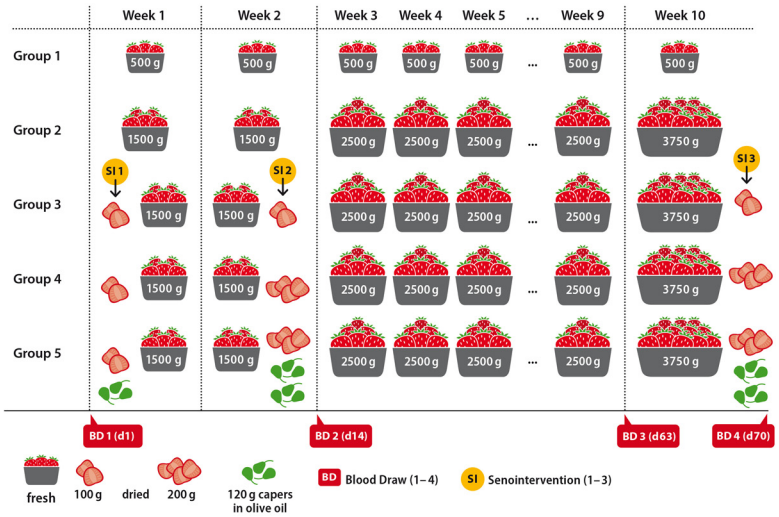
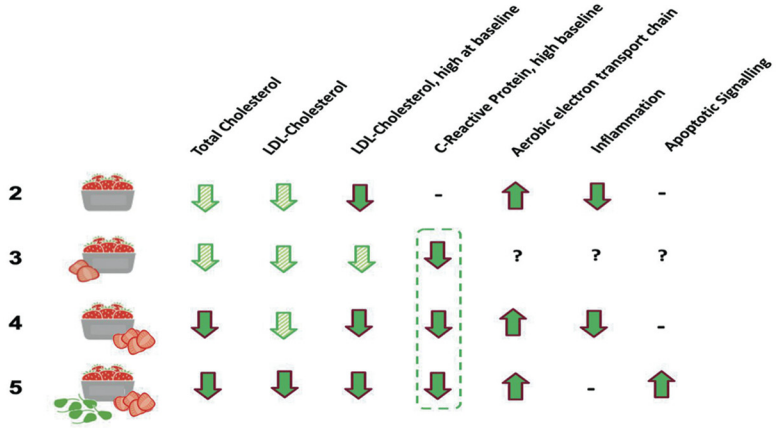
Research Paper

High-Dose Polyphenol-Rich Nutrition Improves Lipid and Inflammation Profiles and Can Trigger Apoptotic Signaling in Healthy Older Adults (the ErdBEHR Study)





Authors

Alexander Hartmann, Riccardo Secci, Juliane Mensch, Kathrin Jäger, Elisabeth Steinhagen-Thiessen, Israel Barrantes, Daniel Palmer, Axel Kowald, Rico Schwarz, Burkhard Hinz, Daniela Weber, Tilman Grune, Verena Heck, Matthias Strauss, Marc Pignitter, Patricia Huebbe, Gerald Rimbach, Henrik Rudolf, Michael Walter, and Georg Fuellen

Correspondence

henrik.rudolf@uni-rostock.de
michael.walter@med.uni-rostock.de
fuellen@uni-rostock.de

• A high-dose polyphenol-rich nutrition decreases LDL and CRP levels in healthy older adults, and at highest dose, gene expression data highlight “positive regulation of apoptotic signaling”.
• Top: Study design, escalating the intervention per group. Senointerventions SI 1, SI 2 and SI 3 were done on single days.
• Bottom: Main study results, suggesting a dose-response pattern. Thick arrows refer to significant findings ($p \leq 0.05$); dashed arrows refer to trends. Results refer to findings in group 2, 3, 4, or 5 when comparing to group 1, for the blood draw at the start of study versus the blood draw at the end of study, after the last intervention; for C-reactive Protein, senointervention groups 3-5 are compared to groups 1 and 2. The “?” refers to no data, the “-” refers to no finding.

Hartmann et al., 2024, *AgingBio*, *1*, 1–15, February 15, 2024 https://doi.org/10.59368/agingbio.20240020

Cite this article as: Hartmann A., et al. (2024). High-Dose Polyphenol-Rich Nutrition Improves Lipid and Inflammation Profiles and Can Trigger Apoptotic Signaling in Healthy Older Adults (the ErdBEHR Study), *AgingBio*, *1*, 1–15. doi: 10.59368/agingbio.20240020.

Research Paper

High-Dose Polyphenol-Rich Nutrition Improves Lipid and Inflammation Profiles and Can Trigger Apoptotic Signaling in Healthy Older Adults (the ErdBEHR Study)

Alexander Hartmann,[1] Riccardo Secci,[2] Juliane Mensch,[1] Kathrin Jäger,[1] Elisabeth Steinhagen-Thiessen,[1] Israel Barrantes,[2] Daniel Palmer,[2] Axel Kowald,[2] Rico Schwarz,[3] Burkhard Hinz,[3] Daniela Weber,[4,5] Tilman Grune,[4,5,6,7] Verena Heck,[6] Matthias Strauss,[6,8] Marc Pignitter,[6] Patricia Huebbe,[9] Gerald Rimbach,[9] Henrik Rudolf,[2,*] Michael Walter,[1,*] and Georg Fuellen[2,10,11,*]

[1]Institute of Clinical Chemistry and Laboratory Medicine, Rostock University Medical Center, Rostock, Germany
[2]Institute for Biostatistics and Informatics in Medicine and Ageing Research, Rostock University Medical Center, Rostock, Germany
[3]Institute of Pharmacology and Toxicology, Rostock University Medical Center, Rostock, Germany
[4]German Institute of Human Nutrition (DIfE) Potsdam-Rehbrücke, Nuthetal, Germany
[5]Food4Future, c/o Leibniz Institute of Vegetable and Ornamental Crops, Grossbeeren, Germany
[6]Institute of Physiological Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria
[7]German Center for Diabetes Research, Neuherberg, Germany
[8]Vienna Doctoral School in Chemistry (DoSChem), University of Vienna, Vienna, Austria
[9]Institute of Human Nutrition and Food Science, University of Kiel, Kiel, Germany
[10]Interdisciplinary Faculty, Department AGIS (Aging of the Individual and Society), University of Rostock, Rostock, Germany
[11]School of Medicine, University College Dublin, Dublin, Ireland
[*]Corresponding authors: henrik.rudolf@uni-rostock.de; michael.walter@med.uni-rostock.de; fuellen@uni-rostock.de
Alexander Hartmann and Riccardo Secci have equally contributed to this research.
https://doi.org/10.59368/agingbio.20240020

Nutritional interventions in healthy individuals may be particularly informative if high, but not excessive, amounts of specific healthy foods are taken to maximize effects without sacrificing safety. We hypothesized that high amounts of polyphenols taken in a single day may affect senescent blood cells. We conducted a 10-week parallel-group controlled randomized open trial with an escalation of consumption of up to ~4 kg of fresh strawberries weekly, plus 200 g of dried strawberries and 240 g of capers in olive oil on three single so-called seno-intervention days (SIDs) in 168 healthy older adults aged 50–80 y. Two primary endpoints, low-density lipoprotein (LDL) cholesterol and high-sensitive C-reactive protein (CRP), were prespecified. We found a significant effect in LDL cholesterol (−0.27 mmol/L, p = 0.007), for the highest-intervention group versus control. All groups with SIDs had a benefit of ~30% in improvement of CRP compared to groups without SIDs (for participants with increased baseline values). LDL levels were dose-dependently reduced by 0.0174 mmol/L for any single 500 g increment in the weekly fresh strawberry intake of the average participant. Gene expression analyses of whole blood suggested improvement of mitochondrial and immunological function, suppression of inflammation (in high-intervention groups), and positive regulation of apoptotic signaling (in the highest-intervention group). Overall, a medium-term nutritional intervention improved lipid and inflammation status and provided specific hints for apoptotic/senolytic effects.

Introduction

Can food be a preventive medicine and a key to longevity? More specifically, are there particular foods or food combinations and corresponding dosing schemes that provide, on the one hand, both outstanding safety and affordability and yet strong health benefits on the other? Can aging-associated processes be slowed down considerably, stopped, or even partially reversed in humans by dietary geroprotection, that is, by foods containing ingredients that promote health? The nutritional "ErdBEHR" ("Erdbeeren [strawberries] for Biomarker identification for the Extension of Health by Rejuvenation") trial reported here was designed to approach these questions for polyphenol-rich food, for which some observational and interventional evidence of positive health effects has been described. Polyphenols are natural organic compounds; frequently studied polyphenols are, for example, catechins, stilbens (e.g., resveratrol), and quercetin, which are found in a variety of plant-based foods. In particular, epidemiologically, health improvements in the Mediterranean and Dietary Approaches to Stop Hypertension (DASH) diets are attributed in

part to their high polyphenol content[1]. Moreover, short- and long-term consumption of polyphenol-rich berries such as strawberries has been associated with a variety of health improvements in previous human studies, with a focus on cardiovascular disease[2].

Here, we studied a food-based high-polyphenol intervention based on strawberries, including capers in olive oil in the highest-intervention group. Previously, strawberries were found to contain the polyphenol fisetin that extends healthspan and lifespan in mice[3], and capers include the closely related polyphenol quercetin[4]. Both polyphenols are frequently mentioned in the literature on interventions aiming at removing senescent cells[5]. As described in **Box 1** (see supplementary files), we found ample amounts of quercetin in both strawberries and capers. Further bioactive ingredients in strawberries are other polyphenols such as pelargonidin (an anthocyanin responsible for red color), fibers, vitamins, and phytosterols[6]. Furthermore, olive oil is also known for its health benefits on lipid metabolism and cardiovascular function and for reducing mortality, due in part to the polyphenols hydroxytyrosol and oleuropein[7].

We had three hypotheses: (a) an intervention with fresh strawberries would trigger improvements in the overall cardiovascular risk profile, specifically cholesterol and inflammation status; (b) the intervention would be further ameliorated by adding "seno-intervention days" (SIDs), on 3 single days over the entire 10-week study participation, whereby large but not excessive amounts of freeze-dried strawberries (and, in the highest-intervention group, also capers in olive oil) are consumed; and (c) SIDs would trigger the apoptosis of senescent blood cells, leading to improvements by eliminating these cells. These improvements could, in part, also be seen as hormetic, triggered by the repair reaction of the body based on the effects of the intervention, resulting in benefits as long as the intervention dose is not too high.

As primary endpoints, we prespecified low-density lipoprotein (LDL) cholesterol and high-sensitive C-reactive protein (hsCRP) in the ethics application (see supplementary files), based on our literature survey[2] and a pilot study (see below). We also measured a set of secondary and exploratory endpoints, including gene expression by next-generation sequencing (transcriptomics). We observed no safety issues except self-reported potential allergy in two cases. We met the primary LDL endpoint in the highest-intervention group, culminating a dose-response trend over all intervention groups, which was even stronger for total cholesterol (TC). We found anti-inflammatory effects in the high-intervention groups, superimposed by a pro-inflammatory pattern in the highest-intervention group, just after the last SID, which we attribute to apoptotic processes based on the gene expression data. Hypothetically, the highest-intervention SID enabled the killing of senescent blood cells, an exciting proposition deserving further research. Since polyphenols may interact with medication[8,9] (**Box 2**, see supplementary files), the study was performed on healthy individuals.

Methods

We follow the Consort reporting guidelines for describing the study.

