and caecum. Body fat mass was measured as mesenteric fat and epidydimal fat tissue of the animals.

Table 1
Composition of the main micro- and macronutrients of the microalgae.

	Nannochloropsis oceanica	*Phaeodactylum tricornutum*	*Chlorella vulgaris*
Crude protein [g/kg dry mass]	387	429	542
Crude lipids [g/kg dry mass]	167	99	104
Crude ash [g/kg dry mass]	237	177	71
Calcium [g/kg dry mass]	2.6	24.1	3.4
Phosphorus [g/kg dry mass]	11.9	27.9	14.0
Caloric value [MJ/kg dry mass]	22.0	19.2	22.6

Shown are mean values.

2.3. Histological analysis

For histological analysis, formalin-fixed tissue samples of the CD and the WSD 15 % supplemented microalgae diets were embedded in paraffin and stained using the hematoxylin-eosin (H & E)-staining as previously described (Neumann, Derwenskus, et al., 2018). Samples of duodenum, jejunum, ileum, and colon were scored for cell infiltration (score 0–3) and tissue damage (score 0–3) as described (Hagenlocher et al., 2016). Ballooning (swelling) of hepatocytes is also a common structural change in nonalcoholic steatohepatitis. The degree of lipid accumulation as well as the degree of ballooning was scored according to the S2k guideline for nonalcoholic fatty liver disease (Roeb et al., 2015). The lipid scores ranging from 0 to 3: 0: under 5 % lipid-accumulation; 1:5–33 % lipid accumulation; 2: 33–66 % lipid-accumulation; 3: over 66 % lipid-accumulation. Liver samples were analyzed for steatosis, infiltration and tissue damage with liver scores ranging from 0 to 3: 0: no damage visible, no steatosis, no inflammatory cell aggregates; 1: single damages, mild steatosis, and few inflammatory cells; 2: increased number of damaged cells, increased steatosis, accumulation of inflammatory cell aggregates; 3: extensive damage, high steatosis, and many inflammatory cells. The thickness of the muscularis externa was measured in colon and ileum with the Axio Vision Rel. 4.8 Software (magnification 200_, Zeiss, Oberkochen, Germany). At least 6 measurements were performed per image for at least 4 pictures per mouse, for 6 animals per group. The percentage area of goblet cells in the colon was analyzed by measurement of the white area within a field area including only villi using the analysis software Axio Vision Rel. 4.8 (Zeiss, Oberkochen, Germany).

2.4. Measurement of triglyceride and fatty acid levels in the liver and feed

The triglyceride content of the liver was measured using a commercial kit for the colorimetric determination of triglycerides (TG) in tissue (Cayman Chemical Triglyceride Colorimetric Assay Kit 10010303). Free fatty acids were analyzed in the liver and feed, using gas chromatography and a flame ionization detector, as described earlier (Neumann, Derwenskus, et al., 2018; Neumann, Louis, et al., 2018).

Table 2
Food composition of the Control Diets and Western Style Diets with or without microalgae added.

Diets/Food composition	Control Diet (CD)				Western-style diet (WSD)			
	CD	CD-CV	CD-NO	CD-PT	WSD	WSD-CV	WSD-NO	WSD-PT
Microalgae suppl. [%]		15	15	15		15	15	15
CP [%]	18.6	18.6	18.6	18.6	18.6	18.6	18.6	18.6
CL [%]	6.1	6.1	6.1	6.1	20.5	20.5	20.5	20.5
CF [%]	7.9	4.8	7.5	6.6	6.8	3.8	6.6	5.7
CHO [kcal %]	65	65	65	65	42	42	42	42
Crude ash [%]	4.8	7.2	5.2	5.7	4.8	7.3	5.2	5.7
Calcium [%]	0.93	0.92	0.92	0.95	0.93	0.92	0.94	0.92
P [%]	0.7	0.7	0.7	0.74	0.7	0.71	0.7	0.74
Energy [MJ/kg]	15.3	15.36	15.3	15.3	18.6	18.6	18.6	18.6
Protein [kcal %]	20	20	20	20	17	17	17	17
Fat [kcal %]	15	15	15	15	41	41	41	41
SFA [mg/g]	7.5	8.7	8.8	9.7	66.8	94.2	67.6	83.3
MUFA [mg/g]	9.6	9.3	10.0	12.2	26.7	42.5	27.3	38.1
PUFA [mg/g]	23.8	28.5	27.4	27.8	14.6	24.1	15.1	13.9
EPA [mg/g]	0.0	0.0	4.6	1.9	0.0	0.0	4.4	2.5

Data shown as Mean. Abbreviations: Suppl, supplementation; CP, crude protein, CL, crude lipid, CF, crude fiber, CHO, carbohydrates; P, phosphorus, MJ: megajoules; SFA, saturated fatty acids, MUFA monounsaturated fatty acids, PUFA, polyunsaturated fatty acids, EPA, eicosapentaenoic acid, CD, control diet; CV, CD + *Chlorella vulgaris*; NO, CD + *Nannochloropsis oceanica*; PT, CD + *Phaeodactylum tricornutum*; WSD, western-style diet; WSD-CV, WSD + *Chlorella vulgaris*; WSD-NO, WSD + *Nannochloropsis oceanica*; WSD-PT, WSD + *Phaeodactylum tricornutum*.

2.5. Determination of blood markers

The Glucose Colourimetric Assay Kit (Cayman, Ann Arbor USA) was used to determine the glucose concentration in the plasma of the test animals. The cholesterol level in the plasma was determined using a fluorometric assay kit (Cayman, Ann Arbor USA). The insulin concentration of the test animals was determined using an enzyme immunoassay (Bertin Pharma, Montigny-le-Bretonneux, France). An enzyme-linked immunosorbent assay kit (abbexa, Cambridge United Kingdom) was used to determine the endotoxin concentration in plasma. Quantification of leptin in the plasma of the mice was performed using an enzyme immunoassay kit (Bertin Pharma, Montigny-le-Bretonneux, France).

2.6. Determination of intestinal markers ileum and colon tissue

Ileum and colon tissue (50–100 mg) was used for RNA extraction through the peqGOLD TriFast method (PEQLAB, Erlangen, Germany), following the manufacturer's guidelines. Subsequently, the extracted RNA underwent cDNA transcription using SuperScript® IV Reverse-Transcriptase (Thermo Fisher Scientific, Darmstadt, Germany) following DNAse treatment (Promega, Madison, WI, USA). In the context of real-time polymerase chain reaction (PCR), the PCR mixture was formulated using EvaGreen® Supermix SsoFastTM (Bio-Rad Laboratories GmbH, Munich, Germany). This mixture, totaling 10 µl, comprised cDNA, SYBR Green Master Mix, and mouse-specific oligonucleotide primers (see Table 3). The 18S gene was used as a reference gene to normalize the cycle threshold values of the markers mentioned. The amplification reaction for all markers took place in an iCycler (BioRad Laboratories). Except for α-and β-defensin, the following was performed: initial holding step at 95 °C for 3 min and 40 cycles of a three-step PCR consisting of 95 °C for 30 s, 60 °C for 10 s and 72 °C for 30 s. A post-PCR dissociation curve was then generated in the temperature range from 65 °C to 95 °C.

For α-defensin, the process began with an initial holding step at 96 °C for 5 min, followed by 45 cycles of a three-step PCR (96 °C for 10 s, 64 °C for 5 s, 72 °C for 15 s). A post-PCR dissociation curve was then generated in the temperature range from 62 °C to 99 °C.

As with β-defensin, the procedure began with an initial holding step at 96 °C for 5 min, followed by 45 cycles of a three-step PCR (96 °C for 10 s, 58 °C for 5 s, 72 °C for 15 s and 76 °C for 5 s). The post-PCR dissociation curve was generated in a temperature range from 56 °C to 95 °C. The relative gene expression was calculated using the ΔΔ-Ct method in comparison to the house keeping gene 18S.

Table 3
Primers used in quantitative real-time PCR.

Gene	Sequence (5′–3′)	
	Forward primer	Reverse primer
TNF-α	ACC ACC ATC AAG GAC TCA	AGG TCT GAA GGT AGG AAG G
IL-1β	ACG GAT TCC ATG GTG AAG TC	GAG TGT GGA TCC CAA GCA AT
IL-6	AGT CAC AGA AGG AGT GGC TA	CTG ACC ACA GTG AGG AAT GT
IL-10	CTT GCA CTA CCA AAG CCA CA	GTT ATT GTC TTC CCG GCT GT
TGF-β	AG CTC TTC CAG ATA CTT CG	GTT GGA CTC TCT CCT CAA CA
IFN-γ	CTG ATG GGA GGA GAT GTC TA	CAC CAG GTG TCA AGT CTC TT
α-Defensin (Ileum mDefa1)	TCA AGA GGC TGC AAA GGA AGA GAA C	TGG TCT CCA TGT TCA GCG ACA GC
β-Defensin (Colon mDefb4)	TCC AAT AAC ATG CAT GAC CA	TCA TGG AGG AAA TTC TG
Muc-2	GAT GGC ACC TAC CTC GTT GT	GTC CTG GCA CTT GTT GGA AT
Occludin	ACT CCT CCA ATG GAC AAG TG	CCC CAC CTG TCG TGT AGT CT
Notch-1	CGTGGTCTTCAAGCGTGATG	GCTCTTCCTCGTGGCCATAG
Reg-III-γ	CGT GCC TAT GGC TCC TAT TGC T	TTC AGC GCC ACT GAG CAC AGA C
18S	GTA ACC CGT TGA ACC CCA TT	CCA TCC AAT CGG TAG TAG CG

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

2.7. Fecal DNA extraction, 16S ribosomal ribonucleic acid (rRNA) gene sequencing and sequence processing

Murine fecal DNA was extracted according to Ehrlich (2015) at the Institute of Nutritional Medicine of the University of Hohenheim and was kept at −20 °C before analysis. 16S Library preparation was performed by a two-step Nextera PCR using the primers 341F (5′- CCT ACG GGN GGC WGC AG-3′) and 805R (5′- GAC TAC HVG GGT ATC TAA TCC-3′), targeting the V3-V4 region (Nextera XT DNA Library Preparation Kit, Illumina, Inc., San Diego, CA, USA). Sequencing was conducted using

the Illumina MiSeq platform. A v2 500 cycles kit (Illumina) was used to sequence the PCR libraries. The produced paired-end reads which passed Illumina's chastity filter were demultiplexed and trimmed using Illumina's real time analysis software included in the MiSeq reporter software version 2.6. The quality of the reads was checked with the software FastQC version 0.11.8 (website: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/).

