High-dose Polyphenol-rich Nutrition Improves Lipid and Inflammation Profiles and Triggers 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[6,9], Marc Pignitter[6], Patricia Hübbe[8], Gerald Rimbach[8], Henrik Rudolf**[2], Michael Walter**[1], Georg Fuellen**[2,10,11]

[1]Institute of Clinical Chemistry and Laboratory Medicine, Rostock University Medical Center, Germany [2]Institute for Biostatistics and Informatics in Medicine and Ageing Research, Rostock University Medical Center, Germany [3]Institute of Pharmacology and Toxicology, Rostock University Medical Center, 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, Austria [7]German Center for Diabetes Research, Neuherberg, Germany [8]Institute of Human Nutrition and Food Science, University of Kiel, Germany [9]Vienna Doctoral School in Chemistry (DoSChem), University of Vienna, Austria [10]Interdisciplinary Faculty, Department AGIS (Aging of the Individual and Society), University of Rostock, Germany [11]School of Medicine, University College Dublin, Dublin, Ireland. * AH and RS: equal contribution; ** corresponding authors: henrik.rudolf@uni-rostock.de, michael.walter@med.uni-rostock.de, fuellen@uni-rostock.de.

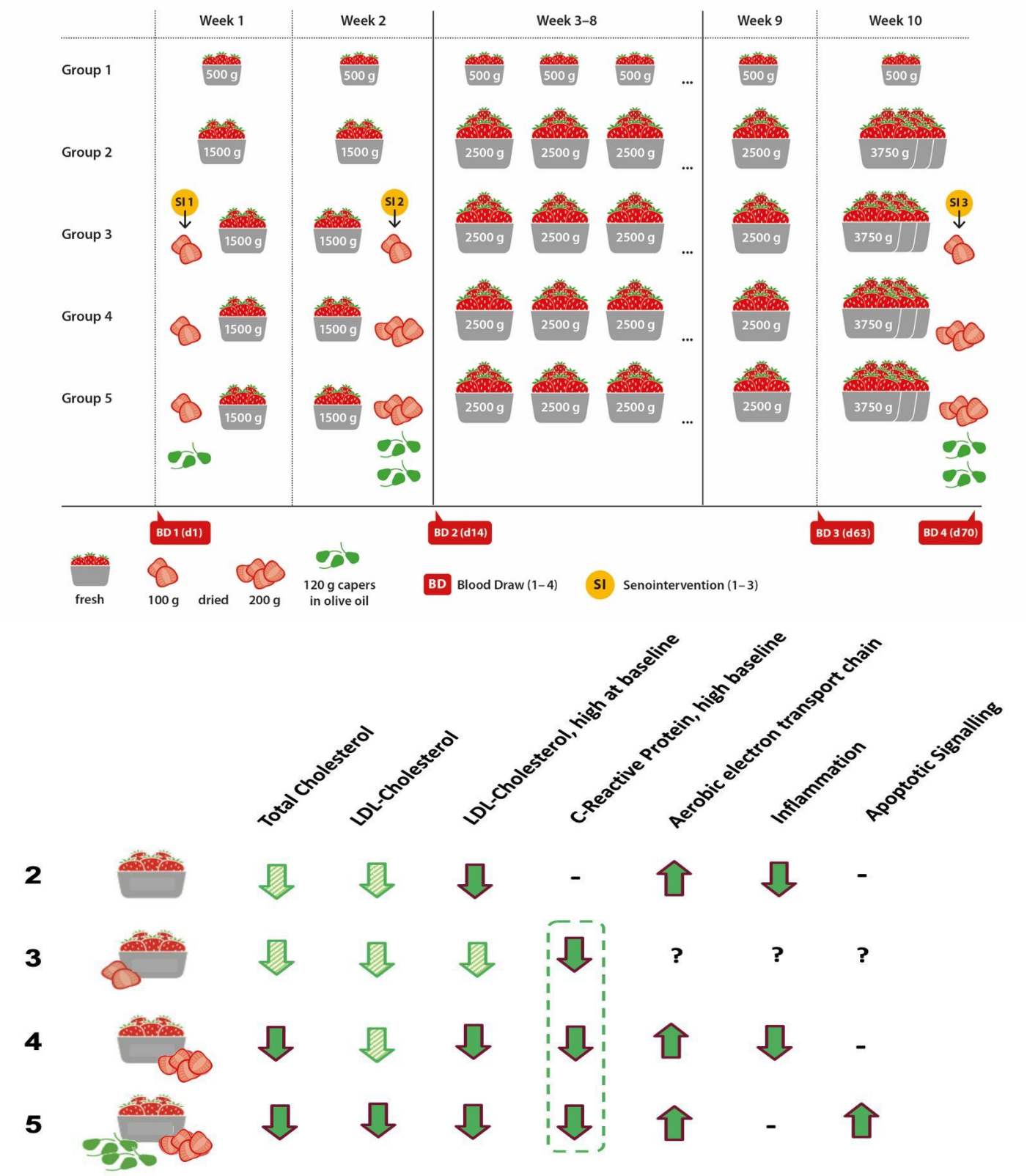
Abstract

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 on single days may affect senescent blood cells. We conducted a ten-week parallel-group controlled randomized open trial with an escalation of consumption, up to ~4kg fresh strawberries weekly, plus 200g dried strawberries and 240g capers in olive oil on three single so-called “seno-intervention” days, in 168 healthy older adults aged 50-80 years. Two primary endpoints, LDL cholesterol and high sensitive CRP, were prespecified. We found a significant decline in LDL cholesterol, and in CRP by ~50% in all groups with seno-intervention days (limited to participants with increased baseline values). LDL levels were dose-dependently reduced by 0.0174 mmol/L for any single 500g-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.

Running Title Nutritional seno-interventions in older adults
Keywords clinical trial, polyphenols, strawberries, capers, olive oil, inflammation, lipids, cellular senescence
Statements relating to relevant ethics and integrity policies, clinical trial registration etc.
Please see “Ethics” in the Methods section
Supplementary Files, Data, Figures and Texts are available (see below).

Key findings

- High-dose polyphenol-rich nutrition decreases LDL and CRP levels in healthy older adults
- At highest dose, gene expression data highlight “positive regulation of apoptotic signaling”
- A clear dose-response pattern based on an escalating intervention design



Graphical Abstract. Top: Study design. Senointerventions SI 1, SI 2 and SI 3 were done on single days. Bottom: Main study results. 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.