Trial design

Overall, we adopted a parallel-group five-arm single-center controlled randomized and open design (Fig. 1); see also the cover figure (top panel). The control group (Group 1) featured a small amount of strawberry consumption (once a week), and the four intervention groups (Groups 2–5) featured a longitudinal escalation over the 10-week participation period. Three intervention groups also included three SIDs each, with an escalating dose of freeze-dried

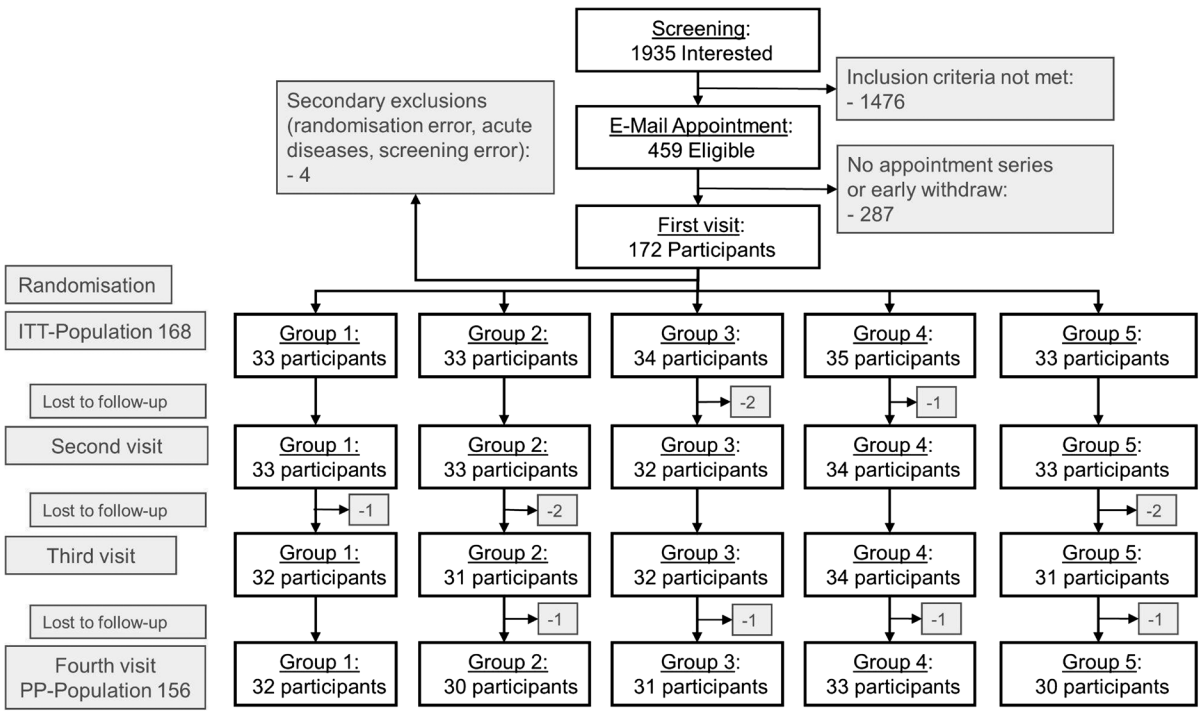


Figure 1. Flowchart of the ErdBEHR study. Abbreviations: ITT, intention to treat; PP, per-protocol.

strawberries and adding capers in olive oil in Group 5. Four visits to the study center were done over the 10 weeks of study participation: at the start (t1), after 2 weeks (day 14, t2), after another 7 weeks (day 63, t3), and at the end (day 70, t4).

Participants

Based on a pilot trial in August 2020, the study was powered as described below. Recruitment of participants was conducted via a recruitment questionnaire run in early 2021 (see supplementary files part A, in German) written by the investigators but run and advertised by the supporting strawberry farm, Karls Erdbeerhof, Rövershagen, Germany. Based on the predefined inclusion and exclusion criteria as outlined in the Ethics Application (see supplementary files part A, in German) and as detailed in the **Supplementary Methods**, respondents were selected; see also the Participant Flow section.

Data collection

Visits to the study center were carried out Tuesdays to Fridays from 7 am to 10 am (avoiding Mondays to mitigate weekend effects) on participation days 1, 14, 63, and 70 of the study. Participants were asked to fast for at least 8 hours (only water and medications, as noted in the **Supplementary Methods**, were allowed). Great care was taken to minimize deviations from the timeplan. Aside from the blood draws, blood pressure, pulse, body weight measurement, and a Chair-Rise-Test were also performed. Participants were asked about their adherence on days 14, 63, and 70, and protocol violations were recorded. Almost all participants filled out the validated EPIC-Potsdam Food Frequency Questionnaire-II (Nothlings et al., 2007)[10], within the first weeks of the study. Clinical data questionnaires (see supplementary files part A, in German) were given to participants on days 1 and 63 of the study, to be returned on days 14 and 70, including quality-of-life questionnaires (EQ-5D-5L and EQ-VAS), a fatigue questionnaire, self-reported anthropometrics, current disease and medication, COVID-19 disease and/or vaccination, vitamin and supplement consumption (regular/in excess of suggested intake), smoking (in pack-years), and exercise. On day 1, the history of disease and direct relatives' disease were also tallied. Questionnaire data were digitized following the four-eyes principle.

Up to 63 mL of blood were drawn and used to measure standard parameters, including platelets, neutrophils, lymphocytes (giving rise to neutrophil-lymphocyte ratio), as well as glucose and insulin/C-peptide (giving rise to HOMA-IR), HbA1c, alkaline phosphatase, electrolytes (calcium, sodium, potassium), liver enzymes (e.g., GGT), albumin, creatinine (giving rise to estimated glomerular filtration rate, eGFR), ferritin, coagulation markers (factor V, factor VIII, factor XII, and D-dimers), and gene expression by next-generation sequencing (see below). Moreover, Luminex protein data of IL6, sTNFr1, and GDF15 were obtained from the serum of 143 compliant non-smoking participants (by whom no difficulties in food consumption were reported in the questionnaires) by using premixed multiplex Human magnetic Luminex® Assay plates (R&D Systems/Bio-Techne, Minneapolis, USA) analyzed on a Luminex® 100/200™ (Burlington, USA). Vitamins and carotenoids (retinol, α-carotene, β-carotene, α-tocopherol, γ-tocopherol, lutein, lycopin, and β-cryptoxanthin) were analyzed in plasma by reversed-phase high-performance liquid chromatography (HPLC) with ultra-violet (UV) detection (internal standard and carotenoids) and fluorescence detection (retinol and tocopherols), respectively[11]. MDA (malondialdehyde) was measured by reverse-phase HPLC and fluorescence detection after derivatization with thiobarbituric acid[12]. Free oxylipins and polyunsaturated fatty acids were isolated from plasma according to Dumlao et al.[13] and then analyzed by a targeted LCMS/MS method based on Wiley et al.[14]; see the **Supplementary Methods** for details. Proteomics, metabolomics, and analyses of the optional skin biopsies and stool microbiomes are pending.

Interventions

The control Group 1 was instructed to eat 500 g of fresh strawberries once a week. A placebo was not available, as a convincing placebo for whole, fresh strawberries is not practical. 500 g of strawberries per week matches the moderate consumption reported in the recruitment questionnaire by all qualifying respondents. Our design further followed a "double-escalation" strategy: the total polyphenol consumption was increased from group to group by introducing specific dietary components; at the same time, all groups except for the control group featured an escalation of dose in time. The standard intervention Group 2 was thus instructed to eat an escalating dose of fresh strawberries: 3 times a week, 500 g over the first 2 weeks, 5 times 500 g each week over the next 7 weeks, and 5 times 750 g over the last week. The high-intervention groups all featured an additional three SIDs of escalating dose. The first SID was scheduled for day 1, *after* the t1 blood draw, with the second and third SIDs scheduled for the day *preceding* the t2 and t4 blood draws, respectively. In Group 3, the SIDs consisted of 100 g of dried strawberries; in Group 4, the first SID featured 100 g, but the second and third featured 200 g of dried strawberries; in Group 5, an additional 120 g of capers in olive oil were added to the first SID and 240 g to the second and third SID. Participants were explicitly allowed to distribute the SID consumption over the two days before the blood draw if, based on their experiences on day 1, they felt that they could not consume all SID food on a single day. See the **Supplementary Methods** for more details on the intervention food.

Outcomes

In the March 2021 ethics application (see supplementary files part A, in German), we prespecified LDL and hsCRP as primary endpoints and the following secondary endpoints: Chair-Rise-Test (number of cycles of rising and sitting accomplished in 30 sec, CR30s), quality-of-life questionnaires (EQ-5D-5L, EQ-VAS, collectively called EQs), high-density lipoprotein (HDL), TC, triglycerides (TG), HOMA-IR, *Phenotypic Age*[15], IL6, sTNFr1, and GDF15. The primary endpoints were motivated by Secci et al.[2] and the pilot study. Our assessment of the literature further motivated the lipid and glucose metabolism markers[16–18], the IL6 and sTNFr1 combination[19], and GDF15[20,21]. *Phenotypic Age*, CR30s, and EQs were recommended in expert interviews.

Sample size, randomization, and blinding

Based on the results of the pilot study, the sample size was planned as described in the **Supplementary Methods**. Investigators were blinded; allocation to groups was not revealed to the physicians or laboratory personnel involved. Participants were not blinded to groups.

Statistical methods

Participant characteristics at baseline were tabled by group assignment with mean (and standard deviations) or count (and percentage). Differences between groups at baseline were

checked by either ANOVA, chi-squared, or fisher test as appropriate. Since repeated measures ANOVA invokes a risk of bias in estimates when used with dropouts, statistical analyses were done by means of linear mixed models (LMM)[22] for all outcomes unless noted otherwise. CRP values were log-transformed, and for Chair-Rise-Test, a generalized model for poisson data was applied. Primary endpoints were examined by LMM, reporting statistical test results for group-by-visit interaction (i.e., for differences in change over time between groups), estimated marginal means for improvements in outcomes (from t1 to t4) for each group, and statistical test results for treatment effects, as the difference in improvement, of Groups 2–5 versus the control Group 1 (t test of coefficients in the LMM). Secondary endpoints were also tested in LMMs (unless stated otherwise), checking group-by-visit interactions first. If these interactions may have triggered a notable difference in time trends between groups, which we deemed possible in the case of $p < 0.2$, effects in groups were examined separately; otherwise, the change in comparison to baseline irrespective of group (i.e., the effect of time considering the fourth visit) was reported. The confirmatory, explorative, and descriptive analyses described above were performed with the statistical software R, along with the R packages lmerTest, emmeans, comparegroups, and ggplot. The significance level was set to 0.025 for the two primary endpoints and 0.05 otherwise, and all p values are two-sided.

Sequencing and bioinformatics methods

We profiled 36 compliant nonsmoking participants by next-generation sequencing of peripheral blood mononuclear cells at timepoints t1 and t4, that is, 5 participants each randomly selected from Groups 1, 2, and 4, and all 21 compliant nonsmoking participants from Group 5. We performed RNA sequencing single-end with a read length of 100 using Salmon, DESeq2, GSEA, and REVIGO for data processing and enrichment analyses; see the **Supplementary Methods** for details.

Ethics

The ethics board of the Rostock University Medical Center approved this study under the registration number A 2021-0096 (see supplementary files part A, in German). The study was registered as DRKS00026998 at the German Register for Clinical Trials (DRKS). We confirm that the study conforms to recognized standards and that participants have given free, prior informed consent.