The locus specific V3-V4 primers were trimmed from the sequencing reads using cutadapt version 2.8 (Martin, 2011). Paired-end reads were discarded if the primer could not be trimmed. Trimmed forward and reverse reads of each paired-end read were merged to in-silico reform the sequenced molecule considering a minimum overlap of 15 bases using the software USEARCH version 11.0.667 (https://doi.org/10.1093/bioinformatics/btq461). Merged sequences were quality filtered allowing a maximum of one expected error per merged read. Reads that contained ambiguous bases or are outliers regarding the amplicon size distribution were also discarded. Samples that resulted in less than 5000 merged reads were discarded, to not distort the statistical analysis. Reads were denoised using the UNOISE algorithm (http://dx.https://doi.org/10.1101/081257) implemented in USEARCH v11.0.667 (website: https://github.com/rcedgar/usearch12) to form operational taxonomic units (OTUs) discarding singletons and chimeras in the process (clustering with 97 % identity). The resulting OTU abundance table was filtered for possible bleed-in contaminations using the UNCROSS algorithm (https://doi.org/10.1101/400762). OTUs were then compared against the reference sequences of the Ribosomal Database Project 16S database (https://doi.org/10.1093/nar/gkt1244) and taxonomies were predicted considering a minimum confidence threshold of 90 % using the SYNTAX algorithm implemented in USEARCH. Alpha diversity was estimated using the total number of observed OTUs and the Shannon index. Alpha diversity calculations and rarefaction analysis were performed with the R software (R Core Team, Vienna, Austria) packages phyloseq version 1.26.1 and vegan version 2.5–5 (McMurdie & Holmes, 2013). All upstream analyses (llibrary preparation, sequencing, and taxonomic determination) were performed by Microsynth AG (Balgach, Switzerland). To omit assessing spurious data in the statistical analyses, we only assessed phyla and genera with a mean relative abundance > 1 %.

2.8. Statistical analysis

Normal distribution was tested with the Kolmogorov–Smirnow test. Except for the microbiota data, all data were normally distributed. For normally distributed data, a one-way ANOVA was used to evaluate statistically significant differences ($p < 0.05$) between groups. The equality of variances was evaluated utilizing Levene's test. For equal variances Tukey's post hoc test was used, for unequal variances Dunnett's post hoc test. For microbiota data, group differences were tested using the Kruskal-Wallis test. To assess microbial beta diversity, Euclidean distance between bacterial communities on genus level was compared by permutational multivariate analysis of variance (PERMANOVA) using the Adonis function in the R software version 3.5.3. All analyses except beta diversity analyses were performed in GraphPad Prism version 10.2.2 (GraphPad Software, La Jolla, California, USA). A $p < 0.05$ was considered as statistically significant, a $p < 0.07$ was considered as a trend.

3. Results

3.1. Effects of the microalgae on energy intake and body weight

Within the normocaloric fed controls, mice fed with CD-CV had higher feed consumption than the mice fed with CD ($p < 0.02$) and CD-PT ($p < 0.05$, Fig. 1A). No difference was observed within the WSD groups (Fig. 1A). The energy content of the diets differed with 15.3 MJ/kg (3.7 kcal/g) in the CD group, while the WSD group had an energy content of 18.6 MJ/kg (4.4 kcal/g) feed. Therefore, the average caloric intake in the WSD groups was 13.6 % higher than in the CD and CD-PT groups ($p < 0.01$). The caloric intake of the mice fed with CD-NO and CD-CV did not differ from the WSD (Fig. 1B). The increased energy

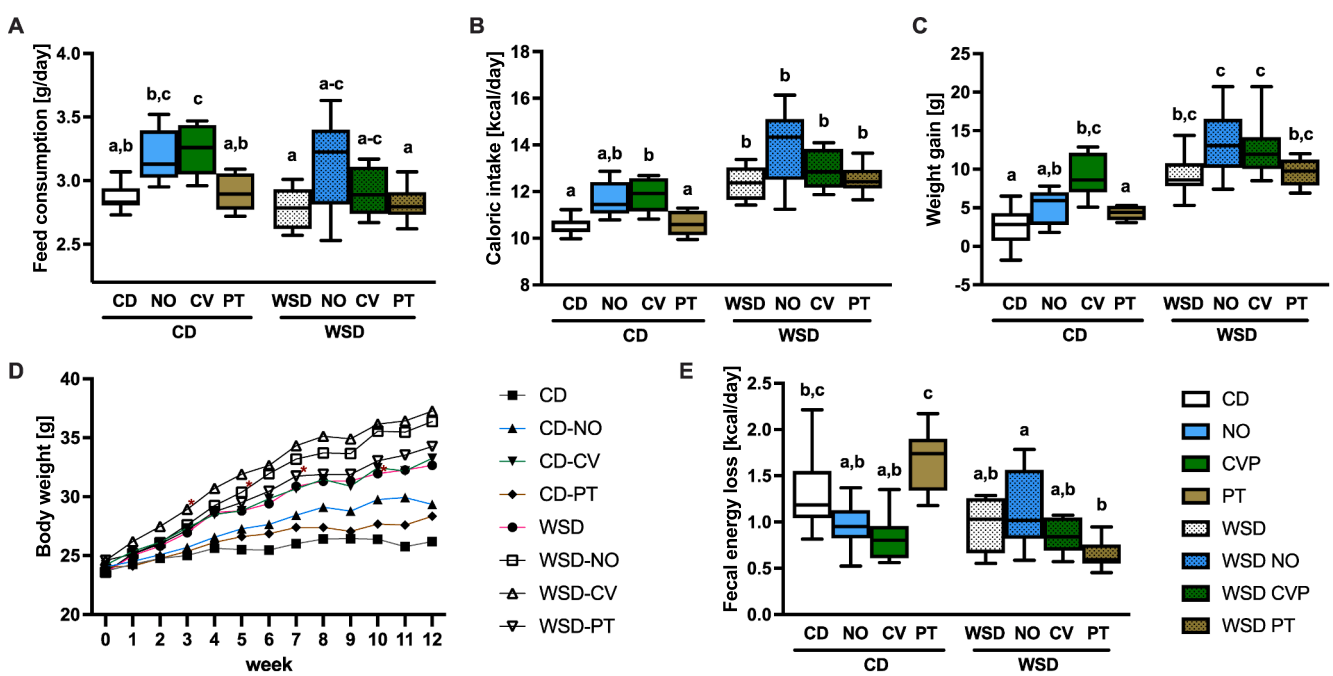

Fig. 1. Feed consumption (A), Caloric intake (B), Weight gain over 12 weeks (C), Body weight development (D), Fecal energy loss is the energy content of feces on day 69 measured by Bomb calorimetry (E). Boxplots and Whiskers (25th and 75th percentiles) are shown (n = 8 per group). Different letters indicate significant differences as analysed by ANOVA with Tukey's or Dunnett's post-hoc test. Abbreviations: CD, Control diet; CD-CV, CD + Chlorella vulgaris; CD-NO, CD + *Nannochloropsis oceanica*; CD-PT, CD + *Phaeodactylum tricornutum*; WSD, Western-style Diet; WSD-CV, WSD + Chlorella vulgaris; WSD-NO, WSD + *Nannochloropsis oceanica*; WSD-PT, WSD + *Phaeodactylum tricornutum*.

intake was accompanied by increased weight gain for all WSD groups and the CD-CV group (ANOVA $p < 0.001$). Within the CD, the CV group had the highest percentage weight gain with 37.8 ± 12.8 % and within the WSD the WSD-NO group had the highest weight gain with 56.9 ± 18.3 % ($p < 0.01$; Fig. 1C). As shown in the weight progression after 3 weeks, the weight of the WSD-CV group was already higher compared to the CD ($p = 0.01$), WSD-CV weight was higher after 5 weeks ($p = 0.02$), CD-PT after 7 weeks ($p = 0.01$) and WSD after 10 weeks higher compared to the CD ($p = 0.04$) (Fig. 1C).

Bomb calorimetry is the gold standard for assessing intestinal absorption capacity (Wierdsma et al., 2014). Therefore, the energy in faeces was measured on day 69 to analyse the bioavailability of the microalgae (Fig. 1E). The energy loss after the CD-PT was different compared to the other microalgae diets, but not different to the CD ($p < 0.05$). If energy loss is less than energy intake, the quotient will be higher. A higher ratio means that more energy is being absorbed from food and less is being lost in feaces.