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 food 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, e.g., catechins, stilbens (e.g., resveratrol) and quercetin, which are found in a variety of plant-based foods. In particular, epidemiologically, health improvements by the Mediterranean and 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 have 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]. Further, 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: 1) an intervention with fresh strawberries would trigger improvements in the overall cardiovascular risk profile, specifically cholesterol and inflammation status; 2) 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; 3) SIDs would trigger the apoptosis of senescent blood cells, leading to hormetic improvements by eliminating these cells. These improvements could thus be 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 LDL cholesterol and 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. 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 with healthy individuals.

Methods

We follow the Consort reporting guidelines for describing the study.

Trial Design

Overall, we adopted a parallel-group 5-arm single-center controlled randomized and open design (Fig. 1). 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 “seno-intervention days” (SIDs) each, with an escalating dose of freeze-dried strawberries, 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 two 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, 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, in German) and as detailed in *Supplementary Methods*, respondents were selected, see also “Participant Flow”, below.

Data Collection

Visits to the study center were carried out Tuesdays to Fridays 7-10h (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 *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 in 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, 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, history of disease and direct relatives’ disease was also tallied. Questionnaire data were digitized following the 4-eyes principle.

Up to 63mL 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, Natrium, Potassium), liver enzymes (e.g., GGT), albumin, creatinine (giving rise to estimated glomerular

filtration rate, eGFR), ferritin, coagulation markers (Factor 5, Factor 8, Factor 12 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, β-cryptoxanthin) were analyzed in plasma by reversed-phase HPLC with 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 *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 500g of fresh strawberries once a week. A placebo was not available, as a convincing placebo for whole, fresh strawberries is not practical. 500g 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 500g over the first two weeks, 5 times 500g each week over the next 7 weeks, and 5 times 750g 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 the third SIDs scheduled for the day *preceding* the t2 and the t4 blood draws, respectively. In group 3, the SIDs consisted of 100g dried strawberries; in group 4, the first SID featured 100g, but the second and third featured 200g of dried strawberries; in group 5, an additional 120g of capers in olive oil were added on the first SID, and 240g on 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 *Supplementary Methods* for more details on the intervention food.

Outcomes

In the March 2021 ethics application (see Supplementary Files, 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 seconds, CR30s), quality-of-life questionnaires (EQ-5D-5L, EQ-VAS, collectively called EQs), HDL cholesterol, total cholesterol (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] [17] [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, sample size was planned as described in *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 were checked by either t-test, chi-squared or fisher test as appropriate. Since repeated measures ANOVA invokes risk of bias in estimates when used with drop-outs, 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 (EMM) 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 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 PBMC 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 *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, 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, in German), 1935 applications were received and screened (Figure 1) in early 2021. According to the inclusion/exclusion criteria, 459 applicants (including smokers) qualified, who 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 appointments series (of 4 visits each) that were available, from June 10 to October 1, 2021, recruiting ~28 participants per week from June 10 to July 23, 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* (ITT) population. 12 participants were lost to follow-up, so that 156 finished the study regularly.

Baseline Data

The baseline characteristics of the 168 participants are shown in Table 1. Average age was ~60 years, 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 Total Cholesterol in healthy older adults

As the first primary endpoint, we pre-specified LDL in the March 2021 ethics application (see Supplementary Files, in German), and we noted a decline in LDL cholesterol after ten weeks in group 5 (–0.148 mmol/L, that is, –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. As the second primary endpoint, we pre-specified 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] [24] [25], are clinically most meaningful. 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, 4 and 5 featuring SIDs had significantly lower CRP levels compared to groups 1 and 2, see Figure 2b and Table 2, even though CRP values are expected to increase over 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 pre-specified 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), triglycerides, IL6 (decreasing in all groups over time, but significantly least so in group 5, Figure 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 30 seconds 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 group 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, i.e., Factor 5, Factor 8, Factor 12 and d-dimers. However, there were two significant changes of 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 0.37µg/ml in group 5, versus 0.15µg/ml in group 1 (p=0.67); the increase in group 3 was of same magnitude as in the highest-intervention group 5. The Supplementary Data contains the list of all lipids measured and the corresponding effect plots and response data plots. Exploratively, we also analyzed the subgroup of participants which 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.

Dose-response analysis of intervention food intake on LDL levels

Food intake was escalated over groups and time. To estimate effects of food doses, we modeled intake in the time period preceding the LDL measurements, and the group variable was decomposed into weekly food intake of fresh strawberries, modeled with unity 500g/week, capers and olive oil (120g/week), and freeze dried strawberries (100g/week). LDL levels were found to be reduced by 0.0174 mmol/L (0.67 mg/dL), for any 500g-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 750g weekly (that is, 3750g weekly); there was no clear signal for dried strawberries, nor for capers in olive oil considered alone.

Gene expression: Dietary intervention suppresses inflammation, but also induces apoptosis and phagocytosis pathways

Enrichments of 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 PCA (Suppl. Fig. 2) tends to group the samples by participant ID, motivating longitudinal analyses. The Supplementary Data include the tables of DEGs, the enrichments (tables and additional figures), as well as additional results and discussion of the control group 1 gene expression data.

Considering group 2 and group 4, we profiled 5 participants each, longitudinally at the first and the last time point. Inspecting GOBP terms, for group 2 we found a significant positive enrichment (that is, 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) (Figure 3a). Terms with a significant negative enrichment (that is, 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 (Figure 3b). The latter were enriched because they include the 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) (Figure 3c). Furthermore, we saw an increase in reactive oxygen species metabolism (matching the increase in mitochondrial respiration), and in metabolic activity, e.g. 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 (Figure 3d). The latter overlap in part with the observations in group 2, e.g. 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).

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 full. 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. “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) (Figure 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)* (Figure 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 given 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 ([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, that is, CD38, FCGR2A, ITGB2, 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 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 ten weeks, specifically demonstrating the health effects of “seno-intervention days” (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. Adding dried fruit on 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 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 of 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 of 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 case of LDL and up to −0.25 mmol/L in 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 increasing 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 in time, in a hormetic fashion. Yet, in subjects with initially elevated CRP values we observed a strong approximately 50% decrease in CRP (by up to around –0.7 mg/L) already after the first two weeks of intervention, but only in the 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 to 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 of 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, which for values above 2 mg/L indicate a need for treatment, even in case 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 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 46 47]. For the highest-intervention group 5,