Results

Participant flow

Via the recruitment questionnaire (see supplementary files part A, in German), 1935 applications were received and screened (Fig. 1) in early 2021. According to the inclusion/exclusion criteria, 459 applicants (including smokers) qualified and were invited consecutively. Series with four appointments per applicant on participation days 1, 14, 63, and 70 were arranged by email. After informed consent, 172 participants could be allocated to one of the 180 appointment series (of 4 visits each) that were available from 10 June to 1 October 2021, recruiting ~28 participants per week from 10 June to 23 July 2021. After baseline assessment, participants received bags packed according to group allocation, containing vouchers for fresh strawberries and the personalized consumption plan, as well as (for Groups 3–5) the additional SID foods to be consumed until the next appointment. There were four secondary exclusions due to randomization error (1), acute disease/accident (2), and screening error (1), leaving 168 participants for analysis in the *intention to treat* population. 12 participants were lost to follow-up, so 156 finished the study regularly.

Baseline data

The baseline characteristics of the 168 participants are shown in Table 1. The average age was ~60 y, and 66% were female. Participants' characteristics were balanced between groups for the clinical attributes and biomarkers at baseline, with the exception of age, which had a slight random imbalance.

Prespecified primary and secondary endpoints: Dietary intervention improves LDL, CRP, and TC in healthy older adults

As the first primary endpoint, we prespecified LDL in the March 2021 ethics application (see supplementary files part A, in German), and we noted a decline in LDL cholesterol after 10 weeks in Group 5 (–0.148 mmol/L, i.e., –5.72 mg/dL), which was a significant improvement over Group 1 ($p = 0.007$); see Figure 2A and Table 2. We also noted a lowering of LDL values for all other intervention groups; see also the cover figure (bottom panel). As the second primary endpoint, we prespecified hsCRP. We found no significant intervention effects in the hsCRP data as recorded (Table 2); we found that these were measured with a detection limit of 1 mg/L, triggering a floor effect (40% of participants had a CRP baseline value recorded at the detection limit). Intervention effects for the subgroup with elevated starting values (>2 mg/L), justifying the use of statins in primary prevention[23–25], are clinically most meaningful. The data of the 43 qualifying participants were thus analyzed exploratively, henceforth referred to as CRP data. While the change over time between groups overall was insignificant, Groups 3–5 featuring SIDs had an improvement of up to −1.43 mg/L (at second visit, ~39% reduction), which was a significant effect compared to Groups 1 and 2 ($p = 0.049$), see Figure 2B and Table 2, even though CRP values are expected to increase over the summer (see Dopico et al.[26], their fig. 5d).

Regarding the prespecified secondary endpoints, TC changes over time were different between groups ($p = 0.091$), and Groups 4 and 5 improved significantly over Group 1 by up to –0.25 mmol/L longitudinally (and by –0.45 mmol/L, Group 5 in comparison to Group 1), see Figure 2C and Table 2. Quality of life could be evaluated in 152 participants, and for EQ-5D-5L (subjective health in five dimensions of interest), no differences between groups were found. For EQ-VAS (subjective health on a scale from 0 to 100), there was no significant group-by-time interaction ($p = 0.115$), but Groups 3 and 4 improved significantly versus Group 1 by up to 6 points (Figure 2D and Table 2). Regarding the further prespecified secondary endpoints, there were no groupwise differences in change over time for *Phenotypic Age* (increasing in all groups by one year, $p = 0.032$, Figure 2E), HOMA-IR, HDL cholesterol (increasing in all groups by 0.042 mmol/L, $p = 0.001$; **Suppl. Fig. 1a**), TG, IL6 (decreasing in all groups over time, but significantly least so in Group 5; Fig. 2F and Table 2), sTNFr1 (decrease in all groups; **Suppl. Fig. 1b**), and GDF15 (decrease in all groups; **Suppl. Fig. 1c**). Chair-Rise-Test data showed that the number of attempts in

Table 1. Baseline data of the ErdBEHR study.

	N = 168	Group 1 N = 33	Group 2 N = 33	Group 3 N = 34	Group 4 N = 35	Group 5 N = 33	p Value
Age	60.7 (7.32)	57.8 (5.66)	61.9 (8.14)	60.1 (6.69)	60.4 (8.15)	63.0 (6.97)	0.044
Sex (female)	110 (65.5%)	20 (60.6%)	25 (75.8%)	25 (73.5%)	20 (57.1%)	20 (60.6%)	0.369
Smoking (yes)	16 (9.52%)	3 (9.09%)	3 (9.09%)	3 (8.82%)	4 (11.4%)	3 (9.09%)	1.000
BMI	25.9 (3.49)	26.1 (2.96)	25.8 (3.41)	25.4 (4.20)	25.7 (2.90)	26.8 (3.86)	0.585
Weight (kg), measured onsite	75.8 (12.1)	77.8 (9.85)	73.7 (11.6)	72.6 (14.7)	77.8 (10.5)	77.2 (13.1)	0.227
Albumin (g/L)	43.4 (2.02)	43.4 (1.62)	43.3 (2.11)	43.3 (1.98)	43.4 (2.11)	43.8 (2.32)	0.883
Alkaline phosphatase (U/L)	76.4 (20.1)	73.6 (17.5)	79.0 (21.7)	76.8 (22.2)	77.0 (19.8)	75.4 (19.5)	0.866
Total cholesterol (mmol/L)	6.04 (1.10)	5.92 (1.16)	6.13 (1.18)	6.01 (1.07)	6.11 (1.08)	6.03 (1.08)	0.941
Creatinine (μmol/L)	73.3 (13.1)	71.1 (12.3)	72.5 (15.4)	73.1 (13.6)	76.0 (13.3)	73.6 (10.7)	0.629
CRP (mg/L)	1.87 (1.51)	1.91 (1.81)	1.96 (1.49)	1.56 (0.94)	1.76 (1.31)	2.20 (1.85)	0.499
Ferritin (μg/L)	156 (138)	162 (125)	134 (128)	146 (130)	148 (93.5)	195 (197)	0.461
GGT (U/L)	27.2 (24.9)	21.1 (11.6)	29.7 (23.6)	24.8 (15.4)	25.4 (18.9)	35.1 (42.3)	0.189
Glucose (mmol/L)	5.71 (0.67)	5.84 (1.00)	5.69 (0.50)	5.76 (0.56)	5.56 (0.44)	5.72 (0.70)	0.535
HbA1c (mmol/mol Hb)	39.3 (4.31)	39.6 (7.13)	38.7 (3.11)	40.8 (3.21)	38.6 (3.34)	39.0 (3.18)	0.220
HDL (mmol/L)	1.72 (0.42)	1.64 (0.34)	1.68 (0.41)	1.88 (0.46)	1.69 (0.48)	1.72 (0.41)	0.175
Interleukin-6 (pg/mL)	3.02 (1.43)	3.03 (1.43)	3.17 (1.44)	3.07 (1.51)	3.15 (1.41)	2.67 (1.33)	0.380
LDL (mmol/L)	3.86 (0.89)	3.85 (0.91)	3.98 (0.96)	3.69 (0.87)	3.96 (0.85)	3.82 (0.87)	0.683
Neutrophil-lymphocyte ratio	1.80 (0.73)	1.73 (0.60)	1.95 (0.85)	1.61 (0.58)	1.92 (0.87)	1.78 (0.69)	0.282
Platelets (10E9/L)	240 (54.3)	246 (57.8)	227 (54.3)	239 (36.8)	253 (57.8)	235 (61.4)	0.342
Triglycerides (mmol/L)	1.16 (0.55)	1.19 (0.42)	1.07 (0.45)	1.13 (0.64)	1.18 (0.61)	1.25 (0.62)	0.765
Statin use	11 (6.55%)	2 (6.06%)	2 (6.06%)	4 (11.76%)	0 (0.0%)	3 (9.09%)	0.300
HOMA-IR (mmol/L)	0.20 (0.09)	0.21 (0.11)	0.20 (0.08)	0.20 (0.09)	0.18 (0.06)	0.20 (0.09)	0.715
eGFR (mL/min)	80.3 (13.8)	85.0 (13.8)	79.4 (15.5)	78.7 (13.6)	78.9 (13.8)	79.7 (12.1)	0.305
PhenoAge (y)	54.9 (8.36)	52.2 (6.81)	56.3 (9.12)	54.3 (7.90)	54.9 (9.27)	56.8 (8.14)	0.219
Health (EQ-5d-5L)	0.94 (0.10)	0.95 (0.07)	0.95 (0.06)	0.91 (0.16)	0.94 (0.11)	0.94 (0.06)	0.362
Health (EQ-VAS)	82.5 (14.6)	83.5 (10.6)	83.74 (10.6)	81.0 (13.4)	83.7 (12.1)	85.9 (9.64)	0.287
30-sec Chair-Stand-Test	13.2 (2.53)	13.8 (2.14)	12.7 (2.11)	13.5 (2.53)	13.6 (2.88)	12.7 (2.80)	0.241

Baseline characteristics and selected primary and derived biomarkers of participants; laboratory biomarkers are listed alphabetically. Numbers show the mean (with standard deviation) or count (with percentage) according to the type of characteristic/biomarker. Abbreviations: BMI, body-mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyltransferase; HbA1c, glycated hemoglobin A1c; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein; PhenoAge: phenotypic age (by Levine); p values represent tests for differences between groups, either ANOVA, chi-squared, or fisher test as appropriate.

30 sec was improved on average by 2 repetitions, with no differences between groups.

Exploratory analyses (fatigue, clinical chemistry, micronutrients, MDA, lipids, LDL elevated at baseline)

Further testing included other biomarkers that may benefit from strawberry intake, according to expert consultations. For eGFR, we found no significant differences in change over time between groups; we found higher values for Groups 4 and 5 though (**Suppl. Fig. 1d**). Other exploratory analyses of fatigue, platelets, neutrophil-lymphocyte ratio, HbA1c, alkaline phosphatase, electrolytes, liver enzymes, albumin, and ferritin showed no group-specific effects, but platelet counts increased in all groups (see **Suppl. Fig. 1e**). Furthermore, we found no significant differences between groups for β-carotene ($p = 0.086$), even though values in Groups 2–5 decreased in comparison to Group 1 (**Suppl. Fig. 1f**). There were no specific findings for the other micronutrients, nor for MDA levels, nor for the coagulation markers, that is, factor V, factor VIII, factor XII, and D-dimers. However, there were two significant changes in the lipid panel, that is, leukotriene B4 (LTB4) increased in Group 3 ($p = 0.0001$), and eicosapentaenoic acid (EPA) decreased in Group 5 ($p = 0.031$). Of note, for the oxylipin 1a,1b-dihomo-15-deoxy-delta-12,14-PGJ2, a potential marker of senolysis[14], we found an insignificant increase of 0.37 μg/ml in Group 5, versus 0.15 μg/ml in Group 1 ($p = 0.67$); the increase in Group 3 was of the same magnitude as in the highest-intervention Group 5. The supplementary data (see Supplementary Files) contains a list of all lipids measured by LCMS/MS and the corresponding effect and response data plots. Exploratively, we also analyzed the subgroup of participants that had high starting values in the primary endpoint LDL, consisting of 72 participants with elevated LDL (>4 mmol/L). Whereas in Group 1, LDL continued to rise, Groups 2, 4, and 5 significantly improved, on average, by –0.2 mmol/L.