3.2. Effects of the microalgae on organ health and liver pathology

The fat weight was measured as epididymal (visceral) and mesenteric (subcutaneous) white adipose tissue. The WSD resulted in an increase in fat compared to the CD ($p < 0.001$, Fig. 2A). The effects of the WSD were clearly visible in the fat accumulation from 2.7 % in the mice fed the CD as compared to 17.7 % in the mice fed with the WSD ($p < 0.004$). Within the normocaloric diets, mice fed with CD-CV had a significantly higher fat weight compared to mice fed the CD ($p = 0.007$). No significant differences in fat weight were found within the WSD groups (Fig. 2A). Mice fed with the WSD-NO showed a lower liver steatosis compared to mice fed with the WSD (4.7 % versus 17.0 %, $p < 0.002$) and mice fed with the WSD-PT by trend showed a reduced liver steatosis compared to the WSD (8.7 %, $p < 0.07$) (Fig. 2B). Looking more closely at the liver, the results of liver damage after the WSD showed the highest value of 1.8 ± 0.5 points (Fig. 2C), which was six times higher than that of the CD group (0.3 ± 0.4, $p < 0.001$, Hematoxylin-eosin staining of the livers (Fig. 2D)). The hypercaloric diets in WSD-PT and WSD-NO fed mice again reduced the liver damage induced by the WSD and were no longer different from the normocaloric diets ($p < 0.001$, Fig. 2C). An imbalance in the supply and removal of fatty acids and abnormal de novo synthesis of TG leads to increased lipid accumulation in the liver. Hepatocyte ballooning (swelling) is also a common structural change in non-alcoholic steatohepatitis. Ballooning occurred in mice fed with the WSD. The negative effect was significantly reduced by consumption of the microalgae-enriched WSD-PT and WSD-NO ($p < 0.001$; Table 4). Another marker, the percentage of goblet cells in the mucosa, which could indicate a weakening of the gut barrier

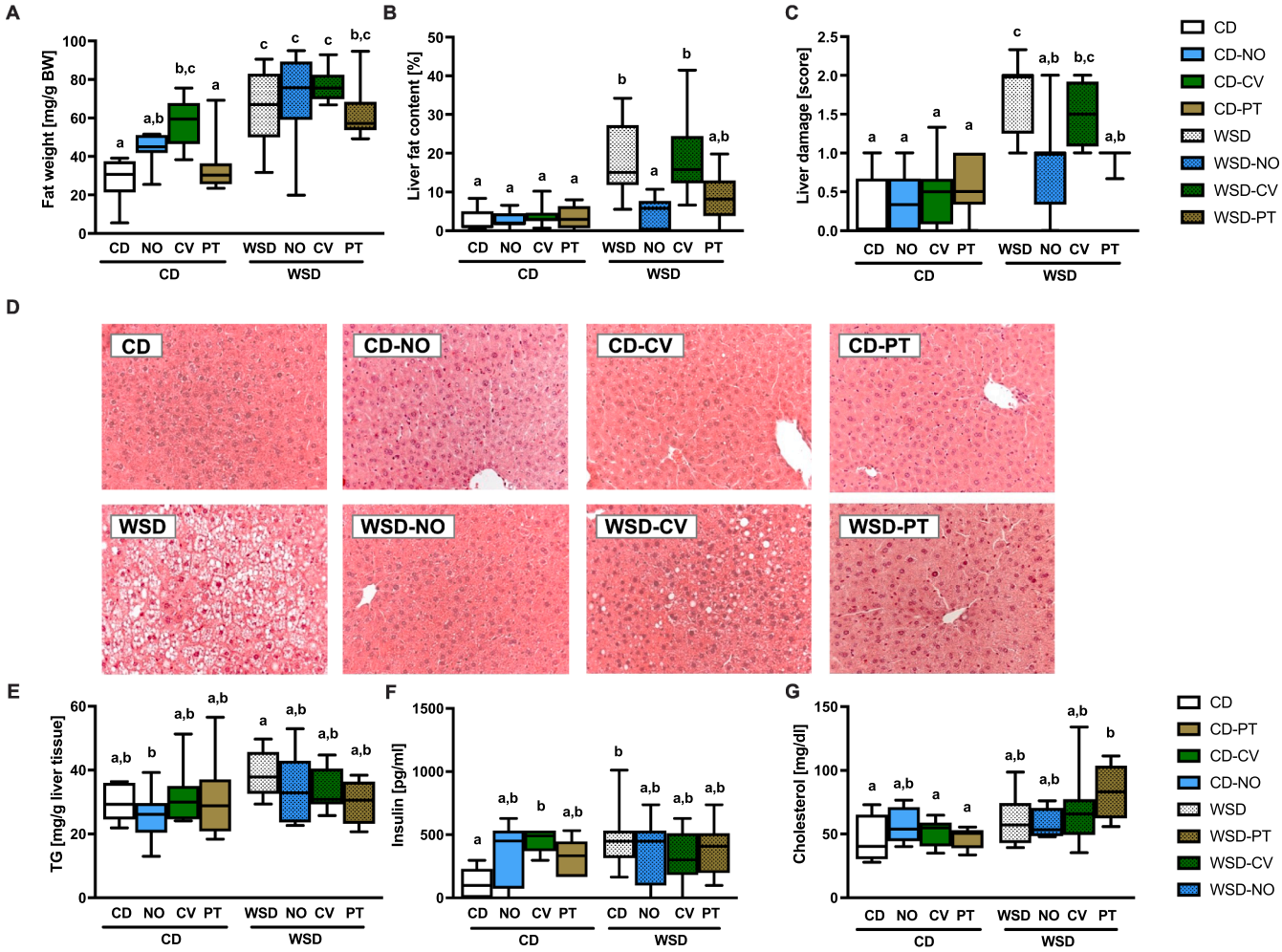

Fig. 2. Body fat mass (A), liver fat content (B) and Score for liver damage (C) in mice after 84 days of microalgae supplementation. Representative H&E stainings of livers of mice after CD and WSD with and without microalgae (D). TG (E), Insulin (F), Serum Cholesterol (G). Boxplots and Whiskers (25th and 75th percentiles) are shown (n = 8 per group). Different letters indicate significant differences as analysed by ANOVA with Tukey's or Dunnett's post-hoc test. Abbreviations: BW, body weight,TG, Triglyceride; CD, Control diet; CD-CV, CD + *Chlorella vulgaris*; CD-NO, CD + *Nannochloropsis oceanica*; CD-PT, CD + *Phaeodactylum tricornutum*; WSD, Western-style diet; WSD-CV, WSD + *Chlorella vulgaris*; WSD-NO, WSD + *Nannochloropsis oceanica*; WSD-PT, WSD + *Phaeodactylum tricornutum*.

Table 4
Histology markers.

Diets/Histology markers	CD	CD-CV	CD-NO	CD-PT	WSD	WSD-CV	WSD-NO	WSD-PT
Degree of swelling (ballooning) of the hepatocytes (Score 0–3)	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	1.2 ± 0.7b	0.4 ± 0.6 a	1.6 ± 0.7 b	0.4 ± 0.6 a
Goblet cell area (%)	14.4 ± 6.1 a	12.9 ± 2.2 a	12.9 ± 2.1 a	12.4 ± 3.0 a	11.5 ± 2.2 a	16.4 ± 2.7 a	14.4 ± 4.1 a	16.0 ± 3.8 a
External Muscle Layer of Colon (μm)	84.1 ± 36 a	74.3 ± 22 a	88.63 ± 31 a	102.1 ± 24 a	78.38 ± 34 a	77.1 ± 29 a	77.5 ± 14 a	104.6 ± 38 a
Infiltration in Colon (Score 0–3)	0.5 ± 0.4 a,b	0.25 ± 0.2 a, b	0.3 ± 0.3 a,b	0.28 ± 0.2 a, b	0.66 ± 0.3 a	0.27 ± 03 a, b	0.34 ± 0.3 a, b	0.20 ± 0.1b
Tissue Damage colon (Score 0–3)	0.03 ± 0.1b	0.00 ± 0b	0.00 ± 0b	0.03 ± 0.1b	0.23 ± 0.2 a	0.03 ± 0.1b	0.11 ± 0.2 a, b	0.0 ± 0.1 a,b
External Muscle Layer of Ileum (μm)	25.0 ± 5 a	31.0 ± 4 a	28.1 ± 3 a	29.5 ± 5 a	33.3 ± 10 a	30.9 ± 7 a	27.4 ± 9 a	30.8 ± 4 a
Infiltration in Ileum (Score 0–3)	0.1 ± 0.2 a	0.13 ± 0.1 a	0.28 ± 0.4 a	0.05 ± 0.1 a	0.14 ± 0.1 a	0.13 ± 0.2 a	0.17 ± 0.2 a	0.16 ± 0.1 a
Tissue Damage in Ileum (Score 0–3)	0.02 ± 0.0 a	0.02 ± 0.4 a	0.19 ± 0.4 a	0.00 ± 0.0 a	0.03 ± 0.1 a	0.11 ± 0.1 a	0.03 ± 0.1 a	0.06 ± 0.1 a

Data shown as mean ± standard deviation. $n = 8$ per group. Different letters indicate significant differences as analysed by ANOVA with Tukey's or Dunnett's post-hoc test. Significant effects of the microalgae diets compared to the control diets are marked in bold. Abbreviations: CD, Control diet; CD-CV, CD + *Chlorella vulgaris*; CD-NO, CD + *Nannochloropsis oceanica*; CD-PT, CD + *Phaeodactylum tricornutum*; WSD, Western-style Diet; WSD-CV, WSD + *Chlorella vulgaris*; WSD-NO, WSD + *Nannochloropsis oceanica*; WSD-PT, WSD + *Phaeodactylum tricornutum*.

function, showed no differences between the groups (Table 4). The infiltration in the colon after the WSD-PT was at the same level as in mice fed with CD and significantly lower than in mice fed with WSD ($p < 0.02$, Table 4). Tissue damage in the colon was lower in mice after the WSD-NO than after the WSD ($p < 0.05$, Table 4). There were no effects of the diets on muscle layer thickness and in the ileum. In relation to body weight, there was an effect of the WSD in reducing the length of the intestine, caecum, and colon, but no improvement with the microalgae diet was found for these parameters (Fig. S1).