gene expression-based enrichments suggest a more complex scenario, as follows. 1) 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]. 2) Immunity is enhanced, including *myeloid leukocyte mediated immunity, phagocytosis,* and *platelet activation*. 3) We also found an enrichment of *cell killing* and *positive regulation of 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]), e.g. 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]. 4) 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 ones, are usually small. We set out to test how modest as well as more radical changes of 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 was already featuring a high level of health at the start of the study, as demonstrated by their *Phenotypic Age*, which was already ~6-8 years younger than reference at baseline (Table 1, Fig. 2e). Nevertheless, we observed multiple health improvements in all groups (*Phenotypic Age,* EQs, HDL, IL6, sTNFr1, GDF-15, 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, e.g. 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 ten weeks, the point estimate for body weight change was –0.10 kg (*p=0.433*), with no group-specific differences, rendering it unlikely that 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 power: The analysis of potential effect modifications was generally limited by statistical power, since it involves 3-fold interaction terms in the LMM. Nevertheless, sex may have modified the effect of the intervention on LDL (*p=0.011* for the three-fold 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 an 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 (see, e.g. [57]), and suggesting that polyphenols (and, specifically, the comparatively large amount of quercetin) of 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 senointervention days; this salt effect may be corroborated by the gene ontology term "vasopressin-related water reabsorption" highlighted 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 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 onwards [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 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 food items can significantly lower LDL and trigger an overall more favorable cardiovascular profile, and potentially a senolytic response. To extend healthy life expectancy, a high quality diet, regular exercise and socio-psychological interventions tend to feature few side effects as well as easy availability under most circumstances. Optimizing these interventions towards a maximum of synergy, 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 some insights towards designing and selecting nutritional interventions for maximum effect, and encourages future work to define high-effect nutritional interventions.

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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 data, analysis and interpretation. M.P, M.S. and V.H. contributed the 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 manuscript with contributions of the other authors.

Conflict of Interest

None declared.

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 Files

In the following, all Supplementary Files, Data, Figures, Texts, and Boxes are listed and described.

Table of Contents:

A) Supplementary Files (Ethics and administrative matters, available at DRKS, the German Clinical Trials Registry, or from the corresponding authors)

1) **Ethics Application, Study Protocol** (03/2021, in German, available at DRKS (link)); the Ethics Application, Study Protocol, with tracked changes (in German, based on the ErdBEHR1 pilot study with Ethics Approval in 2020, is also available at DRKS (link))
2) **Ethics Approval (google drive link)** (04/2021, in German)
3) **Supplementary Documents (google drive link)** (in German)
    a) Recruitment questionnaire
    b) Info sheet (Informationsblatt) ErdBEHR-2-Studie
    c) Clinical data questionnaire (Klinischer Fragebogen ErdBEHR-Studie)
    d) 2nd Clinical data questionnaire (Zweiter Klinischer Fragebogen)
    e) Detailed personalized consumption plan, sample (Verzehrplan Gruppe 5)
4) **Recipes given to the participants (google drive link)** (in German)

B) Supplementary Data

1) PCA

The "PCA" folder (google drive) presents the results of the Principal Component Analysis (PCA) of the gene expression samples, focusing on the top 500 most variable genes.

2) DEG

The “DEG” folder (google drive) contains the results of the differential gene expression (DEG) analysis, which compares the gene expression profiles between different groups. The analysis results are aggregated in the file differentialtable.csv. In addition, there are other files that contain the specific DEGs for each comparison, and their names indicate the comparison groups. These comparisons included all groups (1,2,4 and 5) at timepoint 4 vs timepoint 1, as well as group 5 vs group 1 at timepoint 1 and at timepoint 4.

3) Enrichment_GSEA

The "enrichment_GSEA" folder (google drive) contains the results of the Gene Set Enrichment Analysis (GSEA), based on the DEG data. The results are organized into group-specific subfolders. Each subfolder includes bar plots for the REVIGO and KEGG results, as well as tables that provide the GO, KEGG, and REVIGO results used to generate the plots.

4) Heatmaps

The "heatmaps" folder (google drive) contains heatmaps created from the gene expression data. The heatmaps show the gene expression values of a specific subset of the genes across the samples, as heatmaps of the genes belonging to a selected GO term (see main text; myeloid leukocyte mediated immunity, platelet activation, phagocytosis, aerobic electron transport chain, positive regulation of apoptotic signaling) which is specified in the title of each heatmap. Note that no measurements were done for group 3. Transcript abundance is visualized as the binary logarithm of the read count.

5) Lipid panel

The “lipids” folder (google drive) contains the list of all free oxylipins and polyunsaturated fatty acids measured and the corresponding effect plots and response data plots. Effects in groups (top) are the differences between last visit (t4) and 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 3 and 5 were tested with reference group 1, p-values of t-test for coefficients in the LMM are displayed. Dots in plots (bottom) represent model-based individual response data across groups and timepoints (t1, t2, t3, t4 as available), including their EMM (visualized by a large dot) and confidence interval.

C) Supplementary Figures, Table, Methods, Results and Discussion, and Boxes 1-3

1) Supplementary Figures 1 + 2, and Supplementary Table 1
2) Supplementary Methods
3) Supplementary Results and Discussion, gene expression analyses: Presentation of the group 1 gene expression data.
4) Supplementary Discussion, previous findings: Cholesterol, Inflammation, Interactions of Cholesterol and Inflammation.
5) **Box 1: Quercetin (and fisetin) contents in strawberries, capers and controls**
6) Box 2: Side effects and toxicity
7) Box 3: In-vitro testing of strawberry extract in hepatocytes expressing CRP

Please see the Supplementary Figures, Table, Methods, Results and Discussion, and Boxes 1-3 reproduced below.

Tables and Figures

Table 1. Baseline data of the ErdBEHR study.

		Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	***p-value***
	N=168	***N=33***	***N=33***	***N=34***	***N=35***	***N=33***	
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 phos-phatase (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 [years]	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
30s-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

Table 1: Baseline characteristics, and selected primary and derived biomarkers of participants; laboratory biomarkers are listed alphabetically. Numbers show mean (with standard deviation), or count (with percentage)

according to type of characteristic/biomarker. Abbreviations: BMI: body-mass index; CRP: C-reactive Protein; GGT: gamma-glutamyltransferase; HbA1c: glycated hemoglobin A1c; HDL: high-density lipoproteins cholesterol; LDL: low-density lipoproteins cholesterol; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; eGFR: estimated glomerular filtration rate, PhenoAge: phenotypic age (by Levine).