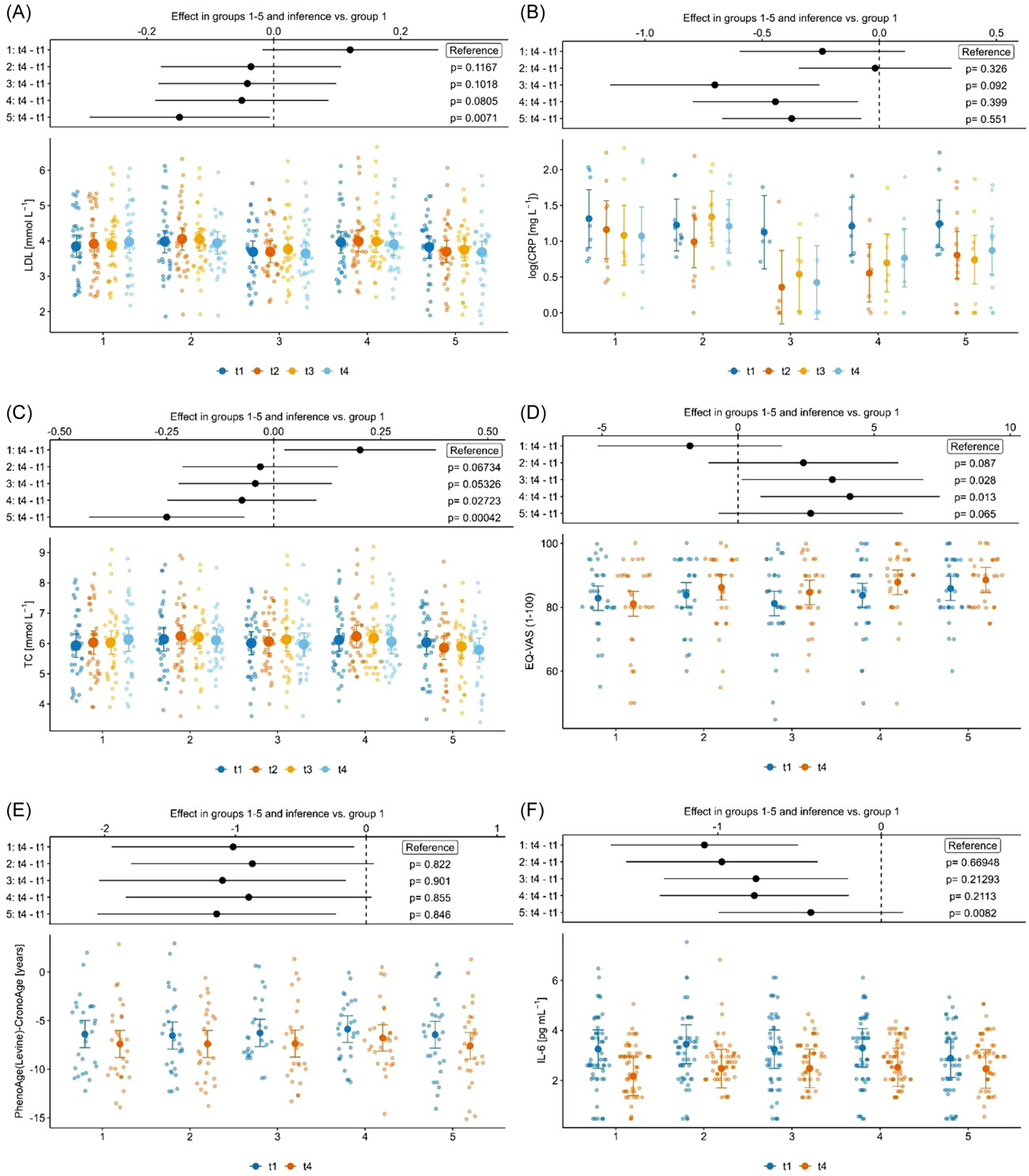


Figure 2. Effect plots and response data plots for primary and selected secondary endpoints. **(A)** low-density lipoprotein (LDL); **(B)** C-reactive protein (CRP) of participants with high baseline level; **(C)** total cholesterol; **(D)** EQ-VAS; **(E)** *Phenotypic Age*; **(F)** IL6. Effects in groups (top) are the differences between the last visit (t4) and the first visit (t1) of estimated marginal means (EMM) with 95% confidence intervals from the linear mixed model (LMM). Differences in change over time (t4–t1) of Groups 2–5 were tested with reference Group 1, and p values of the t test for coefficients in the LMM are displayed. Dots in plots (bottom) represent model-based individual response data across groups 1–5 (on the x-axis) and timepoints (t1, t2, t3, t4, as available), including their EMM (visualized by a large dot) and confidence interval. Exploratively, the effect of seno-intervention days (SID) on participants with elevated CRP starting values (>2 mg/L, panel (B)) was analyzed in a model with pooled groups (3–5 vs. 1–2). A significant group-by-time interaction was found ($p = 0.011$). Considering the log-transformation of CRP, the estimated difference in change over time of −0.342 (3–5 vs. 1–2) corresponds to a benefit of 29% in CRP reduction for SID groups ($\exp(-0.342) = 0.71$).

Table 2. Effect estimates and statistical tests for primary, secondary, and selected exploratory outcomes.

Outcome (Population, n)	Kind of Effect (Interaction Effect, or Effect on Outcome) Being Tested/ Estimated	Effect Estimates; Change at Last Visit vs. First Visit (Standard Error)	p Value
LDL (ITT, n = 168)	Group-by-visit interaction		0.248
	Effect by group at last visit		
	Group 1	0.121 (0.069)	(Ref.)
	Group 2	−0.036 (0.071)	0.117
	Group 3	−0.041 (0.070)	0.101
	Group 4	−0.050 (0.068)	0.081
	Group 5	−0.148 (0.071)	0.007
CRP (log-transf.) (ITT, n = 168)*	Group-by-visit interaction		0.337
CRP (log-transf.) (elevated at baseline: >2 mg/L, n = 43)	Group-by-visit interaction		0.100
	Effect by group at last visit		
	Group 1	−0.243 (0.179)	(Ref.)
	Group 2	−0.018 (0.165)	0.336
	Group 3	−0.702 (0.226)	0.092
	Group 4	−0.443 (0.179)	0.399
	Group 5	−0.374 (0.150)	0.551
TC (n = 168)	Group-by-visit interaction		0.091
	Effect by group at last visit		
	Group 1	0.202 (0.090)	(Ref.)
	Group 2	−0.031 (0.092)	0.072
	Group 3	−0.043 (0.091)	0.057
	Group 4	−0.074 (0.089)	0.030
	Group 5	−0.250 (0.092)	<0.001
EQ-5D-5L (n = 164)	Group-by-visit interaction		0.731
	Effect at last visit**	−0.005 (0.009)	0.599
EQ-VAS (n = 164)	Group-by-visit interaction		0.115
	Effect by group at last visit		
	Group 1	−1.8 (1.71)	(Ref.)
	Group 2	2.4 (1.77)	0.092
	Group 3	3.5 (1.69)	0.031
	Group 4	4.1 (1.67)	0.015
	Group 5	2.7 (1.72)	0.069
Phenotypic Age (n = 143)	Group-by-visit interaction		0.992
	Effect at last visit**	−1.01 (0.207)	<0.001
HOMA-IR (n = 156)	Group-by-visit interaction		0.114
	Effect by group at last visit		
	Group 1	−0.011 (0.008)	(Ref.)
	Group 2	−0.017 (0.008)	0.609
	Group 3	−0.014 (0.008)	0.817
	Group 4	0.010 (0.008)	0.052
	Group 5	−0.008 (0.008)	0.762
Triglycerides (n = 168)	Group-by-visit interaction		0.230
	Effect at last visit**	0.055 (0.050)	0.271
HDL cholesterol (n = 168)	Group-by-visit interaction		0.882
	Effect at last visit**	0.042 (0.013)	0.001
Interleukin-6 (n = 142)	Group-by-visit interaction		0.089

(*Continued on next page*)

Table 2. Continued.

Outcome (Population, n)	Kind of Effect (Interaction Effect, or Effect on Outcome) Being Tested/ Estimated	Effect Estimates; Change at Last Visit vs. First Visit (Standard Error)	p Value
	Effect by group at last visit		
	Group 1	−1.081 (0.281)	(Ref.)
	Group 2	−0.975 (0.288)	0.669
	Group 3	−0.766 (0.288)	0.213
	Group 4	−0.776 (0.284)	0.211
	Group 5	−0.430 (0.278)	0.008
sTNFr1 (n = 143)	Group-by-visit interaction		0.472
	Effect at last visit**	−130.4 (47.95)	0.008
GDF15 (n = 124)	Group-by-visit interaction		0.450
	Effect at last visit**	−47.3 (26.29)	0.081
Chair-Rise-Test (generalized linear mixed model, Poisson, *n = 156*)	Group-by-visit interaction		0.999
	Effect at last visit**	0.16 (0.029)	<0.001

Results for primary, secondary, and selected exploratory analyses. Numbers show estimates of effect at last visit (t4–t1, in units as in Table 1, except for logarithmic transformation) with standard errors. p values are either according to the chi-square test for group-by-visit interaction or the t test for comparisons of the effect at the last visit in Groups 2–5 against the reference Group 1, as specified in the Methods section. Effects on outcomes without hints for group-by-visit interaction are listed in **Supplementary Table 1**. Abbreviations: See Table 1; ITT, intention to treat analysis.
*Detection limit of 1 mg/dL; values below 1 were not set to missing; instead, the value 1 was inserted for ITT analysis of group-by-time interaction.
**The same for all groups.

Dose-response analysis of intervention food intake on LDL levels

Food intake was escalated over groups and time. To estimate the effects of food doses, we modeled intake in the time period preceding the LDL measurements, and the group variable was decomposed into the weekly food intake of fresh strawberries, modeled with unity 500 g/week, capers and olive oil (120 g/week), and freeze-dried strawberries (100 g/week). LDL levels were found to be reduced by 0.0174 mmol/L (0.67 mg/dL) for any 500 g increment in the weekly fresh strawberry intake of an average participant (p = 0.011), corresponding to an expected change of –0.13 mmol/L (–5 mg/dL) for the study maximum intake of up to 5 times 750 g weekly (i.e., 3750 g weekly); there was no clear signal for dried strawberries, nor for capers in olive oil considered alone.

Gene expression: Dietary intervention suppresses inflammation but can also induce apoptosis and phagocytosis pathways

Enrichments of gene ontology (GO) biological processes (GOBP) and KEGG pathways are presented in the following, with a focus on the longitudinal changes in the intervention groups. As expected, the principal component analysis (PCA; **Suppl. Fig. 2**) tends to group the samples by participant ID, motivating longitudinal analyses. The supplementary data include the tables of differentially expressed genes (DEGs), the enrichments (tables and additional figures), as well as additional results and discussion of the control Group 1 gene expression data.

Considering Groups 2 and 4, we profiled 5 participants each longitudinally at the first and last timepoints. Inspecting GOBP terms for Group 2, we found a significant positive enrichment (i.e., enrichment within the upregulated genes, “enriched-up”) of terms related to mitochondrial respiration (aerobic electron transport chain/mitochondrial respiratory chain complex assembly) and coagulation (platelet activation/coagulation) (Fig. 3A). Terms with a significant negative enrichment (i.e., enrichment within the downregulated genes, “enriched-down”) pointed to a reduction in inflammation (toll-like receptor signaling pathway/myeloid leukocyte differentiation/(regulation of) interleukin-6 production). Correspondingly, based on KEGG, oxidative phosphorylation/thermogenesis and platelet activation are “enriched-up” together with cardio-related pathways and general disease pathways (Fig. 3B). The latter were enriched because they include mitochondrial complexes and “housekeeping proteins” such as tubulin, actin, ubiquitin, and histones. In turn, “enriched-down” pathways are associated with lipids (fatty acid metabolism) and inflammation (TNF-, IL-17-, and toll-like signaling and inflammatory diseases). Employing GOBP for Group 4, we again saw an “enriched-up” of terms related to mitochondrial respiration (mitochondrial respiratory chain complex assembly/mitochondrial ATP synthesis coupled electron transport) and additionally a higher activity in some immune-associated processes (myeloid leukocyte-mediated immunity/immune effector process) (Fig. 3C). Furthermore, we saw an increase in reactive oxygen species metabolism (matching the increase in mitochondrial respiration) and in metabolic activity, for example, the catabolism of lipids and carbohydrates. Among the terms reflecting downregulation, we found a variety of terms, many of which related to cellular stress. Using KEGG, we also found upregulation of respiration/metabolism (oxidative phosphorylation/thermogenesis/glycolysis/gluconeogenesis) and related (disease) terms, and we found downregulation related to cancer and (again) to inflammation (Fig. 3D). The latter overlap in part with the observations in Group 2, for example, on *TNF and IL-17 signaling*. Of note, immune-related “enriched-up” terms may be confounded by parallel seasonal effects, while the “enriched-down” terms related to a reduction of inflammation are counter-seasonal[26] (their fig. 5).