3.3. Effects of the microalgae on plasma glucose, cholesterol, and insulin levels

Plasma insulin, cholesterol, TG, and glucose levels were measured to evaluate possible anti-atherosclerotic effects of additional microalgae intake. For TG levels, the CD-NO group had the lowest value (25.4 ± 7.8 mg/g liver), which was lower compared to the WSD group (39.0 ± 7.1 mg/g liver, $p < 0.05$, Fig. 2E). All WSD groups with algae supplementation seemed to have lower TG values than the WSD group, but these differences were not statistically significant. Evaluation of insulin levels showed higher levels after the WSD compared to the CD ($p < 0.05$, Fig. 2F). About cholesterol concentration, the WSD-PT showed higher values compared to the CD-PT ($p < 0.01$, Fig. 2G). There were no differences in glucose levels and other markers such as leptin, urea, β-hydroxybutyrate, malondialdehyde, alanine aminotransferase activity and glutathione (data not shown).

3.4. Effects of the microalgae on the liver fatty acid composition

Mice after CD-NO and CD-PT had lower monounsaturated fatty acids (MUFA) liver concentration compared to WSD-CV ($p < 0.05$) and CD-NO lower compared to CD ($p < 0.001$, Fig. 3A). Polyunsaturated fatty acid (PUFA) levels in the liver of the mice fed with CD-CD and CD-PT were higher compared to mice fed with the CD ($p < 0.01$, (Fig. 3B). Only the diets with NO and PT contained EPA, which is reflected in the liver amounts. The highest levels of EPA in liver tissue were measured in the mice fed with additional NO (CD-NO 1.0 ± 0.3 mg/g liver, WSD-NO 2.2 ± 0.7 mg/g liver) and further shown in Fig. 3C $p < 0.001$). The ratio of omega-6 to omega-3 fatty acids was lower after the CD than after the WSD ($p < 0.001$). Mice fed with CD-CV had a higher omega-6 to omega-3 ratio compared to mice fed with the CD ($p < 0.001$, Fig. 3D). Mice fed with a WSD, WSD-CV and CD-CV had the highest omega-6 to omega-3 ratio differs as shown in Fig. 3D ($p < 0.05$).

3.5. Effects of the microalgae on the mRNA expression of selected gut barrier markers

In the ileum and colon, mRNA expression of the pro-inflammatory cytokines TNF-α, IL-1β, IL-6 and TGF-β did not show significant changes with the microalgae diets (data not shown). However, the WSD-CV group showed higher IL-10 mRNA expression compared to the WSD ($p < 0.05$, Fig. 3E). Evaluation of the mRNA expression of α-defensin and Notch-1 as well as of the gut barrier function markers Muc-2 and occludin, showed no differences between the diet groups in either the colon or ileum (data not shown). In all animals of the respective groups, significantly lower plasma endotoxin concentrations were measured in the mice fed with CD compared to the corresponding WSD groups ($p < 0.01$) (Fig. 3F), indicating impaired gut barrier function by the WSD.

3.6. Effects of microalgae on the gut microbiota composition

Gut microbiota composition was assessed by 16S next-generation sequencing in fecal samples of all 64 mice. As shown in Fig. 4A and in detail in Table S1, supplementation of the microalgae NO was associated with a lower alpha diversity. In detail, mice fed with CD-NO showed a lower number of observed OTUs compared to mice fed with the CD ($p < 0.05$). Consistently, mice fed with WSD-NO showed a lower number of observed OTUs compared to mice fed with WSD ($p < 0.05$) (Fig. 4A). There was no difference in the alpha diversity, if assessed by the alpha diversity marker Shannon index (all $p > 0.05$). We furthermore investigated possible differences in beta diversity on genus level between the groups using PERMANOVA analyses, showing no results (all $p > 0.05$, data not shown). Looking at the abundance of different bacterial genera, we found several differences between the groups, as shown in Fig. 4B-F, and shown in detail in Table S1. For example, mice fed with a WSD had a lower abundance of *Bacteroides* compared to mice fed with the CD ($p < 0.05$) (Fig. 4B). Interestingly, the abundance of *Bacteroides* was higher in mice fed with WSD-NO compared to mice fed with the WSD ($p < 0.05$). Mice fed with WSD-PT had a higher abundance of *Clostridium* cluster IV compared to mice fed with the WSD ($p < 0.05$) (Fig. 4C). In contrast, mice fed with WSD-CV had a lower abundance of *Clostridium* cluster XIVa compared to mice fed with the WSD ($p < 0.05$). Also, mice fed with WSD-PT had a higher abundance of *Flavonifractor* (Fig. 4E) and a lower abundance of *Olsenella* (Fig. 4F) than mice fed with the WSD (both $p < 0.05$).

4. Discussion

Nutrient-rich microalgae have the potential to contribute to a more balanced diet and mitigate the health risks associated with the western lifestyle (Gille et al., 2018). Furthermore, microalgae are a sustainable food and energy source, which can help to combat upcoming issues derived from climate change and a growing world population (Molotoks et al., 2021). There is a growing number of studies showing beneficial health effects of microalgae and their bioactive components are being

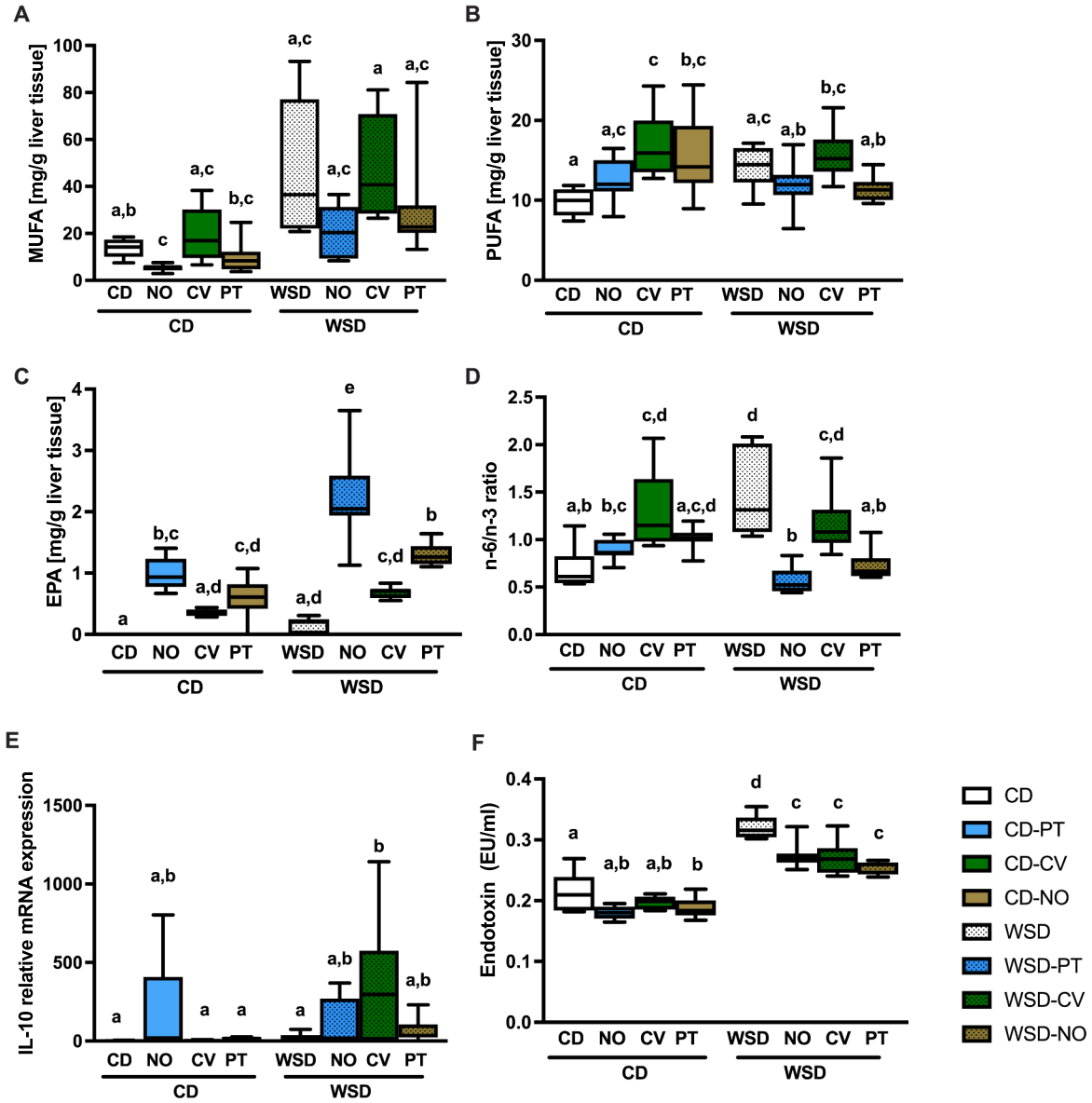

Fig. 3. Measured fatty acids in mice livers after 84 days of microalgae supplementation. MUFA, monounsaturated fatty acids (A), PUFA, polyunsaturated fatty acids (B), EPA, eicosapentaenoic acid (D), omega-6 to omega-3 ratio. mRNA expression of Interleucin-10 in the Ileum (E) and endotoxin measured in the blood in the fasting state (F). Different letters indicate significant differences as analysed by ANOVA with Dunnett's post-hoc test. Boxplots and Whiskers (25th and 75th percentiles) are shown (n = 8 per group). Abbreviations: CD, Control diet; CD-CV, CD + *Chlorella vulgaris*; CD-NO, CD + *Nannochloropsis oceanica*; CD-PT, CD + *Phaeodactylum tricornutum*; WSD, Western-style diet; WSD-CV, WSD + *Chlorella vulgaris*; WSD-NO, WSD + *Nannochloropsis oceanica*; WSD-PT, WSD + *Phaeodactylum tricornutum*.

recognised as promising implements in nutraceuticals and pharmaceuticals (Kaur et al., 2023). The present study adds to the present knowledge, showing that microalgae supplementation may be a novel sustainable approach to combat disease associated with the WSD, most dominantly to combat liver-associated disease. Importantly, the Intake of all three microalgae, added as 15 % of the diet, showed no adverse effects or unexpected behavior. This might be a valuable information for future microalgae-related novel-food-applications.