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 group 2 group 3 group 4 group 5	 0.121 (0.069) -0.036 (0.071) -0.041 (0.070) -0.050 (0.068) -0.148 (0.071)	 (Ref.) 0.117 0.101 0.081 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 group 2 group 3 group 4 group 5	 -0.243 (0.179) -0.018 (0.165) -0.702 (0.226) -0.443 (0.179) -0.374 (0.150)	 (Ref.) 0.336 0.092 0.399 0.551
TC (*n=168*)	Group-by-visit Interaction		0.091
	Effect by group at last visit group 1 group 2 group 3 group 4 group 5	 0.202 (0.090) -0.031 (0.092) -0.043 (0.091) -0.074 (0.089) -0.250 (0.092)	 (Ref.) 0.072 0.057 0.030 <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 group 2 group 3 group 4 group 5	-1.8 (1.71) 2.4 (1.77) 3.5 (1.69) 4.1 (1.67) 2.7 (1.72)	(Ref.) 0.092 0.031 0.015 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 group 2 group 3 group 4 group 5	 -0.011 (0.008) -0.017 (0.008) -0.014 (0.008) 0.010 (0.008) -0.008 (0.008)	 (Ref.) 0.609 0.817 0.052 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
	Effect by group at last visit group 1 group 2 group 3 group 4 group 5	 -1.081 (0.281) -0.975 (0.288) -0.766 (0.288) -0.776 (0.284) -0.430 (0.278)	 (Ref.) 0.669 0.213 0.211 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

Table 2: 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 chisquare-test for group-by-visit interaction or t-test for comparisons of the effect at last visit in groups 2-5 against the reference group 1, as specified in the Methods section. Effects for 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. ** Same for all groups.

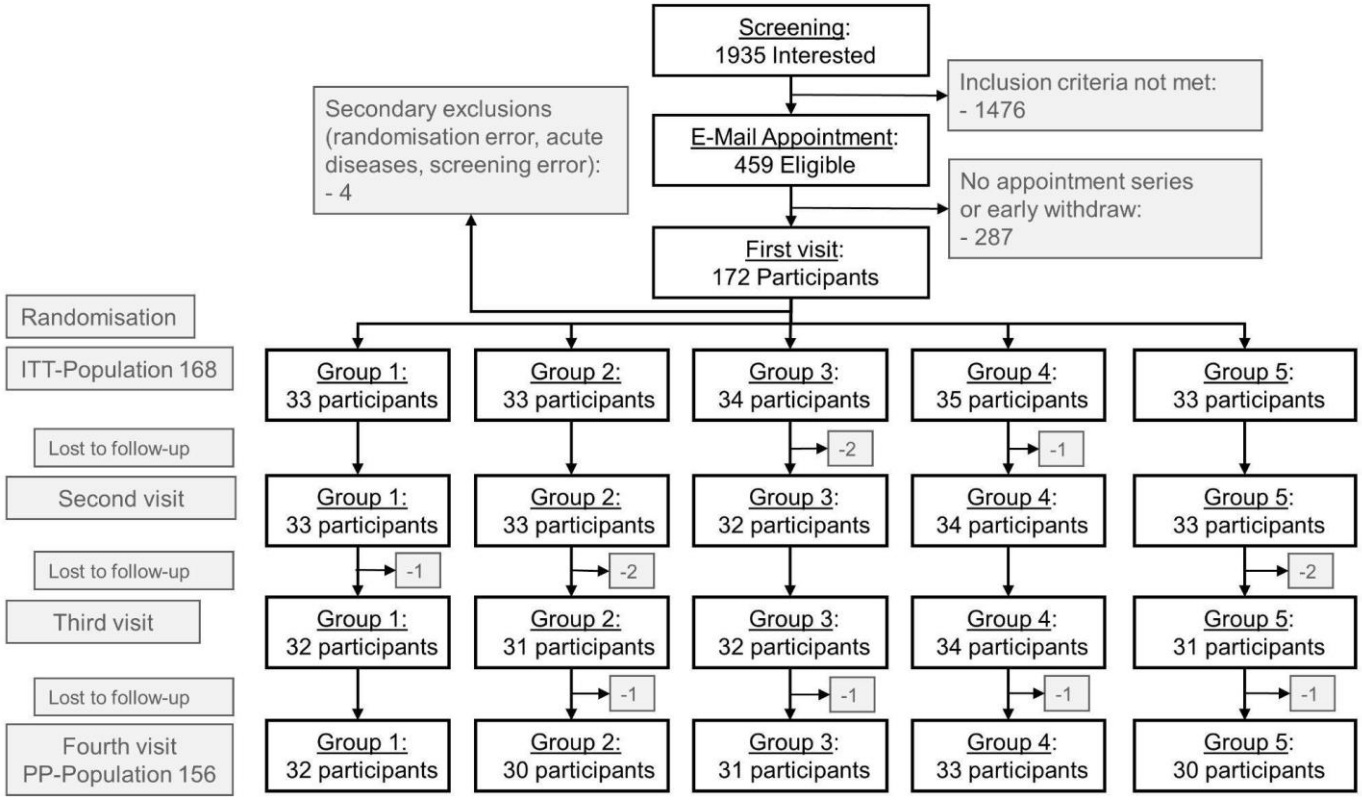


Figure 1. Flowchart of the ErdBEHR study. Abbreviations: ITT Intention to treat, PP per-protocol.