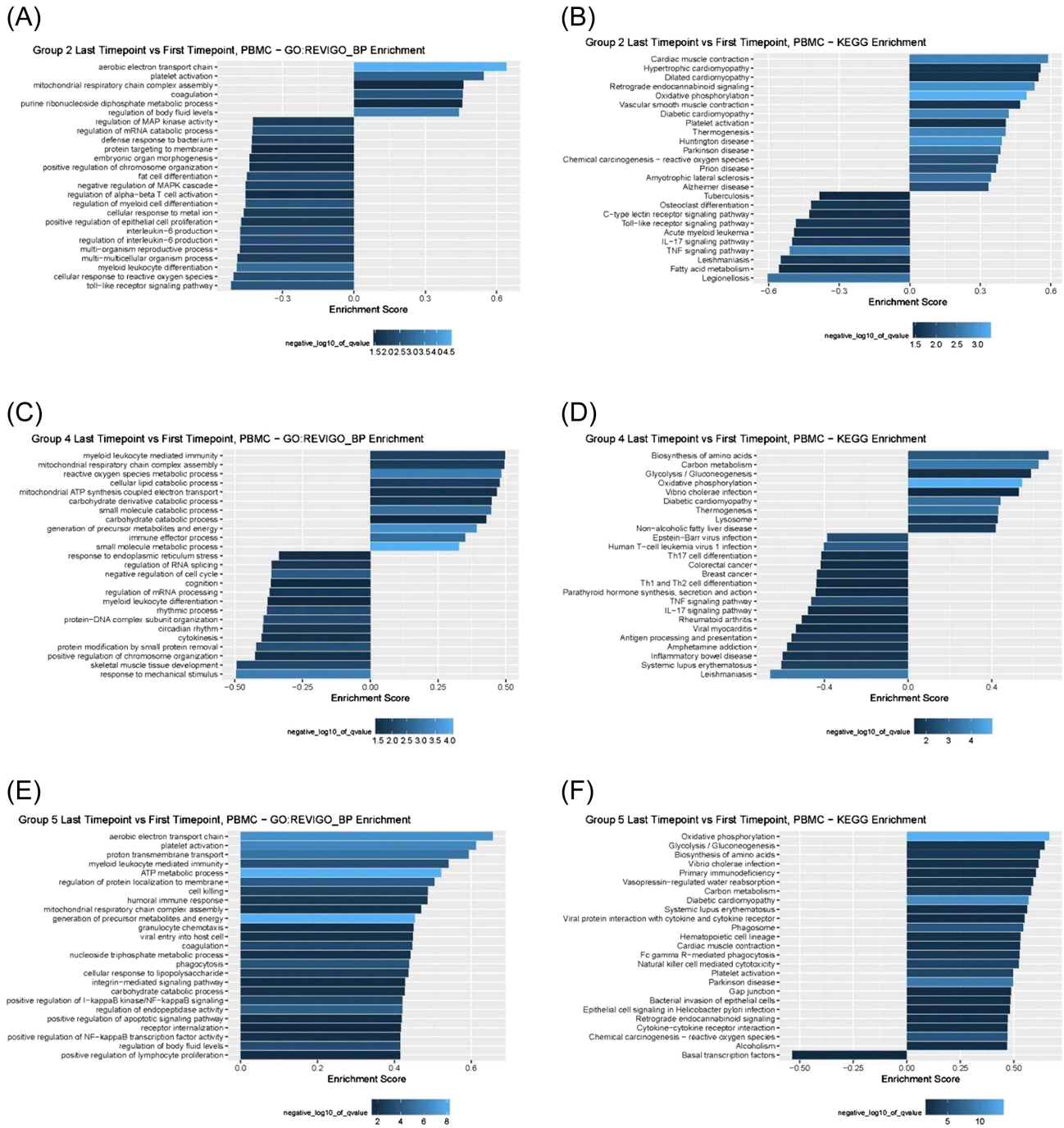


Figure 3. Bar charts illustrate the functional enrichment results of the genes that are longitudinally differentially expressed between timepoints 1 and 4 across the groups. Each pair of subplots, (**A**,**B**) for Group 2, (**C**,**D**) for Group 4, and (**E**,**F**) for Group 5, represents the enrichment results of the longitudinally differentially expressed genes for biological processes (Revigo_BP, left) and pathways (KEGG pathways, right). The size of each bar represents the *Enrichment score* and the color indicates the significance of the enrichment, measured as the negative log10 of the *q-value* (i.e., the adjusted p value used to control for the false discovery rate). Gene expression data were obtained from PBMC (peripheral blood mononuclear cells).

Considering our hypothesis that specific seno-therapeutic effects may be obtained due to the SIDs, we were most interested in the longitudinal change of gene expression in the highest-intervention Group 5, which we profiled in more depth. Since capers in olive oil were consumed in addition to strawberries by Group 5, the following enrichments pertain to the whole high-polyphenol diet with three SIDs, which may specifically reflect hormetic responses to the last highest-intervention SID just before the last blood draw. Heatmaps for the GO terms marked by (*) in the following are provided; see supplementary data (part of the Supplementary Files). “Enriched-up” in GOBP are terms indicating improvement of mitochondrial (aerobic electron transport chain*/proton transmembrane transport/ATP metabolic process/mitochondrial respiratory chain complex assembly), immune (myeloid leukocyte-mediated immunity*/humoral immune response/granulocyte chemotaxis/positive regulation of lymphocyte proliferation) and platelet function (platelet activation*/coagulation), and of apoptosis and associated processes (cell

killing/positive regulation of apoptotic signaling pathway*/phagocytosis*/positive regulation of NF-kappaB transcription factor activity) (Fig. 3E). All "enriched-down" GOBP terms are below the significance threshold for reporting. For "enriched-up" in KEGG, we again found mitochondrial and metabolism-related pathways (oxidative phosphorylation, glycolysis/gluconeogenesis), apoptosis-associated processes (phagosome/(Fc gamma R-mediated) phagocytosis/natural killer cell-mediated cytotoxicity), and platelet-associated processes (platelet activation) (Fig. 3F). Of note, *platelet activation* is likely reflecting not an activation but an increase in the number of cells expressing platelet-specific genes, given the increase in the number of platelets in Group 5 (as in all other groups, **Suppl. Fig. 1e**) and the dominance of constitutive proteins in the underlying list of genes, including actin beta, integrin subunit alpha 2b, Fc epsilon receptor Ig, myosin light chain, as well as membrane proteins and enzymes. Of interest, the platelet effect was likely counter-seasonal (Dopico et al.[26], their fig. 4b), while some immune/inflammation-related terms and also the related term *phagocytosis* may have been confounded by parallel seasonal effects (Dopico et al.[26], their fig. 5a; about half of the seasonal phagosomal genes listed in their supplementary fig. 8, i.e., CD38, FCGR2A, ITGB2, and ITGAM coincide with the genes that we see differentially regulated in the *phagocytosis* KEGG pathway). Finally, KEGG enrichments again highlighted a wide set of (infectious) disease pathways because these feature mitochondrial complexes, tubulin, actin, ubiquitin, histones, or immune-related surface marker proteins; the only "enriched-down" KEGG term refers to basal transcription.

Harms

No severe side effects were reported by the participants, except for two cases of self-reported strawberry allergy, though without clinical confirmation. Furthermore, 8 participants reported mild intolerance reactions or difficulties consuming the comparatively large amount of extra food on SIDs. (For a review of the potential harms of SIDs, specifically regarding potential food-drug interactions, see **Box 2**, supplementary files.)

Discussion

A strawberry-based intervention improves lipid profiles and inflammation markers

Here, we tested an escalating intervention scheme over 10 weeks, specifically demonstrating the health effects of SIDs that feature high amounts of polyphenol-rich food. Our medium-term dietary intervention dose-dependently lowered LDL and CRP in healthy older adults, associated with a specific response with respect to inflammation and immunity. Moreover, effects got stronger as participants continued consumption of the test food, demonstrating convincing dose-response relationships. Significant LDL improvement in participants with elevated LDL, as well as mitochondrial improvements and attenuated inflammation (as reflected by the transcriptome), were already triggered by fresh strawberries alone. By adding dried fruit to SIDs, CRP (in participants with a high baseline level) improved. A higher level of dried fruit triggered significant TC improvements (secondary endpoint). Finally, LDL improvements were the strongest and statistically significant in the highest-intervention group (primary endpoint, $p < 0.007$), which also featured putative senolytic effects (specifically in the transcriptome; see below). We can thus claim a health improvement effect in healthy (and well-nourished) older adults when considering risk biomarkers as proxies of health. Moreover, for the nutritional intervention tested here, we noted high self-reported compliance and no safety issues, despite high amounts of polyphenol-rich food in the highest-intervention group.

Past studies of polyphenols

The effects of a polyphenol-rich diet in general and (straw-)berries in particular are well known for both lipid metabolism (specifically LDL and TC) and inflammation (specifically CRP and IL6)[2,6]. Many active ingredients in a polyphenol-rich diet, such as quercetin, are considered to mediate these effects, and the host microbiome is involved since it metabolizes polyphenols (as well as fibers) and also changes in its composition based on diet, giving rise to a complex network of molecular mechanisms[27]. Lists of notable strawberry components are available[6]; we singled out quercetin because other polyphenol ingredients in strawberries are less well understood or less frequently reported to be found in notable amounts; furthermore, we explicitly identified quercetin in the intervention food (**Box 1**, see supplementary files). In particular, human studies on pelargonidin, the anthocyanin considered responsible for the red color of strawberries, are scarce; we identified one recent epidemiological study demonstrating neuroprotective effects[28]. Nevertheless, supplementation with anthocyanins has been shown to improve LDL levels in dyslipidemic patients[29], while anthocyanin-rich foods are known for some of the effects we found, including improvements in LDL, CRP, and TC[2]. Fisetin is comparatively well researched, but we could not identify it in the strawberry samples we investigated (**Box 1**, see supplementary files).

Cholesterol

In our study, the positive cholesterol-related effects were stronger for TC than for LDL in all intervention groups, with the effects being visible in Group 2 (fresh strawberries only) and increasing monotonously in size with each escalation step for both markers (Table 2), up to −0.148 mmol/L in the case of LDL and up to −0.25 mmol/L in the case of TC. As described in the **Supplementary Discussion**, this reduction of LDL cholesterol and TC is comparable to what was reported in the literature. Such a clear dose-response relationship is not always found; most recently, it was reported that the lower dose, but not the higher dose, of strawberry powder was effective in reducing LDL for unclear reasons[30]. HDL and triglycerides showed no intervention-related pattern in our study; however, HDL improved overall.