4.1. Supplementation of microalgae to the WSD did not attenuate weight gain

Contrary to our expectations, the supplementation of microalgae to a WSD did not result in attenuation of weight gain and body fat mass, but rather resulted in a trend towards an increased weight gain. These results are consistent with our previous studies showing that CV, NO and PT contribute to weight gain through increased consumption (Neumann, Derwenskus, et al., 2018; Stiefvatter et al., 2022). Other studies have shown a decrease in body weight gain in rats and mice after WSD, mostly after PT supplementation (Mayer et al., 2019; Kim et al., 2016; Lü et al., 2024), which was attributed to fucoxanthin and the induction of thermogenic activity (Winarto et al., 2023). The reason for this difference is currently unknown but might derive from different nutrient profiles of the microalgae used and/or from different feed. We assume that CV supplementation in the CD group and that NO and CV supplementation in the WSD group made the food more attractive to the mice, leading to increased consumption and weight gain. WSD-NO showed comparable effects on body weight gain as WSD, but showed the lowest liver fat accumulation among all WSD groups.

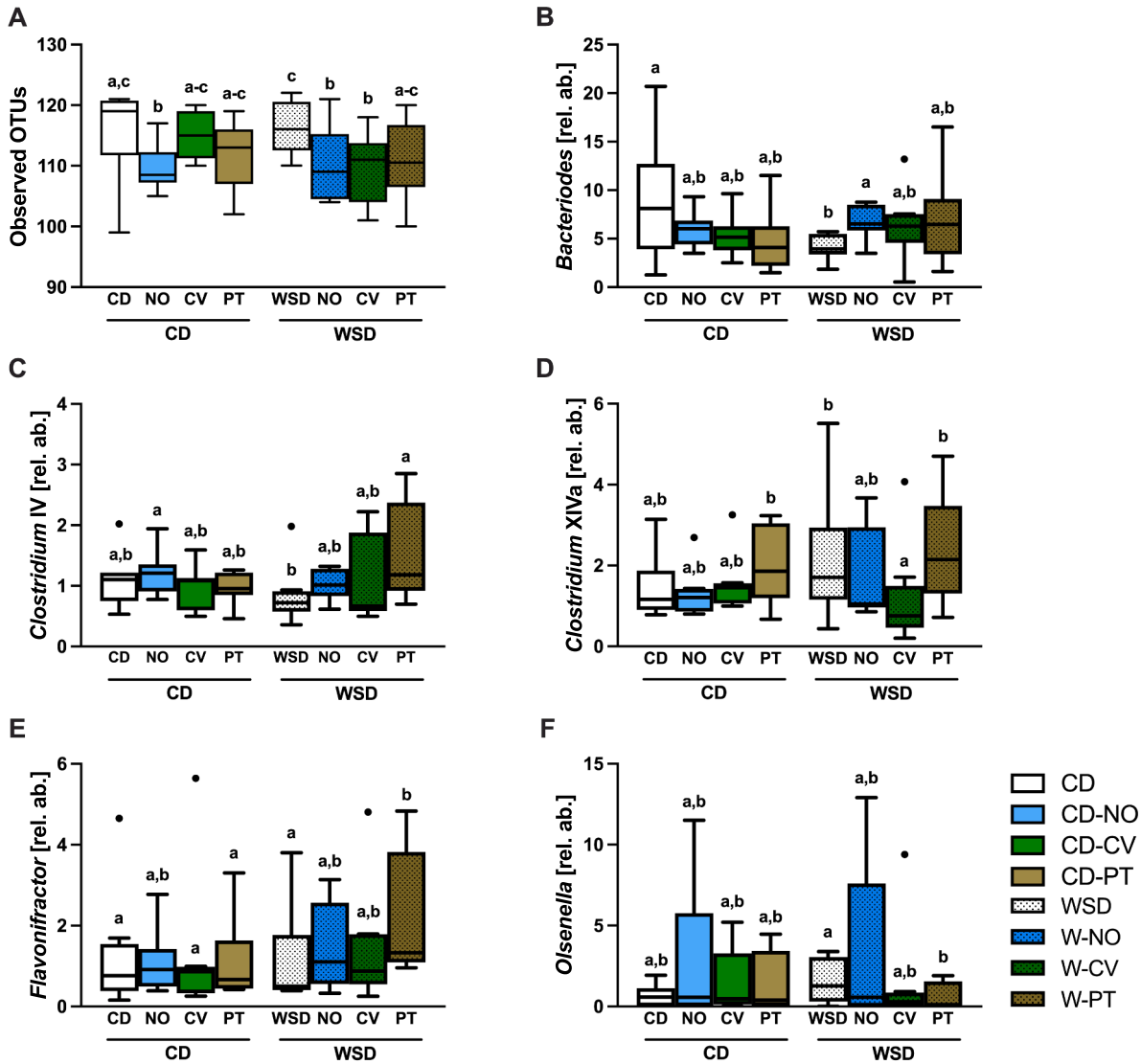

Fig. 4. Alpha diversity measures (operational taxonomic units, OTUs, A) and relative abundance (rel. ab., B-F) of bacterial genera after 69 days of microalgae supplementation. Different letters indicate significant differences, analysed by Kruskal-Wallis tests (non-adjusted individual p values are shown; $p < 0.05$). Boxplots and Whiskers (25th and 75th percentiles) are shown (n = 8 per group). Abbreviations: CD, Control diet; CD-CV, CD + *Chlorella vulgaris*; CD-NO, CD + *Nannochloropsis oceanica*; CD-PT, CD + *Phaeodactylum tricornutum*; rel. ab., relative abundance; WSD, Western-style Diet; WSD-CV, WSD + *Chlorella vulgaris*; WSD-NO, WSD + *Nannochloropsis oceanica*; WSD-PT, WSD + Phaeodactylum tricornutum. This figure summarized the findings shown in Table S1.

4.2. NO and PT supplementation improve liver health in hypercaloric-fed mice

There is a growing number of studies investigating the effect of microalgae as a health supplement (Tamel Selvan et al., 2023). In particular, NO and PT have attracted scientific attention due to the high content of bioactive components. In the present study, mice fed a WSD showed no change in liver weight, but showed liver fat accumulation, which is typical of a hypercaloric diet in both mice and humans (Bischoff et al., 2017; Li et al., 2008). Interestingly, supplementation with the microalgae NO reduced liver fat accumulation in hypercaloric fed mice in our study. This improvement was reflected in reduced liver damage, as evidenced by lower liver damage scores and fewer ballooning hepatocytes, conditions which are commonly found in mice fed a high-fat diet (Ohashi et al., 2018). Reduced liver damage was also shown after WSD-PT. Our findings are supported by a previous study in which the size of fat vacuoles in the liver of male Wistar rats was reduced after 16 weeks of a WSD with 5 % NO supplementation, suggesting an improvement in lipid metabolism (du Preez et al., 2021). Other studies also highlighted the protective effect of PT on liver health. A reduction in liver weight had already been shown in rats fed a WSD containing 12 % PT (Mayer et al., 2019) and mice fed a WSD + PT extract containing 3.5–6 % fucoxanthin (Koo et al., 2019).

Our data are in line with results from others, showing that PT and NO offer benefits for liver health, which may be partly due to the high EPA content, as EPA has a positive effect on the regulation of lipid metabolism and helps prevent dyslipidaemia and related diseases (Mayer et al., 2019; Tanaka et al., 2008). The efficacy of α-Linolenic acid, EPA and docosahexaenoic acid in reducing heart and liver inflammation, cardiac fibrosis, and hepatic steatosis was demonstrated in rats with metabolic syndrome induced by a high-fat diet (Poudyal et al., 2013). Meta-analyses of randomised controlled trials also showed an improvement in hepatic steatosis and a reduction in TG levels after supplementation with omega-3 fatty acids in humans (Lu et al., 2016; Zhang et al., 2021). Although our study found no significant effects of the microalgae on TG accumulation in the liver, insulin and cholesterol

levels, other studies suggest an effect on lipid metabolism. For example, in diabetic rats, NO supplementation was observed to reduce serum concentrations of glucose, cholesterol, TG and LDL (Nasirian et al., 2019). And for PT, supplementation in rats and mice prevented hepatic lipid accumulation (Koo et al., 2019; Mayer et al., 2019). It altered the hydroxymethylglutaryl-CoA reductase pathway (Kim et al., 2016), which is the target of statins to lower blood cholesterol levels and treat cardiovascular disease (Sharpe & Brown, 2013). Since PT in general contains larger amounts of fucoxanthin (Celi et al., 2022), the effect could also be attributed to this bioactive ingredient, which can inhibit pancreatic lipase activity and suppress the absorption of triglycerides (Eilam et al., 2022; Matsumoto et al., 2010). In addition, chrysolaminarin, a beta-glucan, and further bioactive ingredients in PT have been shown to be associated with improved hypercholesterolaemia in a zebrafish model (Gora et al., 2022). As hepatic steatosis is closely associated with obesity and other diseases such as MAFLD, often caused by the WSD, supplementation with NO and PT could protect the liver from steatosis.

4.3. NO supplementation to the WSD induced high weight gain, yet particularly low liver fat accumulation and liver damage

Of interest, mice fed with WSD-NO showed a non-significant but noticeable higher caloric intake and weight gain than mice fed with the WSD. Yet surprisingly, the liver fat content and the liver damage score were significantly lower in the WSD-NO group than in the WSD group. This indicates that even though NO increased caloric intake and weight gain, it protected from liver damage.