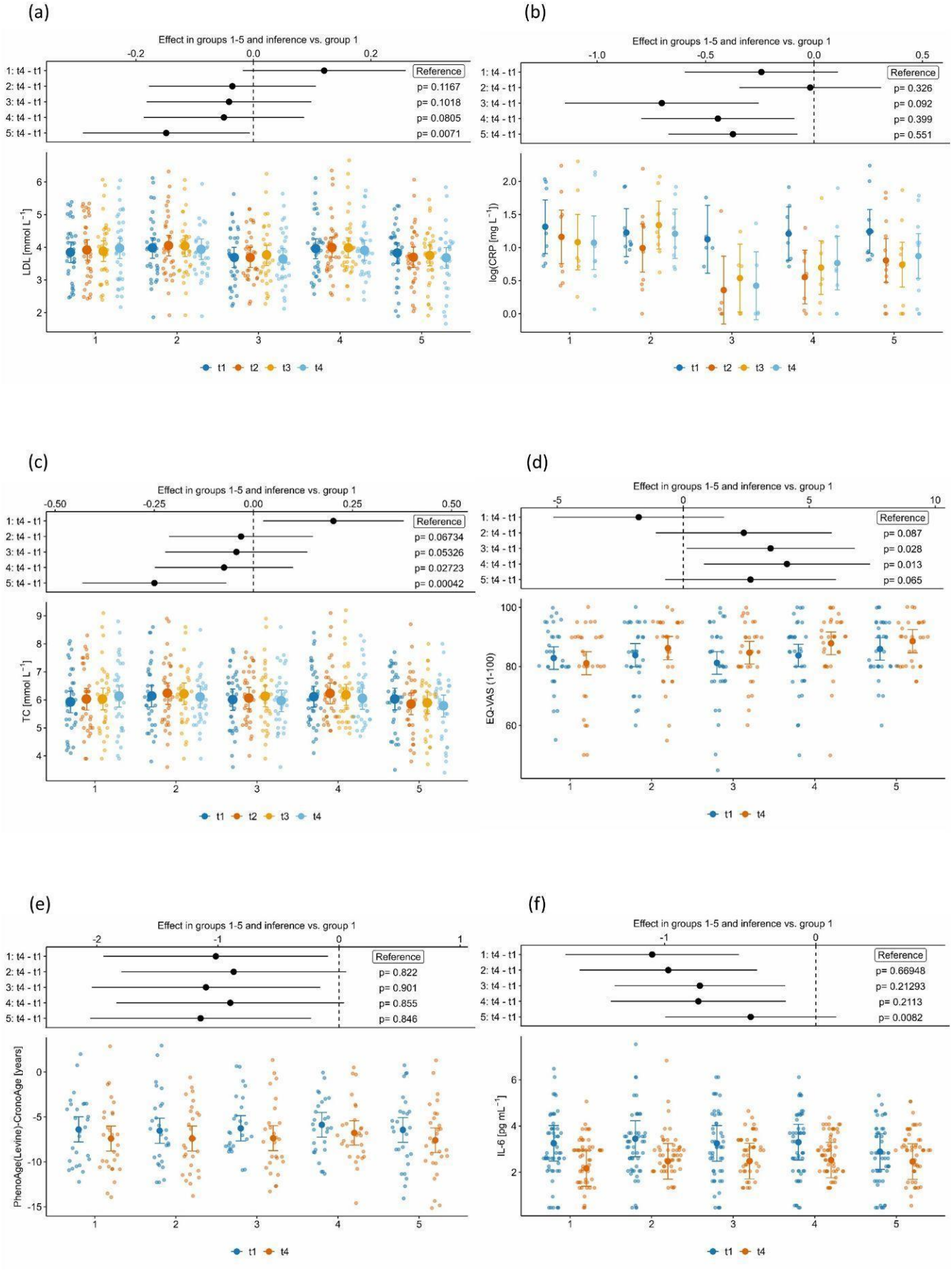
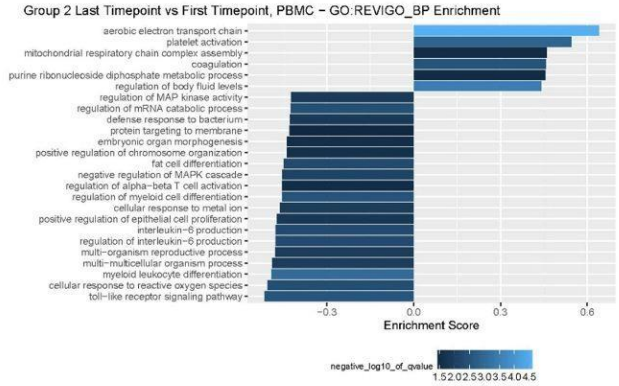
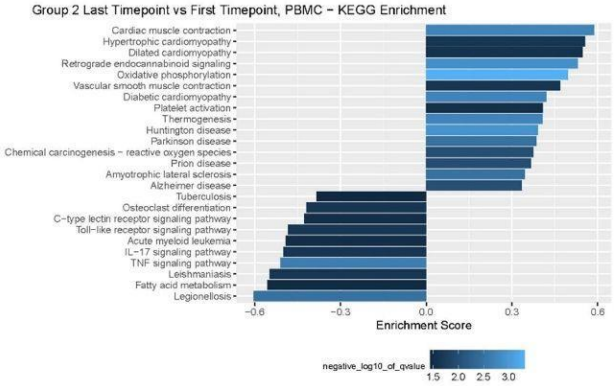

(a)
Effect in groups 1-5 and inference vs. group 1
1: t4 - t1
2: t4 - t1
3: t4 - t1
4: t4 - t1
5: t4 - t1
Reference
p= 0.1167
p= 0.1018
p= 0.0805
p= 0.0071
LDL [mmol L⁻¹]
t1 t2 t3 t4
(b)
Effect in groups 1-5 and inference vs. group 1
Reference
p= 0.326
p= 0.092
p= 0.399
p= 0.551
log(CRP [mg L⁻¹])
t1 t2 t3 t4
(c)
Effect in groups 1-5 and inference vs. group 1
Reference
p= 0.06734
p= 0.05326
p= 0.02723
p= 0.00042
TC [mmol L⁻¹]
t1 t2 t3 t4
(d)
Effect in groups 1-5 and inference vs. group 1
Reference
p= 0.087
p= 0.028
p= 0.013
p= 0.065
EQ-VAS (1-100)
t1 t4
(e)
Effect in groups 1-5 and inference vs. group 1
Reference
p= 0.822
p= 0.901
p= 0.855
p= 0.846
PhenoAge[Levine)-CronoAge [years]
t1 t4
(f)
Effect in groups 1-5 and inference vs. group 1
Reference
p= 0.66948
p= 0.21293
p= 0.2113
p= 0.0082
IL-6 [pg mL⁻¹]
t1 t4

Figure 2. Effect plots and response data plots for primary and selected secondary endpoints. (a) LDL; (b) 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 last visit (t4) and 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, p-values of t-test for coefficients in the LMM are displayed. Dots in plots (bottom) represent model-based individual response data across groups 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, figure 2b) 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) .

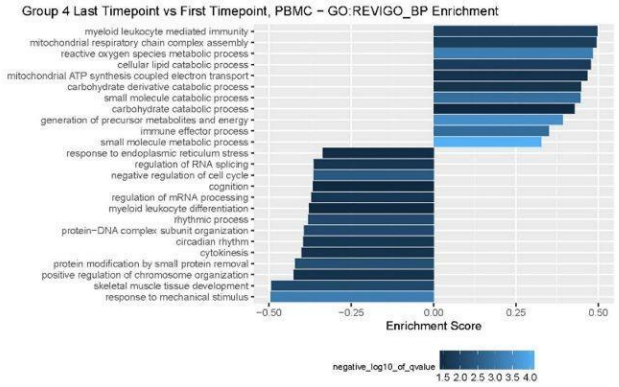
(a)