Mechanistically, cholesterol effects by (straw-)berries and a polyphenol-rich diet in general and by quercetin in particular may be explained by the inhibition of cholesterol uptake through competition and by a direct influence on membrane properties and cellular signaling cascades[31]. Interestingly, the decreases in TC in our study were even more pronounced than in LDL cholesterol, which could (together with positive effects on HDL) point to a decrease of atherogenic small dense LDL and/or other atherogenic particles. The downregulation of PCSK9 and the upregulation of ABCA1 shown in mice after quercetin treatment are other possible explanations for lower LDL (and higher HDL)[31]. Also, phytosterols from strawberries have been shown to lower LDL and LDL-like particles by competing with cholesterol absorption[6].

Inflammation

Inflammation-related effects were visible in our study, although these are more dynamic. More specifically, the interventions (in particular the SIDs in Groups 3–5) may feature acute

versus chronic effects over time, in a hormetic fashion. Yet, in subjects with initially elevated CRP values, we observed a strong, approximately 30% decrease in CRP (by up to around –0.7 mg/L) already after the first two weeks of intervention, but only in SID Groups 3–5. For IL6, we observed a decrease in all groups overshadowed by an intervention effect in Group 5 only, where the decrease was significantly less than in all other groups, including Group 1. We thus postulate that in all high-intervention groups, a chronic anti-inflammatory effect is present, while in Group 5, an acute immune reaction with a relative increase in inflammatory markers co-occurs one day after the SID, possibly triggered by apoptotic/senolytic processes in blood cells. This scenario is motivated and supported by the gene expression data (see below); however, we also note that the salt content of the capers in olive oil may have triggered an inflammatory response (see below). Overall, the anti-inflammatory pattern may be further influenced by seasonal effects; these would have been in a pro-inflammatory direction[26] for the duration of our study, making an even stronger anti-inflammatory effect of the intervention necessary to yield a visible anti-inflammatory effect. As described in the **Supplementary Discussion**, the anti-inflammatory effects we report for CRP are comparable to previous findings in the literature.

Mechanistically, a plethora of anti-inflammatory effects are attributed to (straw-) berries/polyphenols in general and quercetin in particular, and they center around an inflammatory cytokine axis featuring TNFα, IL6, and CRP[2,32,33]. CRP is an important acute-phase protein of hepatic origin that binds to lysophosphatidylcholine expressed on the surface of certain types of bacteria or dying cells to activate the complement system and eliminate these bacteria as well as dead cells. The role of CRP and its mediators is difficult to pin down and is multifunctional. For example, in combination with complement components, CRP can promote the noninflammatory clearance of apoptotic cells[34]; CRP-induced apoptosis was also observed[35]. Of note, CRP is known to be inhibited by quercetin in vitro[36]. Moreover, strawberry extract and pelargonidin-3-O-glucoside were shown to downregulate TNFα and IL6 in a mouse model of inflammation, and in vitro, pelargonidin-3-O-glucoside showed similar effects and also inhibited NFκB and MAPK signaling[37]. Testing strawberry extract effects ourselves, we stimulated cultured hepatocytes with IL1β/IL6, triggering a manifold increase of the CRP mRNA level compared to untreated cells, and a water-soluble strawberry extract indeed impeded CRP induction in a dose-dependent manner (see **Box 3**, supplementary files). Furthermore, in the gene expression data (see below), anti-inflammatory changes were found, with a superimposed immune-inflammatory effect in the highest-intervention group. As an aside and supported by the gene expression data, we found an increase in platelet number within the healthy physiological range for all groups (**Suppl. Fig. 1e**), despite the seasonally expected decrease[26]; this increase is moderate and far within the U-shaped reference range positively predictive for health[38], and a variety of *anti-inflammatory* properties of platelets were described in addition to pro-inflammatory ones[39].

LDL and CRP display various synergistic effects on the cardiovascular systems (described in detail in the **Supplementary Discussion**). Strict target values have been defined for LDL, depending on the global risk, and moderately elevated CRP values belong to the so-called emerging risk factors, whose values above 2 mg/L indicate a need for treatment, even in cases of only moderately elevated LDL[40,41]. Lowering LDL with statins has side effects, and therefore additional dietary LDL lowering, both in primary and secondary prevention, is important to reduce the required statin doses. Therapeutic CRP reduction has even more side effects[42] and is not an option for primary intervention, which makes the combinatorial addressing of LDL and CRP via diet a highly promising approach.

A strawberry-based intervention improves mitochondrial, immune, and platelet function and may clear senescent blood cells

The GOBP and KEGG findings for Groups 2 and 4 (Group 3 was not investigated) yielded many processes and pathways demonstrating improvement of mitochondrial, immunity, and platelet function, as well as suppression of inflammation; these observations match the hypothesis that a polyphenol-rich intervention may not just attenuate inflammation as described above but may also improve mitochondrial metabolism[43,44] and immunity[45–47]. For the highest-intervention Group 5, gene expression-based enrichments suggest a more complex scenario, as follows: (a) Mitochondrial energy metabolism is enhanced in many ways, potentially contributing to better immunity and hormetic inflammation. Preclinical and a few clinical studies specifically support mitochondrial improvements by polyphenols[43,44]. (b) Immunity is enhanced, including *myeloid leukocyte-mediated immunity, phagocytosis*, and *platelet activation*. (c) We also found an enrichment of *cell killing* and *positive regulation of the apoptotic signaling pathway*, associated with *positive regulation of NFkB activity*. This may be an indication of senolysis of senescent blood cells, with a corresponding hormetic activation of some inflammation and immunity processes. Accordingly, IL6 *protein* expression *went down the least* in Group 5, compared to Group 1. Furthermore, in vitro, quercetin alone (as well as oleuropein from olive oil) displays anti-senescence activity[48,49], while also triggering apoptotic and anti-proliferative activity (potentially by acting as a DNA methylation and histone deacetylation inhibitor[50]), for example, in tumor cells (which are, however, not usually senescent) in vitro, and in some animal models[51]. While not statistically significant, in the lipid panel, the oxylipin 1a,1b-dihomo-15-deoxy-delta-12,14-PGJ2 increased correspondingly, and its increase could, in principle, be due to senolysis[14]. (d) We also find an enrichment for the term *phagocytosis*, which may be an event downstream of apoptosis. Alternatively, though, *phagocytosis* and related processes may just be downstream of the immune activation we observe in all high-intervention groups. Correspondingly, in the context of bovine mastitis, quercetin was shown to enhance *phagocytosis* and *bacterial killing*[52], and it promotes the expression of genes involved in phagocytosis in bovine neutrophils[53]. Overall, the gene expression data align with the inflammation marker data, adding mitochondrial and immune aspects, and they suggest apoptosis and related processes triggered in the highest-intervention group, possibly due to the senolysis of senescent blood cells.

In terms of gene expression data, as the closest match to any meta-analysis, an integrative analysis of five studies reported positive effects of polyphenols on cardiometabolic health in humans[54], implicating processes such as cell adhesion and mobility, immune system, metabolism, or cell signaling. More specifically, *TNF and toll-like receptor signaling* and *oxidative phosphorylation* were found, as we did for the high-intervention Groups 2 and 4, and *natural killer cell-mediated cytotoxicity* and the *phagosome*, as we did for the highest-intervention Group 5. A few studies have investigated the effects of an intervention with quercetin on human gene expression, while (straw)berry studies

are lacking. In particular, Boomgaarden *et al.*[55] showed that daily supplementation with quercetin in healthy individuals led to changes in the gene expression profiles of human monocytes; 62 subjects were enrolled in two studies. Overall, quercetin triggered small yet significant changes in the expression of genes related to immune function, nucleic acid metabolism, and cell death, matching some of our findings.

Limitations and strengths: Nutritional trial interpretation and potential sources of bias

Nutritional geroprotective interventions are attractive because of the outstanding safety attributed to changes in diet. A long history of dietary changes due to human and environmental evolution and adaptation, as well as more recent epidemiological data, confirm that effects, positive as well as negative, are usually small. We set out to test how modest as well as more radical changes in diet may affect health when aiming for a specific polyphenol-rich diet, adding strawberries and capers in olive oil to the standard diet of healthy older adults. We note that any dietary intervention is poorly defined because the exact composition of food in terms of ingredients can be quite variable. We checked food samples for two polyphenols, identifying quercetin but not fisetin (**Box 1**, see supplementary files). We expect that a plethora of active ingredients contributed to the effects we are reporting, potentially in synergistic ways[56].

Inclusion bias and placebo effects

Our selected study population already featured a high level of health at the start of the study, as demonstrated by their *Phenotypic Age*, which was already ~6–8 y younger than reference at baseline (Table 1, Fig. 2E). Nevertheless, we observed multiple health improvements in all groups (*Phenotypic Age,* EQs, HDL, IL6, sTNFr1, GDF15, Chair-Rise-Test); just participating in the study (inclusion bias) may have triggered these positive effects. Moreover, placebo effects need to be considered in nutritional studies. No placebo was employed here, as fresh strawberries cannot be "faked." In fact, the instructions to participants explicitly stated that the intervention food was chosen based on well-known knowledge about its health-promoting effects. We thus acknowledge that all effects reported are a mix of true intervention effects and placebo (and other confounder) effects.

Replacement effects

Another source of bias in dietary intervention studies are replacement effects, for example, the high strawberry intake may have implied a lower intake of other fruit (and vegetables), but it may as well have implied a lower intake of less healthy foods, and we did not attempt to find out. Nevertheless, foods triggering high LDL may have been replaced by the intervention food. After 10 weeks, the point estimate for body weight change was –0.10 kg ($p = 0.433$), with no group-specific differences, rendering it unlikely that the effects observed were due to caloric restriction. (As in most nutrition studies, we did not scale the dose of intervention food by body weight or BMI, for reasons of practicality.)

Statistical considerations

The analysis of potential effect modifications was generally limited by statistical power since it involved threefold interaction terms in the LMM. Nevertheless, sex may have modified the effect of the intervention on LDL ($p = 0.011$ for the threefold interaction group:visit:sex), and the difference in LDL change to baseline between women and men was highest in Group 5 (up to –0.472 mmol/L at visit 3), reflecting a larger decrease in women. We found no other specific effects of age group, sex, smoking, or marital status.

While some effects may, at least in part, be due to bias, it is also possible that effects were missed, for example, by insufficient coverage of effects by the proxy biomarkers or sampling timepoints. Detecting the effects of the SIDs is particularly challenging; we sampled blood one day after the SID intervention, and we found evidence that hormetic inflammatory responses were still dominating by then. The SID response is expected to be a mix of responses, and the comparatively high values of IL6 in the highest-intervention group hint at a temporary inflammatory peak, potentially triggered by the apoptosis of cells and downstream consequences, such as phagocytosis[57], and suggest that polyphenols (and, specifically, the comparatively large amount of quercetin) in the SID may have triggered the apoptosis of senescent blood cells. Since the number of senescent cells is usually limited to a small percentage of the total number of cells, the resulting elimination of cells is not expected to be visible in standard cell count data. However, it is also possible that non-senescent cells underwent apoptosis. Also, an alternative explanation for the lack of an anti-inflammatory effect in Group 5 may be the inflammatory effect of the higher consumption of salt (found in the capers in olive oil), even though we instructed the participants in this group to reduce other sources of salt on SIDs; this salt effect may be corroborated by the gene ontology term "vasopressin-related water reabsorption" found in Group 5. Moreover, given that Group 5 features both strawberries and capers, effects due to one of the foods cannot be delineated from the other. We also note that terms related to mitochondria, immunity, cell-killing (phagocytosis), metabolism, and stress response were also found to be upregulated in Group 1, although the signal is much weaker in Group 1 compared to Groups 2, 4, and 5, as described in the **Supplementary Results and Discussion**; we suggest that many of these effects are seasonal. In particular, immune challenges surface from the end of August onward[26], explaining a weak phagocytosis signal.