To some degree this finding might be due to the fact that also fecal energy loss seemed to be higher in the WSD-NO group compared to the WSD group, yet this was not statistically significant. Besides this, this effect could be explained to a large degree by beneficial effects of long-chain omega-3 PUFAs, which are contained in large quantities in NO (Zanella & Vianello, 2020). EPA and DHA increase the activity of brown and beige fat by increasing energy expenditure and oxygen consumption (Jia et al., 2023; Kim et al., 2015; Laiglesia et al., 2016). A *meta*-analysis of 18 randomised clinical trials with MAFLD patients also showed that omega-3 fatty acids are associated with an improvement in fat accumulation in the liver (Bołdys et al., 2023; Yan et al., 2018). Since NO contains large amounts of EPA, reduction of liver fat and liver damage could be explained by the effects of EPA.

4.4. Bioavailability of EPA from NO and PT supplementation and implication for biological effects

The bioactive components of the microalgae such as omega-3 fatty acids and other fatty acids are bioavailable according to our data. Thus, the way of microalgae preparation with ball milling seems to be suitable for obtaining fatty acid-induced biomedical effects in mice in particular, as shown in other studies (Gille et al., 2018, 2019; Neumann, Derwenskus, et al., 2018; Stiefvatter et al., 2022). The current study shows an increase of fatty acids in the liver tissue, which proves a good bioavailability, e.g. of EPA from NO and PT, which confirms the results of a previous study (Neumann, Derwenskus, et al., 2018). Supplementation with 15 % NO resulted in EPA levels in liver tissue comparable to those obtained in previous studies with 10 % fish oil supplementation (Riediger et al., 2008), while supplementation with PT resulted in EPA levels of around 4 %. This suggests that certain strains of microalgae may compete with fish oil in increasing EPA levels in liver tissue, which is supported by our study, previous mouse studies (Neumann, Derwenskus, et al., 2018; Stiefvatter et al., 2022), and human study showing comparable increases in EPA levels with PT supplementation (Stiefvatter et al., 2021). Furthermore, PT supplementation has been shown to improve liver fatty acid composition, as evidenced by a decrease in MUFA levels in liver due to a reduction in Δ9-desaturase levels (Mayer et al., 2019). Regarding the omega-6 to omega-3 fatty acid ratio, which is often elevated in WSD, it has been shown in mice that reducing the ratio significantly attenuates steatohepatitis induced by a high-fat diet (Lazic et al., 2014). Although the ratio was low in all groups in the present study, it was reduced by microalgae supplementation after WSD, which has also been shown in other studies in mice (Mayer et al., 2019; Neumann, Derwenskus, et al., 2018; Stiefvatter et al., 2022). Given the central role of EPA and docosahexaenoic acid in the formation of eicosanoids, which are essential for the regulation of blood pressure, blood clotting and inflammation (Calder, 2015), supplementation with NO and PT seems to efficiently restore a healthy ratio of omega-6 to omega-3 fatty acids with potential health benefits.

4.5. Effect of the microalgae supplementation on gut morphology and gut barrier function

Previous research suggests that bioactive compounds from microalgae may beneficially affect gut health and the gut-liver axis (Eilam et al., 2023). In the current study, we observed elevated levels of endotoxins in mice fed with the WSD. Endotoxins, which are derived from the cell walls of commensal bacteria such as *Escherichia coli*, can trigger immune responses and inflammation when released into the bloodstream. High-fat diets and obesity are known to increase the translocation of bacterial toxins, particularly lipopolysaccharide (LPS), which is a critical factor in the development of non-alcoholic fatty liver disease in mice (Jin et al., 2017) and humans (Kanuri et al., 2015). Notably, in the present study mice fed with a WSD supplemented with one of the three microalgae (CV, NO or PT) showed reduced levels of plasma endotoxin, suggesting an improvement in gut barrier function. However, it must be noted that other markers of gut barrier function, such as occludin and MUC-2 were not altered by the supplementation of the microalgae. Also, further factors besides increased gut barrier permeability can affect proinflammatory cytokine and to some degree plasma endotoxin levels, including infections, impaired liver function, and hyperreactivity of immune cells. Therefore, the effect of microalgae supplementation on gut barrier function needs to be further investigated.

4.6. Microalgae supplementation induced changes in the gut microbiota composition

Our data show that microalgae intake induced changes in the gut microbiota composition. First, we did not see a difference in alpha diversity between mice fed with the CD and mice fed with the WSD. In contrast, Lü et al. (2024) showed that mice fed with a high-fat diet had a lower alpha diversity than mice fed with a low-fat diet. We assume that this difference is due to different meal compositions. For example, the high-fat diet used by Lü et al. contained more fat (50 % vs. 41 kcal% fat) and less carbohydrates (30 % vs. 42 kcal% carbohydrates) that the diet we used. In our study, supplementation of NO to the CD and the WSD induced a statistically significant decrease in alpha diversity. Because NO is especially rich in fibre, these findings are in line with findings in humans showing that supplementation of dietary fibre mostly decreases alpha diversity (Cantu-Jungles & Hamaker, 2023). We did not see a statistically significant effect of PT supplementation on alpha diversity. In contrast, Lü et al. reported that by supplementing PT oil, the low alpha diversity induced by the high-fat diet returned to the level of the low-fat diet (Lü et al., 2024). Therefore, the way of PT supplementation (whole microalgae vs. microalgae oil) seems to have an effect on the gut microbiota. These findings may derive from the fact that not only fibre, but also fatty acids, especially omega-3 fatty acids, and polyphenols can affect the gut microbial composition (Parolini, 2019). So far, the effect of fatty acids and polyphenols on the gut microbiota is not sufficiently investigated.

Our finding that NO added to the WSD increased the abundance of *Bacteroides* is in line with findings from du Preez et al. (2021), who described similar effects in rats. The role of *Bacteroides* species in human health is not sufficiently understood, yet these bacteria exhibit a vast

range of beneficial health effects on the host, e.g. by providing protection from pathogens (Zafar & Saier, 2021). Our data further showed that supplementing PT to the WSD increased the abundance of *Clostridium* cluster IV. *Clostridium* species are carbohydrate- and protein-fermenting bacteria which exert several beneficial health effects including reduced anti-inflammatory effects (Guo et al., 2020). Our previous findings showing an increase in fecal short-chain fatty acid levels by supplementing PT suggest further beneficial effects of PT supplementation (Stiefvatter et al., 2022). These findings are in line with the recent findings from Lü et al., who reported an increase in fecal short-chain fatty acid levels by adding PT oil to low- and high-fat diets in mice (Lü et al., 2024). In summary, our data show effects of the microalgae on the abundance of beneficial bacterial genera. In the future, clinical trials are needed to investigate whether these effects are also found in humans, and which clinical relevance they have.

5. Limitations and strengths

Our study offers several limitations and strengths. One limitation was that we only included male mice. This was due to the fact that female mice show biological alterations due to hormonal alterations, which increases inter-individual variability. Future studies should include both genders to make global assumptions of the biological effects on the microalgae. Even though we have measured important micro- and macronutrients in the microalgae we supplemented, we did not measure all of them, e.g fucoxanthin and chrysolaminarin, for which we refer to published data. There was only a mild mean increase in body weight in the group fed with the CD. This was mainly due to a dampening effect of one mouse which lost weight in the second half of the experiment and one mouse which showed a very low increase in body weight of only 0.6 g. Both mice did not show signs of disease, and the other parameters were in the normal range. The reason for the weight loss and the lack of weight gain in these mice is unknown.

A major strength of our study is its design, including two diets as well as three different microalgae. Also, we performed comprehensive assessments, including histological analyses, organ and tissue health, fatty acid composition, blood and intestinal markers, and gut microbiota composition.

6. Conclusion

In the present study, we showed that supplementation of the microalgae NO reduced liver steatosis induced by the WSD. We furthermore showed that both NO and PT reduced WSD-induced liver damage. Supplementation of NO, CV, and PT also lowered WSD-induced increased blood endotoxin levels. Taken together, our data suggest that a regular consumption of microalgae, in particular NO, could be a sustainable way to combat WSD-related liver steatosis and liver damage. Our findings could pave the way to use microalgae for new types of functional foods and nutraceuticals in the future.

Research involving animals

All animal experiments were conducted in accordance with the EU Directive 2010/63/EU on animal experiments or the guidelines of the National Research Council. All experiments were approved by the local Institutional Animal Care and Use Committee (Regierungspräsidium Stuttgart, authorisation number V330/17 EM).

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CRediT authorship contribution statement

Lena Kopp: Writing – original draft, Visualization, Validation, Formal analysis, Data curation. **Benjamin Seethaler:** Writing – original draft, Visualization, Validation, Formal analysis, Data curation. **Ulrike Neumann:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Stephan C. Bischoff:** Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2024.106429.