Group 2 Last Timepoint vs First Timepoint, PBMC – GO:REVIGO_BP Enrichment
aerobic electron transport chain
platelet activation
mitochondrial respiratory chain complex assembly
coagulation
purine ribonucleoside diphosphate metabolic process
regulation of body fluid levels
regulation of MAP kinase activity
regulation of mRNA catabolic process
defense response to bacterium
protein targeting to membrane
embryonic organ morphogenesis
positive regulation of chromosome organization
fat cell differentiation
negative regulation of MAPK cascade
regulation of alpha–beta T cell activation
regulation of myeloid cell differentiation
cellular response to metal ion
positive regulation of epithelial cell proliferation
interleukin–6 production
regulation of interleukin–6 production
multi–organism reproductive process
multi–multicellular organism process
myeloid leukocyte differentiation
cellular response to reactive oxygen species
toll–like receptor signaling pathway
−0.3
0.0
0.3
0.6
Enrichment Score
negative_log10_of_qvalue
1.5 2.0 2.5 3.0 3.5 4.0 4.5

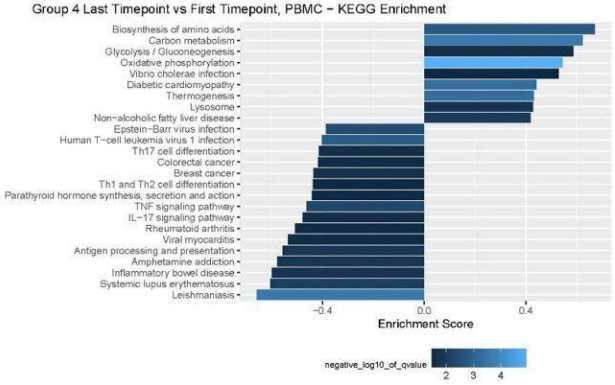
(b)


Group 2 Last Timepoint vs First Timepoint, PBMC – KEGG Enrichment
Cardiac muscle contraction
Hypertrophic cardiomyopathy
Dilated cardiomyopathy
Retrograde endocannabinoid signaling
Oxidative phosphorylation
Vascular smooth muscle contraction
Diabetic cardiomyopathy
Platelet activation
Thermogenesis
Huntington disease
Parkinson disease
Chemical carcinogenesis – reactive oxygen species
Prion disease
Amyotrophic lateral sclerosis
Alzheimer disease
Tuberculosis
Osteoclast differentiation
C–type lectin receptor signaling pathway
Toll–like receptor signaling pathway
Acute myeloid leukemia
IL–17 signaling pathway
TNF signaling pathway
Leishmaniasis
Fatty acid metabolism
Legionellosis
−0.6
−0.3
0.0
0.3
0.6
Enrichment Score
negative_log10_of_qvalue
1.5 2.0 2.5 3.0

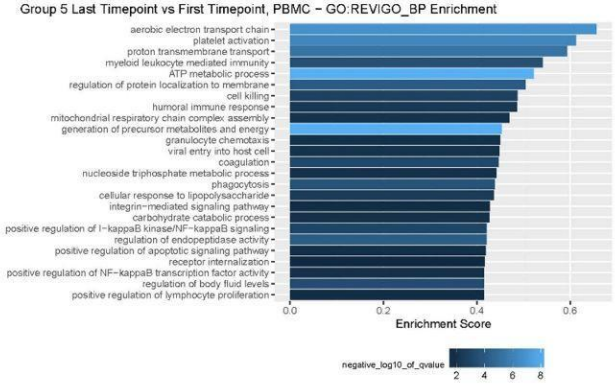
(c)


Group 4 Last Timepoint vs First Timepoint, PBMC – GO:REVIGO_BP Enrichment
myeloid leukocyte mediated immunity
mitochondrial respiratory chain complex assembly
reactive oxygen species metabolic process
cellular lipid catabolic process
mitochondrial ATP synthesis coupled electron transport
carbohydrate derivative catabolic process
small molecule catabolic process
carbohydrate catabolic process
generation of precursor metabolites and energy
immune effector process
small molecule metabolic process
response to endoplasmic reticulum stress
regulation of RNA splicing
negative regulation of cell cycle
cognition
regulation of mRNA processing
myeloid leukocyte differentiation
rhythmic process
protein–DNA complex subunit organization
circadian rhythm
cytokinesis
protein modification by small protein removal
positive regulation of chromosome organization
skeletal muscle tissue development
response to mechanical stimulus
−0.50
−0.25
0.00
0.25
0.50
Enrichment Score
negative_log10_of_qvalue
1.5 2.0 2.5 3.0 3.5 4.0

(d)


Group 4 Last Timepoint vs First Timepoint, PBMC – KEGG Enrichment
Biosynthesis of amino acids
Carbon metabolism
Glycolysis / Gluconeogenesis
Oxidative phosphorylation
Vibrio cholerae infection
Diabetic cardiomyopathy
Thermogenesis
Lysosome
Non–alcoholic fatty liver disease
Epstein–Barr virus infection
Human T–cell leukemia virus 1 infection
Th17 cell differentiation
Colorectal cancer
Breast cancer
Th1 and Th2 cell differentiation
Parathyroid hormone synthesis, secretion and action
TNF signaling pathway
IL–17 signaling pathway
Rheumatoid arthritis
Viral myocarditis
Antigen processing and presentation
Amphetamine addiction
Inflammatory bowel disease
Systemic lupus erythematosus
Leishmaniasis
−0.4
0.0
0.4
Enrichment Score
negative_log10_of_qvalue
2 3 4

(e)


Group 5 Last Timepoint vs First Timepoint, PBMC – GO:REVIGO_BP Enrichment
aerobic electron transport chain
platelet activation
proton transmembrane transport
myeloid leukocyte mediated immunity
ATP metabolic process
regulation of protein localization to membrane
cell killing
humoral immune response
mitochondrial respiratory chain complex assembly
generation of precursor metabolites and energy
granulocyte chemotaxis
viral entry into host cell
coagulation
nucleoside triphosphate metabolic process
phagocytosis
cellular response to lipopolysaccharide
integrin–mediated signaling pathway
carbohydrate catabolic process
positive regulation of I–kappaB kinase/NF–kappaB signaling
regulation of endopeptidase activity
positive regulation of apoptotic signaling pathway
receptor internalization
positive regulation of NF–kappaB transcription factor activity
regulation of body fluid levels
positive regulation of lymphocyte proliferation
0.0
0.2
0.4
0.6
Enrichment Score
negative_log10_of_qvalue
2 4 6 8

(f)