The strengths of our study lie in the large sample size, exceeding previous reports for strawberries, in the dose-escalation design, and in the intervention foods and comprehensive biomarker measurements, some of which were selected specifically based on our hypotheses regarding the role of lipids, inflammation, and apoptosis/senescence for (cardiovascular) health.

Conclusion

We found that a relatively short-term but intense diet with polyphenol-rich foods can significantly lower LDL, trigger an overall more favorable cardiovascular profile, and potentially trigger a senolytic response. To extend healthy life expectancy, a high-quality diet, regular exercise, and sociopsychological interventions tend to feature few side effects as well as easy availability under most circumstances. Optimizing nutritional interventions toward a maximum synergy of its constituents, ideally based on easily affordable biomarkers[58], and thus tailoring them to specific subpopulations or even specific individuals requires robust observational and interventional studies. Our study contributes important insights toward designing and selecting nutritional interventions for maximum effect and encourages future work to define high-effect nutritional interventions.

Acknowledgments

The study was funded in part by Karls Erdbeerhof (Karl's strawberry farm). The authors thank the Interdisziplinäre Fakultät of the University of Rostock (Altern des Individuums und der Gesellschaft) for intramural financial support. The authors also thank Siemens Healthcare for donating reagents. The authors thank the European Fund for Regional Development (EFRE; GHS-15-0019, 2016–2021) and the *Core Facility for Cell Sorting and Analysis* for their infrastructural support. The mass spectrometric analyses of quercetin and fisetin were performed with a triple quadrupole mass spectrometer, LCMS-8050, co-funded by the Deutsche Forschungsgemeinschaft (DFG; INST 264/169-1 FUGG). The analysis of micronutrients was funded by the BMBF (FKZ 031B0731E) as part of the joint project "f4f—food for future." G.F., A.K., and D.P. are further supported by the BMBF (FKZ 01ZX1903A and 1DS19049). The analysis of the oxylipins and polyunsaturated fatty acids was financially supported by the Austrian Science Fund FWF Project P34512. The authors thank all participants, all expert advisors consulted before the study, and all helpful people at Karls. The authors also thank the local teams at the IBIMA and ILAB (A. Brauer, F. Thiesen, S. Schenk, R. Krause, E. Kauffold, L. Werner, A. Werderitsch, A.-L. Roßmann, R. Müller, Z. Setayeshmehr, C. Raßmus, A. Beier, and T. Freitag). The funders had no role in study design, data collection, data analysis, data interpretation, or the writing of the report.

Author Contributions

A.H. and M.W. implemented the clinical study. R.Se. analyzed the gene expression data. H.R. contributed the biostatistics analysis of the clinical data. J.M. and K.J. contributed to implementing the study. A.H., E.S.-T., I.B., D.P., and A.K. contributed to analyzing and interpreting the data. R.Schw. and B.H. contributed the mass spectrometry data, analysis, and interpretation. D.W. and T.G. contributed the micronutrient and MDA (malondialdehyde) data, analysis, and interpretation. M.P., M.S., and V.H. contributed the LCMS/MS lipid panel. P.H. and G.R. contributed the in-vitro data, analysis, and interpretation. G.F., M.W., and H.R. conceived the study, analyzed and interpreted the data, and wrote the article with the contributions of the other authors.

Data Availability Statement

Aggregated data that do not require managed access are provided in the supplementary files. Other data are available from the authors upon request.

Supplementary Materials

Supplemental information can be found online at https://doi.org/10.59368/agingbio.20240020.

Accepted December 20, 2023
Published February 15, 2024

References

1. Esposito S., Gialluisi A., Costanzo S., Di Castelnuovo A., Ruggiero E., De Curtis A., … Study I. (2022). Mediterranean diet and other dietary patterns in association with biological aging in the Moli-sani Study cohort. *Clin. Nutr.* **41**(5), 1025–1033. PMID: 35390726; doi: 10.1016/j.clnu.2022.02.023.
2. Secci R., Hartmann A., Walter M., Grabe H.J., Van der Auwera-Palitschka S., Kowald A., … Barrantes I. (2023). Biomarkers of geroprotection and cardiovascular health: An overview of omics studies and established clinical biomarkers in the context of diet. *Crit. Rev. Food. Sci. Nutr.* **63**(15), 2426–2446. PMID: 34648415; doi: 10.1080/10408398.2021.1975638.
3. Yousefzadeh M.J., Zhu Y., McGowan S.J., Angelini L., Fuhrmann-Stroissnigg H., Xu M., … Niedernhofer L.J. (2018). Fisetin is a senotherapeutic that extends health and lifespan. *EBioMedicine* **36**, 18–28. PMID: 30279143; doi: 10.1016/j.ebiom.2018.09.015.
4. USDA Database for the Flavonoid Content of Selected Foods Release 3.3 [Internet] 2018. URL: https://agdatacommons.nal.usda.gov/articles/dataset/USDA_Database_for_the_Flavonoid_Content_of_Selected_Foods_Release_3_1_May_2014_/24659802, last accessed January 2023.
5. Wissler Gerdes E.O., Misra A., Netto J.M.E., Tchkonia T., & Kirkland J.L. (2021). Strategies for late phase preclinical and early clinical trials of senolytics. *Mech. Ageing Dev.* **200**, 111591. PMID: 34699859; doi: 10.1016/j.mad.2021.111591.
6. Basu A., Nguyen A., Betts N.M., & Lyons T.J. (2014). Strawberry as a functional food: An evidence-based review. *Crit. Rev. Food Sci. Nutr.* **54**(6), 790–806. PMID: 24345049; doi: 10.1080/10408398.2011.608174.
7. Guasch-Ferre M., Li Y., Willett W.C., Sun Q., Sampson L., Salas-Salvado J., … Hu F.B. (2022). Consumption of olive oil and risk of total and cause-specific mortality among U.S. adults. *J. Am. Coll. Cardiol.* **79**(2), 101–112. PMID: 35027106; doi: 10.1016/j.jacc.2021.10.041.
8. Lopes M., Coimbra M.A., Costa M.D.C., & Ramos F. (2023). Food supplement vitamins, minerals, amino-acids, fatty acids, phenolic and alkaloid-based substances: An overview of their interaction with drugs. *Crit. Rev. Food Sci. Nutr.* **63**(19), 4106–4140. PMID: 34792411; doi: 10.1080/10408398.2021.1997909.
9. Lopez-Yerena A., Perez M., Vallverdu-Queralt A., & Escribano-Ferrer E. (2020). Insights into the binding of dietary phenolic compounds to human serum albumin and food-drug interactions. *Pharmaceutics* **12**(11), 1123. PMID: 33233356; doi: 10.3390/pharmaceutics12111123.
10. Freese J., Feller S., Harttig U., Kleiser C., Linseisen J., Fischer B., … Nothlings U. (2014). Development and evaluation of a short 24-h food list as part of a blended dietary assessment strategy in large-scale cohort studies. *Eur. J. Clin. Nutr.* **68**(3), 324–329. PMID: 24398637; doi: 10.1038/ejcn.2013.274.
11. Stuetz W., Weber D., Dolle M.E., Jansen E., Grubeck-Loebenstein B., Fiegl S., … Burkle A. (2016). Plasma carotenoids, tocopherols, and retinol in the age-stratified (35-74 years) general population: A cross-sectional study in six European countries. *Nutrients* **8**(10), 614. PMID: 27706032; doi: 10.3390/nu8100614.
12. Weber D., Stuetz W., Toussaint O., Debacq-Chainiaux F., Dolle M.E.T., Jansen E., … Grune T. (2017). Associations between specific redox biomarkers and age in a large European cohort: The MARK-AGE project. *Oxid Med. Cell. Longev.* **2017**, 1401452. PMID: 28804532; doi: 10.1155/2017/1401452.
13. Dumlao D.S., Buczynski M.W., Norris P.C., Harkewicz R., & Dennis E.A. (2011). High-throughput lipidomic analysis of fatty acid derived eicosanoids and N-acylethanolamines. *Biochim. Biophys. Acta* **1811**(11), 724–736. PMID: 21689782; doi: 10.1016/j.bbalip.2011.06.005.
14. Wiley C.D., Sharma R., Davis S.S., Lopez-Dominguez J.A., Mitchell K.P., Wiley S., … Campisi J. (2021). Oxylipin biosynthesis reinforces cellular senescence and allows detection of senolysis. *Cell Metab.* **33**(6), 1124–36e5. PMID: 33811820; doi: 10.1016/j.cmet.2021.03.008.
15. Levine M.E. (2013). Modeling the rate of senescence: can estimated biological age predict mortality more accurately than chronological age? *J. Gerontol. A. Biol. Sci. Med. Sci.* **68**(6), 667–674. PMID: 23213031; doi: 10.1093/gerona/gls233.
16. Daneshzad E., Shab-Bidar S., Mohammadpour Z., & Djafarian K. (2019). Effect of anthocyanin supplementation on cardio-metabolic biomarkers: A

systematic review and meta-analysis of randomized controlled trials. *Clin. Nutr.* **38**(3), 1153–1165. PMID: 30007479; doi: 10.1016/j.clnu.2018.06.979.