References

Bischoff, S. C., Boirie, Y., Cederholm, T., Chourdakis, M., Cuerda, C., Delzenne, N. M., Deutz, N. E., Fouque, D., Genton, L., Gil, C., Koletzko, B., Leon-Sanz, M., Shamir, R., Singer, J., Singer, P., Stroebele-Benschop, N., Thorell, A., Weimann, A., & Barazzoni, R. (2017). Towards a multidisciplinary approach to understand and manage obesity and related diseases. *Clinical Nutrition, 36*(4), 917–938. https://doi.org/10.1016/j.clnu.2016.11.007

Bito, T., Okumura, E., Fujishima, M., & Watanabe, F. (2020). Potential of chlorella as a dietary supplement to promote human health. *Nutrients, 12*(9), 9. https://doi.org/10.3390/nu12092524

Bołdys, A., Bułdak, Ł., Maligłówka, M., Surma, S., & Okopień, B. (2023). Potential therapeutic strategies in the treatment of metabolic-associated fatty liver disease. *Medicina, 59*(10), Article 1789. https://doi.org/10.3390/medicina59101789

Calder, P. C. (2015). Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 1851*(4), 469–484. https://doi.org/10.1016/j.bbalip.2014.08.010

Cantu-Jungles, T. M., & Hamaker, B. R. (2023). Tuning expectations to reality: Don't expect increased gut microbiota diversity with dietary fiber. *The Journal of Nutrition, 153*(11), 3156–3163. https://doi.org/10.1016/j.tjnut.2023.09.001

Celi, C., Fino, D., & Savorani, F. (2022). Phaeodactylum tricornutum as a source of value-added products: A review on recent developments in cultivation and extraction technologies. *Bioresource Technology Reports, 19*, Article 101122. https://doi.org/10.1016/j.biteb.2022.101122

Clemente-Suárez, V. J., Beltrán-Velasco, A. I., Redondo-Flórez, L., Martín-Rodríguez, A., & Tornero-Aguilera, J. F. (2023). Global impacts of western diet and its effects on metabolism and health: A narrative review. *Nutrients, 15*(12), Article 2749. https://doi.org/10.3390/nu15122749

du Preez, R., Majzoub, M. E., Thomas, T., Panchal, S. K., & Brown, L. (2021). Nannochloropsis oceanica as a microalgal food intervention in diet-induced metabolic syndrome in rats. *Nutrients, 13*(11), 11. https://doi.org/10.3390/nu13113991

Ehrlich, J. (2015). *DNA Extraktions Protokoll, in International Human Microbiome Standard (Hrsg.): Standard Operating pocedure for fecal, in Protocol H (S.1-20). (2015)*.

Eilam, Y., Khattib, H., Pintel, N., & Avni, D. (2023). Microalgae—Sustainable source for alternative proteins and functional ingredients promoting gut and liver health. *Global Challenges, 7*(5), Article 2200177. https://doi.org/10.1002/gch2.202200177

Eilam, Y., Pintel, N., Khattib, H., Shagug, N., Taha, R., & Avni, D. (2022). Regulation of cholesterol metabolism by phytochemicals derived from algae and edible mushrooms in non-alcoholic fatty liver disease. *International Journal of Molecular Sciences, 23*(22), 22. https://doi.org/10.3390/ijms232213667

Fabiani, R., Minelli, L., Bertarelli, G., & Bacci, S. (2016). A western dietary pattern increases prostate cancer risk: A systematic review and meta-analysis. *Nutrients, 8*(10), 10. https://doi.org/10.3390/nu8100626

Gille, A., Hollenbach, R., Trautmann, A., Posten, C., & Briviba, K. (2019). Effect of sonication on bioaccessibility and cellular uptake of carotenoids from preparations of photoautotrophic Phaeodactylum tricornutum. *Food Research International, 118*, 40–48. https://doi.org/10.1016/j.foodres.2017.12.040

Gille, A., Neumann, U., Louis, S., Bischoff, S. C., & Briviba, K. (2018). Microalgae as a potential source of carotenoids: Comparative results of an in vitro digestion method

and a feeding experiment with C57BL/6J mice. *Journal of Functional Foods, 49*, 285–294. https://doi.org/10.1016/j.jff.2018.08.039

González-Arceo, M., Gómez-Zorita, S., Aguirre, L., & Portillo, M. P. (2021). Effect of microalgae and macroalgae extracts on non-alcoholic fatty liver disease. *Nutrients, 13*(6), 6. https://doi.org/10.3390/nu13062017

Gora, A. H., Rehman, S., Kiron, V., Dias, J., Fernandes, J. M. O., Olsvik, P. A., Siriyappagouder, P., Vatsos, I., Schmid-Staiger, U., Frick, K., & Cardoso, M. (2022). Management of hypercholesterolemia through dietary ß-glucans–insights from a zebrafish model. *Frontiers in Nutrition, 8*, Article 797452. https://doi.org/10.3389/fnut.2021.797452

Guo, P., Zhang, K., Ma, X., & He, P. (2020). Clostridium species as probiotics: Potentials and challenges. *Journal of Animal Science and Biotechnology, 11*(1), 24. https://doi.org/10.1186/s40104-019-0402-1

Ha, A. W., Na, S. J., & Kim, W. K. (2013). Antioxidant effects of fucoxanthin rich powder in rats fed with high fat diet. *Nutrition Research and Practice, 7*(6), 475–480. https://doi.org/10.4162/nrp.2013.7.6.475

Hagenlocher, Hösel, Bischoff, & Lorentz. (2016). Cinnamon extract reduces symptoms, inflammatory mediators and mast cell markers in murine IL-10(-/-) colitis. *The Journal of Nutritional Biochemistry, 30*. https://doi.org/10.1016/j.jnutbio.2015.11.015

Jia, M., Xu, T., Xu, Y.-J., & Liu, Y. (2023). Dietary fatty acids activate or deactivate brown and beige fat. *Life Sciences, 330*, Article 121978. https://doi.org/10.1016/j.lfs.2023.121978

Jin, C. J., Engstler, A. J., Ziegenhardt, D., Bischoff, S. C., Trautwein, C., & Bergheim, I. (2017). Loss of lipopolysaccharide-binding protein attenuates the development of diet-induced non-alcoholic fatty liver disease in mice. *Journal of Gastroenterology and Hepatology, 32*(3), 708–715. https://doi.org/10.1111/jgh.13488

Johnson, C. S. C., Shively, C. A., Michalson, K. T., Lea, A. J., DeBo, R. J., Howard, T. D., Hawkins, G. A., Appt, S. E., Liu, Y., McCall, C. E., Herrington, D. M., Ip, E. H., Register, T. C., & Snyder-Mackler, N. (2021). Contrasting effects of Western vs Mediterranean diets on monocyte inflammatory gene expression and social behavior in a primate model. *eLife, 10*, Article e68293. https://doi.org/10.7554/eLife.68293

Kanuri, G., Ladurner, R., Skibovskaya, J., Spruss, A., Königsrainer, A., Bischoff, S. C., & Bergheim, I. (2015). Expression of toll-like receptors 1–5 but not TLR 6–10 is elevated in livers of patients with non-alcoholic fatty liver disease. *Liver International: Official Journal of the International Association for the Study of the Liver, 35*(2), 562–568. https://doi.org/10.1111/liv.12442

Kaur, M., Bhatia, S., Gupta, U., Decker, E., Tak, Y., Bali, M., Gupta, V. K., Dar, R. A., & Bala, S. (2023). Microalgal bioactive metabolites as promising implements in nutraceuticals and pharmaceuticals: Inspiring therapy for health benefits. *Phytochemistry Reviews, 22*(4), 903–933. https://doi.org/10.1007/s11101-022-09848-7

Kim, M., Goto, T., Yu, R., Uchida, K., Tominaga, M., Kano, Y., Takahashi, N., & Kawada, T. (2015). Fish oil intake induces UCP1 upregulation in brown and white adipose tissue via the sympathetic nervous system. *Scientific Reports, 5*(1), Article 18013. https://doi.org/10.1038/srep18013

Kim, J. H., Kim, S. M., Cha, K. H., Mok, I.-K., Koo, S. Y., Pan, C.-H., & Lee, J. K. (2016). Evaluation of the anti-obesity effect of the microalga Phaeodactylum tricornutum. *Applied Biological Chemistry, 59*(2), 283–290. https://doi.org/10.1007/s13765-016-0151-1

Koo, S. Y., Hwang, J.-H., Yang, S.-H., Um, J.-I., Hong, K. W., Kang, K., Pan, C.-H., Hwang, K. T., & Kim, S. M. (2019). Anti-obesity effect of standardized extract of microalga Phaeodactylum tricornutum containing fucoxanthin. *Marine Drugs, 17*(5), 5. https://doi.org/10.3390/md17050311

Kumari, A., Garima, & Bharadvaja, N. (2023). A comprehensive review on algal nutraceuticals as prospective therapeutic agent for different diseases. *3 Biotech, 13*(2), Article 44. https://doi.org/10.1007/s13205-022-03454-2

Laiglesia, L. M., Lorente-Cebrián, S., Prieto-Hontoria, P. L., Fernández-Galilea, M., Ribeiro, S. M. R., Sáinz, N., Martínez, J. A., & Moreno-Aliaga, M. J. (2016). Eicosapentaenoic acid promotes mitochondrial biogenesis and beige-like features in subcutaneous adipocytes from overweight subjects. *The Journal of Nutritional Biochemistry, 37*, 76–82. https://doi.org/10.1016/j.jnutbio.2016.07.019

Lazic, M., Inzaugarat, M. E., Povero, D., Zhao, I. C., Chen, M., Nalbandian, M., Miller, Y. I., Cherñavsky, A. C., Feldstein, A. E., & Sears, D. D. (2014). Reduced dietary Omega-6 to Omega-3 fatty acid ratio and 12/15-lipoxygenase deficiency are protective against chronic high fat diet-induced steatohepatitis. *PLoS One, 9*(9). https://doi.org/10.1371/journal.pone.0107658

Li, H., Lelliott, C., Håkansson, P., Ploj, K., Tuneld, A., Verolin-Johansson, M., Benthem, L., Carlsson, B., Storlien, L., & Michaëlsson, E. (2008). Intestinal, adipose, and liver inflammation in diet-induced obese mice. *Metabolism, 57*(12), 1704–1710. https://doi.org/10.1016/j.metabol.2008.07.029

Lü, L., Li, S.-X., Li, X.-Y., Ma, J.-R., He, J.-X., Xing, H.-Z., Chen, C., Miyashita, K., Yang, Y.-H., & Du, L. (2024). Marine algae oils from *Phaeodactylum tricornutum* and *Laminaria japonica* alleviate obesity, insulin resistance, and gut microbiota dysbiosis in high-fat diet-fed mice. *Journal of Functional Foods, 117*, Article 106234. https://doi.org/10.1016/j.jff.2024.106234