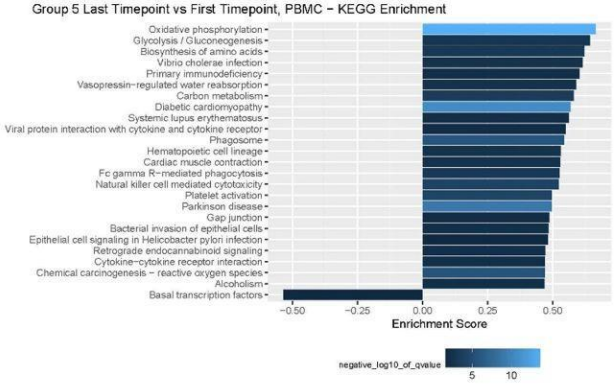

Group 5 Last Timepoint vs First Timepoint, PBMC – KEGG Enrichment
Oxidative phosphorylation
Glycolysis / Gluconeogenesis
Biosynthesis of amino acids
Vibrio cholerae infection
Primary immunodeficiency
Vasopressin–regulated water reabsorption
Carbon metabolism
Diabetic cardiomyopathy
Systemic lupus erythematosus
Viral protein interaction with cytokine and cytokine receptor
Phagosome
Hematopoietic cell lineage
Cardiac muscle contraction
Fc gamma R–mediated phagocytosis
Natural killer cell mediated cytotoxicity
Platelet activation
Parkinson disease
Gap junction
Bacterial invasion of epithelial cells
Epithelial cell signaling in Helicobacter pylori infection
Retrograde endocannabinoid signaling
Cytokine–cytokine receptor interaction
Chemical carcinogenesis – reactive oxygen species
Alcoholism
Basal transcription factors
−0.50
−0.25
0.00
0.25
0.50
Enrichment Score
negative_log10_of_qvalue
5 10

Figure 3. Bar charts illustrating the functional enrichment results of the genes that are longitudinally differentially expressed between timepoint 1 and timepoint 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* (that is, the adjusted p-value used to control for the false discovery rate). Gene expression data were obtained from PBMC (peripheral blood mononuclear cells).

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Cohort: The MARK-AGE Project. Oxid Med Cell Longev. 2017;2017:1401452. Epub 2017/08/15. doi: 10.1155/2017/1401452. PubMed PMID: 28804532; PMCID: PMC5539926.
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Supplementary Figures, Table, Methods, Results and Discussion, and Boxes 1-3

1) Supplementary Figures 1 + 2, and Supplementary Table 1

2) Supplementary Methods

3) Supplementary Results and Discussion, gene expression analyses: Presentation of the group 1 gene expression data.

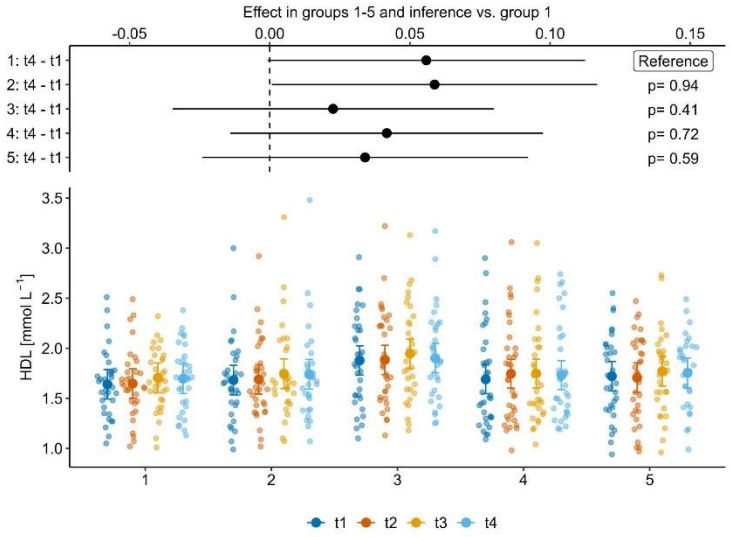
4) Supplementary Discussion, previous findings: Cholesterol, Inflammation, Interactions of Cholesterol and Inflammation.