17. Fallah A.A., Sarmast E., & Jafari T. (2020). Effect of dietary anthocyanins on biomarkers of glycemic control and glucose metabolism: A systematic review and meta-analysis of randomized clinical trials. *Food Res. Int.* **137**, 109379. PMID: 33233081; doi: 10.1016/j.foodres.2020.109379.
18. Shah K., & Shah P. (2018). Effect of anthocyanin supplementations on lipid profile and inflammatory markers: A systematic review and meta-analysis of randomized controlled trials. *Cholesterol* **2018**, 8450793. PMID: 29850238; doi: 10.1155/2018/8450793.
19. Justice J.N., Ferrucci L., Newman A.B., Aroda V.R., Bahnson J.L., Divers J., … Kuchel G.A. (2018). A framework for selection of blood-based biomarkers for geroscience-guided clinical trials: Report from the TAME Biomarkers Workgroup. *Geroscience* **40**(5–6), 419–436. PMID: 30151729; doi: 10.1007/s11357-018-0042-y.
20. Schafer M.J., Zhang X., Kumar A., Atkinson E.J., Zhu Y., Jachim S., … LeBrasseur N.K. (2020). The senescence-associated secretome as an indicator of age and medical risk. *JCI Insight* **5**(12), e133668. PMID: 32554926; doi: 10.1172/jci.insight.133668.
21. Tanaka T., Biancotto A., Moaddel R., Moore A.Z., Gonzalez-Freire M., Aon M.A., … Fantoni G. (2018). Consortium CHI, Semba RD, Ferrucci L. Plasma proteomic signature of age in healthy humans. *Aging Cell* **17** (5), e12799. PMID: 29992704; doi: 10.1111/acel.12799.
22. Cnaan A., Laird N.M., & Slasor P. (1997). Using the general linear mixed model to analyse unbalanced repeated measures and longitudinal data. *Stat. Med.* **16**(20), 2349–2380. PMID: 9351170; doi: 10.1002/(sici)1097-0258(19971030)16:20<2349::aid-sim667>3.0.co;2-e.
23. Kaptoge S., Di Angelantonio E., Lowe G., Pepys M.B., Thompson S.G., Collins R., & Danesh J. (2010). C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: An individual participant meta-analysis. *Lancet* **375**(9709), 132–140. PMID: 20031199; doi: 10.1016/S0140-6736(09)61717-7.
24. Buckley D.I., Fu R., Freeman M., Rogers K., & Helfand M. (2009). C-reactive protein as a risk factor for coronary heart disease: A systematic review and meta-analyses for the U.S. Preventive Services Task Force. *Ann. Intern. Med.* **151**(7), 483–495. PMID: 19805771; doi: 10.7326/0003-4819-151-7-200910060-00009.
25. Helfand M., Buckley D.I., Freeman M., Fu R., Rogers K., Fleming C., & Humphrey L.L. (2009). Emerging risk factors for coronary heart disease: A summary of systematic reviews conducted for the U.S. Preventive Services Task Force. *Ann. Intern. Med.* **151**(7), 496–507. PMID: 19805772; doi: 10.7326/0003-4819-151-7-200910060-00010.
26. Dopico X.C., Evangelou M., Ferreira R.C., Guo H., Pekalski M.L., Smyth D.J., … Todd J.A. (2015). Widespread seasonal gene expression reveals annual differences in human immunity and physiology. *Nat. Commun.* **6**, 7000. PMID: 25965853; doi: 10.1038/ncomms8000.
27. Banerjee A., & Dhar P. (2019). Amalgamation of polyphenols and probiotics induce health promotion. *Crit. Rev. Food Sci. Nutr.* **59**(18), 2903–2926. PMID: 29787290; doi: 10.1080/10408398.2018.1478795.
28. Agarwal P., Holland T.M., James B.D., Cherian L.J., Aggarwal N.T., Leurgans S.E., … Schneider J.A. (2022). Pelargonidin and Berry Intake Association with Alzheimer's disease neuropathology: A community-based study. *J. Alzheimers Dis.* **88**(2), 653–661. PMID: 35694918; doi: 10.3233/JAD-215600.
29. Qin Y., Xia M., Ma J., Hao Y., Liu J., Mou H., … Ling W. (2009). Anthocyanin supplementation improves serum LDL- and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects. *Am. J. Clin. Nutr.* **90**(3), 485–492. PMID: 19640950; doi: 10.3945/ajcn.2009.27814.
30. Richter C.K., Skulas-Ray A.C., Gaugler T.L., Meily S., Petersen K.S., & Kris-Etherton P.M. (2022). Randomized double-blind controlled trial of freeze-dried strawberry powder supplementation in adults with overweight or obesity and elevated cholesterol. *J. Am. Nutr. Assoc.* 1–11. PMID: 35512768; doi: 10.1080/07315724.2021.2014369.
31. Jia Q., Cao H., Shen D., Li S., Yan L., Chen C., … Dou F. (2019). Quercetin protects against atherosclerosis by regulating the expression of PCSK9, CD36, PPARgamma, LXRalpha and ABCA1. *Int. J. Mol. Med.* **44**(3), 893–902. PMID: 31524223; doi: 10.3892/ijmm.2019.4263.
32. Ou Q., Zheng Z., Zhao Y., & Lin W. (2020). Impact of quercetin on systemic levels of inflammation: a meta-analysis of randomised controlled human trials. *Int. J. Food Sci. Nutr.* **71**(2), 152–163. PMID: 31213101; doi: 10.1080/09637486.2019.1627515.
33. Serino A., & Salazar G. (2018). Protective role of polyphenols against vascular inflammation, aging and cardiovascular disease. *Nutrients* **11**(1), 53. PMID: 30597847; doi: 10.3390/nu11010053.
34. Gershov D., Kim S., Brot N., & Elkon K.B. (2000). C-reactive protein binds to apoptotic cells, protects the cells from assembly of the terminal complement components, and sustains an antiinflammatory innate immune response: Implications for systemic autoimmunity. *J. Exp. Med.* **192**(9), 1353–1364. PMID: 11067883; doi: 10.1084/jem.192.9.1353.
35. Blaschke F., Bruemmer D., Yin F., Takata Y., Wang W., Fishbein M.C., … Law R.E. (2004). C-reactive protein induces apoptosis in human coronary vascular smooth muscle cells. *Circulation* **110**(5), 579–587. PMID: 15277326; doi: 10.1161/01.CIR.0000136999.77584.A2.
36. Garcia-Mediavilla V., Crespo I., Collado P.S., Esteller A., Sanchez-Campos S., Tunon M.J., Gonzalez-Gallego J. (2007). The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and down-regulation of the nuclear factor kappaB pathway in Chang Liver cells. *Eur. J. Pharmacol.* **557**(2–3), 221–229. PMID: 17184768; doi: 10.1016/j.ejphar.2006.11.014.
37. Duarte L.J., Chaves V.C., Nascimento M., Calvete E., Li M., Ciraolo E., … Dalmarco E.M. (2018). Molecular mechanism of action of Pelargonidin-3-O-glucoside, the main anthocyanin responsible for the anti-inflammatory effect of strawberry fruits. *Food Chem.* **247**, 56–65. PMID: 29277228; doi: 10.1016/j.foodchem.2017.12.015.
38. Tsai M.T., Chen Y.T., Lin C.H., Huang T.P., & Tarng D.C. (2015) Taiwan Geriatric Kidney Disease Research G. U-shaped mortality curve associated with platelet count among older people: a community-based cohort study. *Blood* **126**(13), 1633–1635. PMID: 26265696; doi: 10.1182/blood-2015-06-654764.
39. Ludwig N., Hilger A., Zarbock A., & Rossaint J. (2022). Platelets at the crossroads of pro-inflammatory and resolution pathways during inflammation. *Cells* **11**(12), 1957. PMID: 35741086; doi: 10.3390/cells11121957.
40. Ridker P.M., Danielson E., Fonseca F.A., Genest J., Gotto A.M. Jr., Kastelein J.J., … Group J.S. (2008). Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N. Engl. J. Med.* **359**(21), 2195–2207. PMID: 18997196; doi: 10.1056/NEJMoa0807646.
41. Choudhry N.K., Patrick A.R., Glynn R.J., & Avorn J. (2011). The cost-effectiveness of C-reactive protein testing and rosuvastatin treatment for patients with normal cholesterol levels. *J. Am. Coll. Cardiol.* **57**(7), 784–791. PMID: 21310313; doi: 10.1016/j.jacc.2010.07.059.
42. Ridker P.M., Everett B.M., Thuren T., MacFadyen J.G., Chang W.H., Ballantyne C., … Group C.T. (2017). Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N. Engl. J. Med.* **377**(12), 1119–1131. PMID: 28845751; doi: 10.1056/NEJMoa1707914.
43. Vasquez-Reyes S., Velazquez-Villegas L.., Vargas-Castillo A., Noriega L.G., Torres N., & Tovar A.R. (2021). Dietary bioactive compounds as modulators of mitochondrial function. *J. Nutr. Biochem.* **96**, 108768. PMID: 34000412; doi: 10.1016/j.jnutbio.2021.108768.
44. Chodari L., Dilsiz Aytemir M., Vahedi P., Alipour M., Vahed S.Z., Khatibi S.M.H., … Eftekhari A. (2021). Targeting mitochondrial biogenesis with polyphenol compounds. *Oxid. Med. Cell Longev.* **2021**, 4946711. PMID: 34336094; doi: 10.1155/2021/4946711.
45. Sangwan N.S., Farag M.A., & Modolo L.V. (2022). *Plants and Phytomolecules for Immunomodulation - Recent Trends and Advances*. New York: Springer.
46. Pahwa H., & Sharan K. (2022). Food and nutrition as modifiers of the immune system: A mechanistic overview. *Trends Food Sci. Technol.* **123**, 393–403. doi: 10.1016/j.tifs.2022.03.017.
47. Basak S., & Gokhale J. (2022). Immunity boosting nutraceuticals: Current trends and challenges. *J. Food Biochem.* **46**(3), e13902. PMID: 34467553; doi: 10.1111/jfbc.13902.

48. Maria J., & Ingrid Z. (2017). Effects of bioactive compounds on senescence and components of senescence associated secretory phenotypes in vitro. *Food Funct.* **8**(7), 2394–2418. PMID: 28665427; doi: 10.1039/c7fo00161d.

49. Varela-Eirin M., Carpintero-Fernandez P., Sanchez-Temprano A., Varela-Vazquez A., Paino C.L., Casado-Diaz A., … Mayan M.D. (2020). Senolytic activity of small molecular polyphenols from olive restores chondrocyte redifferentiation and promotes a pro-regenerative environment in osteoarthritis. *Aging (Albany NY)* **12**(16), 15882–15905. PMID: 32745074; doi: 10.18632/aging.103801.

50. Yessenkyzy A., Saliev T., Zhanaliyeva M., Masoud A.R., Umbayev B., Sergazy S., … Nurgozhin T. (2020). Polyphenols as caloric-restriction mimetics and autophagy inducers in aging research. *Nutrients* **12**(5), 1344. PMID: 32397145; doi: 10.3390/nu12051344.

51. Ghafouri-Fard S., Shoorei H., Khanbabapour Sasi A., Taheri M., & Ayatollahi S.A. (2021). The impact of the phytotherapeutic agent quercetin on expression of genes and activity of signaling pathways. *Biomed. Pharmacother.* **141**, 111847. PMID: 34198048; doi: 10.1016/j.biopha.2021.111847.

52. Disbanchong P., Punmanee W., Srithanasuwan A., Pangprasit N., Wongsawan K., Suriyasathaporn W., & Chuammitri P. (2021). Immunomodulatory effects of herbal compounds quercetin and curcumin on cellular and molecular functions of bovine-milk-isolated neutrophils toward streptococcus agalactiae infection. *Animals (Basel)* **11**(11), 3286. PMID: 34828017; doi: 10.3390/ani11113286.

53. Srikok S., Nambut S., Wongsawan K., & Shuammitri P. (2017). Quercetin promotes the expression of genes involved in phagocytosis in bovine neutrophils. *Am. J. Anim. Vet. Sci.* **12**(2), 85–95. doi: 10.3844/ajavsp.2017.85.95.

54. Ruskovska T., Budic-Leto I., Corral-Jara K.F., Ajdzanovic V., Arola-Arnal A., Bravo F.I., … Milenkovic D. (2022). Systematic analysis of nutri-genomic effects of polyphenols related to cardiometabolic health in humans - Evidence from untargeted mRNA and miRNA studies. *Ageing Res. Rev.* **79**, 101649. PMID: 35595185; doi: 10.1016/j.arr.2022.101649.

55. Boomgaarden I., Egert S., Rimbach G., Wolffram S., Muller M.J., & Doring F. (2010). Quercetin supplementation and its effect on human monocyte gene expression profiles in vivo. *Br. J. Nutr.* **104**(3), 336–345. PMID: 20416132; doi: 10.1017/S0007114510000711.

56. van Breda S.G.J., & de Kok T.M.C.M. (2018). Smart combinations of bioactive compounds in fruits and vegetables may guide new strategies for personalized prevention of chronic diseases. *Mol. Nutr. Food Res.* **62** (1), 1700597. PMID: 29108107; doi: 10.1002/mnfr.201700597.

57. Barnes P.J. (2021). Targeting cellular senescence as a new approach to chronic obstructive pulmonary disease therapy. *Curr. Opin. Pharmacol.* **56**, 68–73. PMID: 33326912; doi: 10.1016/j.coph.2020.11.004.

58. Moqri M., Herzog C., Poganik J.R., Biomarkers of Aging C., Justice J., Belsky D.W., … Gladyshev V.N. (2023). Biomarkers of aging for the identification and evaluation of longevity interventions. *Cell* **186**(18), 3758–3775. PMID: 37657418; doi: 10.1016/j.cell.2023.08.003.