Lu, W., Li, S., Li, J., Wang, J., Zhang, R., Zhou, Y., Yin, Q., Zheng, Y., Wang, F., Xia, Y., Chen, K., Liu, T., Lu, J., Zhou, Y., & Guo, C. (2016). Effects of Omega-3 fatty acid in nonalcoholic fatty liver disease: A meta-analysis. *Gastroenterology Research and Practice, 2016*, Article e1459790. https://doi.org/10.1155/2016/1459790

Maeda, H., Hosokawa, M., Sashima, T., Funayama, K., & Miyashita, K. (2005). Fucoxanthin from edible seaweed, Undaria pinnatifida, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochemical and Biophysical Research Communications, 332*(2), 392–397. https://doi.org/10.1016/j.bbrc.2005.05.002

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet Journal, 17*(1), 1. https://doi.org/10.14806/ej.17.1.200

Martinez, K. B., Leone, V., & Chang, E. B. (2017). Western diets, gut dysbiosis, and metabolic diseases: Are they linked? *Gut Microbes, 8*(2), 130–142. https://doi.org/10.1080/19490976.2016.1270811

Matsumoto, M., Hosokawa, M., Matsukawa, N., Hagio, M., Shinoki, A., Nishimukai, M., Miyashita, K., Yajima, T., & Hara, H. (2010). Suppressive effects of the marine carotenoids, fucoxanthin and fucoxanthinol on triglyceride absorption in lymph duct-cannulated rats. *European Journal of Nutrition, 49*(4), 243–249. https://doi.org/10.1007/s00394-009-0078-y

Mayer, C., Côme, M., Ulmann, L., Chini Zittelli, G., Faraloni, C., Nazih, H., Ouguerram, K., Chénais, B., & Mimouni, V. (2019). Preventive effects of the marine microalga Phaeodactylum tricornutum, used as a food supplement, on risk factors associated with metabolic syndrome in Wistar rats. *Nutrients, 11*(5), 5. https://doi.org/10.3390/nu11051069

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One, 8*(4), Article e61217. https://doi.org/10.1371/journal.pone.0061217

Molotoks, A., Smith, P., & Dawson, T. P. (2021). Impacts of land use, population, and climate change on global food security. *Food and Energy Security, 10*(1), Article e261. https://doi.org/10.1002/fes3.261

Nasirian, F., Sarir, H., & Moradi-Kor, N. (2019). Antihyperglycemic and antihyperlipidemic activities of Nannochloropsis oculata microalgae in Streptozotocin-induced diabetic rats. *Biomolecular Concepts, 10*(1), 37–43. https://doi.org/10.1515/bmc-2019-0004

Neumann, U., Derwenskus, F., Gille, A., Louis, S., Schmid-Staiger, U., Briviba, K., & Bischoff, S. (2018). Bioavailability and safety of nutrients from the microalgae Chlorella vulgaris, Nannochloropsis oceanica and Phaeodactylum tricornutum in C57BL/6 Mice. *Nutrients, 10*(8), Article 965. https://doi.org/10.3390/nu10080965

Neumann, U., Louis, S., Gille, A., Derwenskus, F., Schmid-Staiger, U., Briviba, K., & Bischoff, S. C. (2018). Anti-inflammatory effects of Phaeodactylum tricornutum extracts on human blood mononuclear cells and murine macrophages. *Journal of Applied Phycology, 30*(5), 2837–2846. https://doi.org/10.1007/s10811-017-1352-7

Odermatt, A. (2011). The Western-style diet: A major risk factor for impaired kidney function and chronic kidney disease. *American Journal of Physiology-Renal Physiology*. https://journals.physiology.org/doi/full/10.1152/ajprenal.00068.2011?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub%3Dpubmed&.

Ohashi, T., Kato, M., Yamasaki, A., Kuwano, A., Suzuki, H., Kohjima, M., & Ogawa, Y. (2018). Effects of high fructose intake on liver injury progression in high fat diet induced fatty liver disease in ovariectomized female mice. *Food and Chemical Toxicology, 118*, 190–197. https://doi.org/10.1016/j.fct.2018.05.006

Parolini, C. (2019). Effects of fish n-3 PUFAs on intestinal microbiota and immune system. *Marine Drugs, 17*(6). https://doi.org/10.3390/md17060374

Poudyal, H., Panchal, S. K., Ward, L. C., & Brown, L. (2013). Effects of ALA, EPA and DHA in high-carbohydrate, high-fat diet-induced metabolic syndrome in rats. *The Journal of Nutritional Biochemistry, 24*(6), 1041–1052. https://doi.org/10.1016/j.jnutbio.2012.07.014

Riediger, N. D., Othman, R., Fitz, E., Pierce, G. N., Suh, M., & Moghadasian, M. H. (2008). Low n-6:N-3 fatty acid ratio, with fish- or flaxseed oil, in a high fat diet improves plasma lipids and beneficially alters tissue fatty acid composition in mice. *European Journal of Nutrition, 47*(3), 153–160. https://doi.org/10.1007/s00394-008-0709-8

Roberts, M. D., Mobley, C. B., Toedebush, R. G., Heese, A. J., Zhu, C., Krieger, A. E., Cruthirds, C. L., Lockwood, C. M., Hofheins, J. C., Wiedmeyer, C. E., Leidy, H. J., Booth, F. W., & Rector, R. S. (2015). Western diet-induced hepatic steatosis and alterations in the liver transcriptome in adult Brown-Norway rats. *BMC Gastroenterology, 15*(1), 151. https://doi.org/10.1186/s12876-015-0382-3

Roeb, E., Steffen, H. M., Bantel, H., Baumann, U., Canbay, A., Demir, M., Drebber, U., Geier, A., Hampe, J., Hellerbrand, C., Pathil-Warth, A., Schattenberg, J. M., Schramm, C., Seitz, H. K., Stefan, N., Tacke, F., Tannapfel, A., Jansen, P. L., & Bojunga, J. (2015). S2k-Leitlinie nicht alkoholische Fettlebererkrankungen. *Zeitschrift für Gastroenterologie, 53*(7), 668–723. https://doi.org/10.1055/s-0035-1553193

Sharpe, L. J., & Brown, A. J. (2013). Controlling cholesterol synthesis beyond 3-Hydroxy-3-methylglutaryl-CoA reductase (HMGCR) *. *Journal of Biological Chemistry, 288*(26), 18707–18715. https://doi.org/10.1074/jbc.R113.479808

Stiefvatter, L., Lehnert, K., Frick, K., Montoya-Arroyo, A., Frank, J., Vetter, W., Schmid-Staiger, U., & Bischoff, S. C. (2021). Oral bioavailability of Omega-3 fatty acids and carotenoids from the microalgae Phaeodactylum tricornutum in healthy young adults. *Marine Drugs, 19*(12), 12. https://doi.org/10.3390/md19120700

Stiefvatter, L., Neumann, U., Rings, A., Frick, K., Schmid-Staiger, U., & Bischoff, S. C. (2022). 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, 14*(12), 12. https://doi.org/10.3390/nu14122504

Tamel Selvan, K., Goon, J. A., Makpol, S., & Tan, J. K. (2023). Effects of microalgae on metabolic syndrome. *Antioxidants, 12*(2), 2. https://doi.org/10.3390/antiox12020449

Tanaka, N., Sano, K., Horiuchi, A., Tanaka, E., Kiyosawa, K., & Aoyama, T. (2008). Highly purified eicosapentaenoic acid treatment improves nonalcoholic steatohepatitis. *Journal of Clinical Gastroenterology, 42*(4), 413–418. https://doi.org/10.1097/MCG.0b013e31815591aa

Wierdsma, N. J., Peters, J. H. C., van Bokhorst-de van der Schueren, M. E., Mulder, C. J. J., Metgod, I., & van Bodegraven, A. A. (2014). Bomb calorimetry, the gold standard for assessment of intestinal absorption capacity: Normative values in healthy ambulant adults. *Journal of Human Nutrition and Dietetics: The Official Journal*

of the British Dietetic Association, 27(Suppl. 2), 57–64. https://doi.org/10.1111/jhn.12113

Winarto, J., Song, D.-G., & Pan, C.-H. (2023). The role of fucoxanthin in non-alcoholic fatty liver disease. *International Journal of Molecular Sciences, 24*(9), 9. https://doi.org/10.3390/ijms24098203

Xia, D., Qiu, W., Wang, X., & Liu, J. (2021). Recent advancements and future perspectives of microalgae-derived pharmaceuticals. *Marine Drugs, 19*(12), Article 703. https://doi.org/10.3390/md19120703

Yan, J.-H., Guan, B.-J., Gao, H.-Y., & Peng, X.-E. (2018). Omega-3 polyunsaturated fatty acid supplementation and non-alcoholic fatty liver disease: A meta-analysis of randomized controlled trials. *Medicine, 97*(37), Article e12271. https://doi.org/10.1097/MD.0000000000012271

Zafar, H., & Saier, M. H. (2021). Gut Bacteroides species in health and disease. *Gut Microbes, 13*(1), Article 1848158. https://doi.org/10.1080/19490976.2020.1848158

Zanella, L., & Vianello, F. (2020). Microalgae of the genus *Nannochloropsis*: Chemical composition and functional implications for human nutrition. *Journal of Functional Foods, 68*, Article 103919. https://doi.org/10.1016/j.jff.2020.103919

Zhang, H.-J., Gao, X., Guo, X.-F., Li, K.-L., Li, S., Sinclair, A. J., & Li, D. (2021). Effects of dietary eicosapentaenoic acid and docosahexaenoic acid supplementation on metabolic syndrome: A systematic review and meta-analysis of data from 33 randomized controlled trials. *Clinical Nutrition (Edinburgh, Scotland), 40*(7), 4538–4550. https://doi.org/10.1016/j.clnu.2021.05.025

Zinöcker, M. K., & Lindseth, I. A. (2018). The western diet–microbiome-host interaction and its role in metabolic disease. *Nutrients, 10*(3), 3. https://doi.org/10.3390/nu10030365