5) Box 1: Quercetin (and fisetin) contents in strawberries, capers and controls

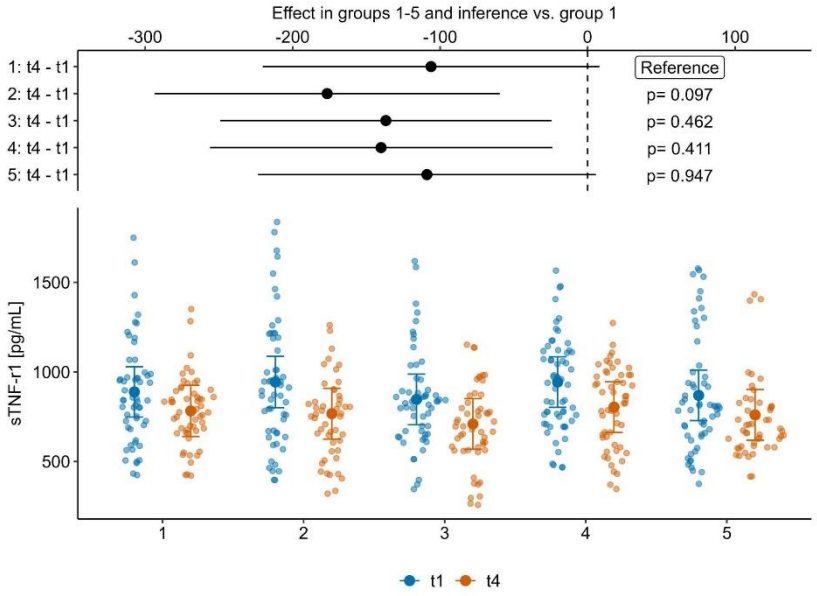
6) Box 2: Side effects and toxicity

7) Box 3: In-vitro testing of strawberry extract in hepatocytes expressing CRP

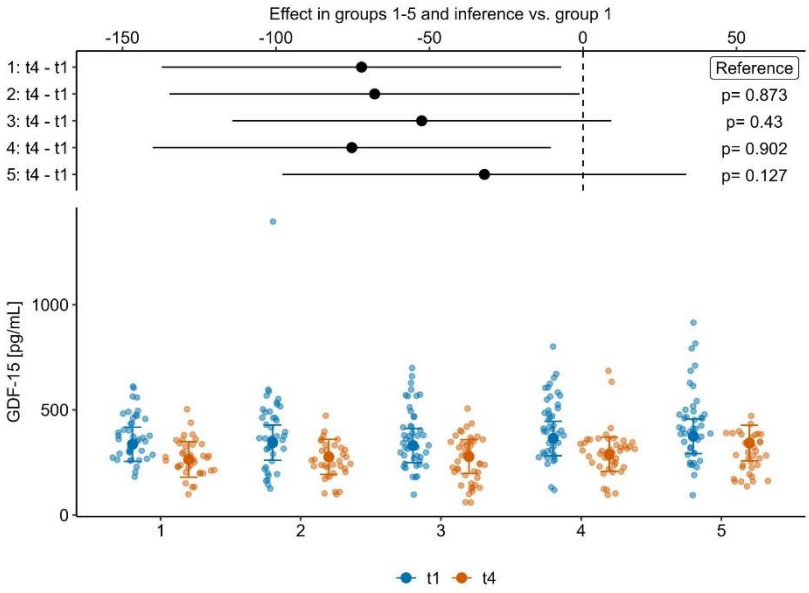
Suppl. Figure 1a: HDL



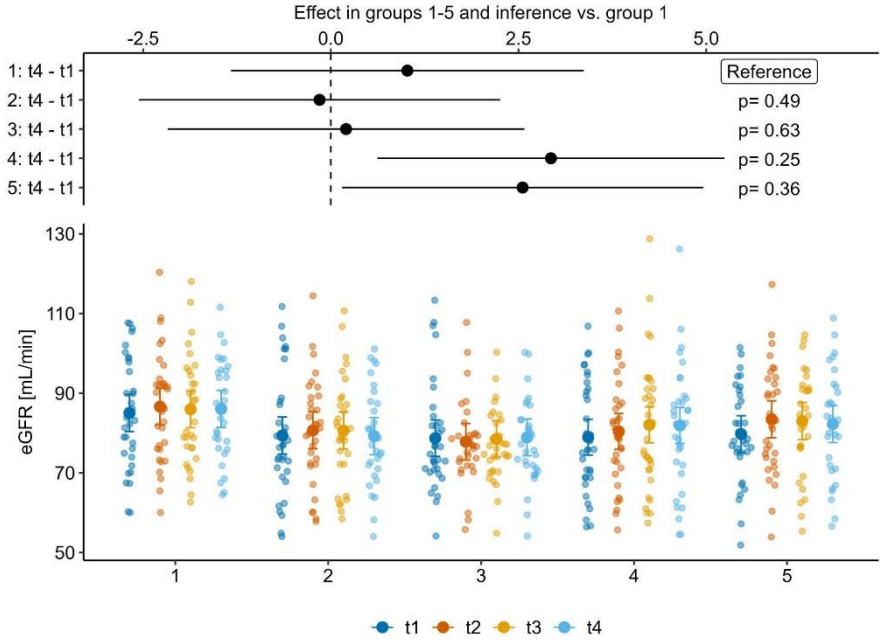
Suppl. Figure 1b: sTNFr1



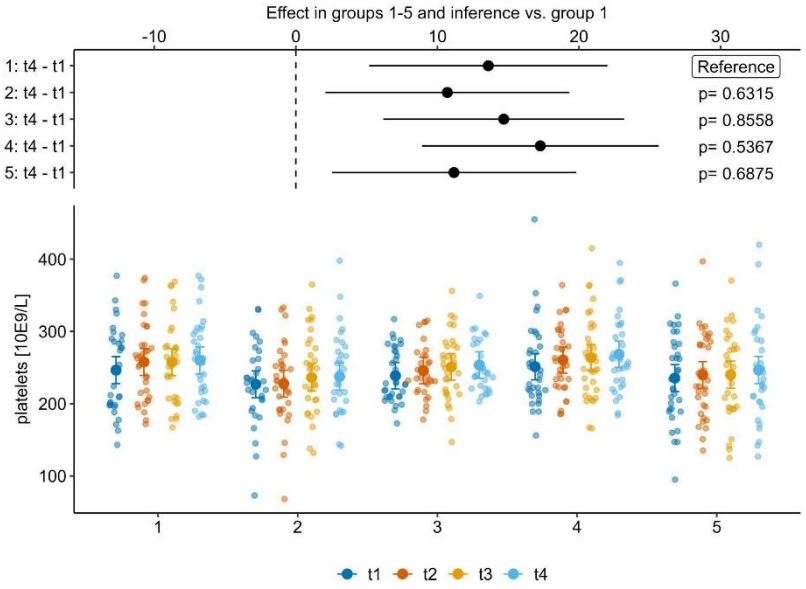
Suppl. Figure 1c: GDF-15



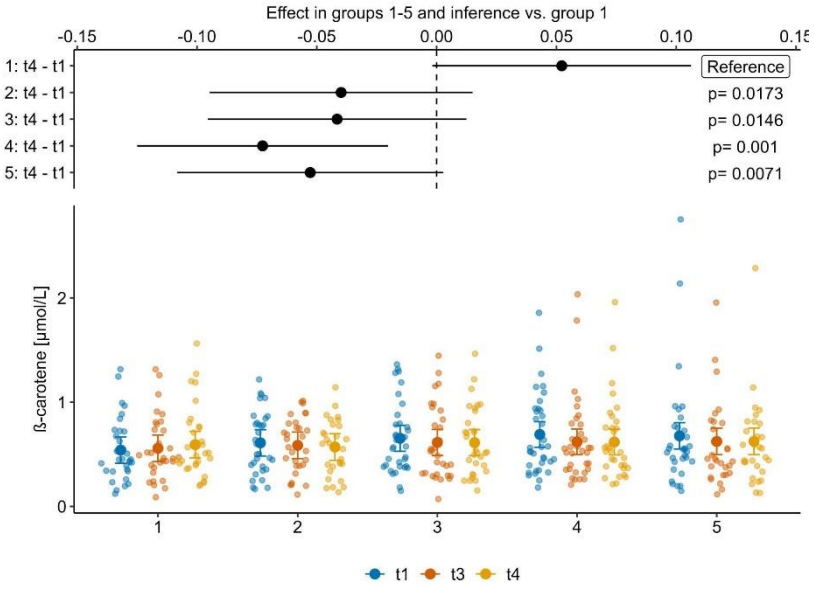
Suppl. Figure 1d: eGFR



Suppl. Figure 1e: platelet counts



Suppl. Figure 1f: β-carotene



Suppl. Fig. 1. Effect plots and response data plots for selected secondary and for exploratory endpoints. (a) HDL; (b) sTNFr1; (c) GDF-15; (d) eGFR; (e) platelet counts; (f) β-carotene. See Figure 2 for further details.

Suppl. Fig. 2. Principal Component Analysis of all NGS samples (taking into account only the top 500 variable genes across the samples). The color represents the time-point, the shape represents the group to which the samples belong.

Outcome	Effects (and standard errors) by group at last visit				
	Group 1	Group 2	Group 3	Group 4	Group 5
EQ-5D-5L (*n=164*)	-0.027 (0.021)	-0.006 (0.021)	0.014 (0.021)	-0.003 (0.021)	-0.003 (0.021)
Phenotypic Age (*n=143*)	-1.018 (0.469)	-0.871 (0.469)	-1.010 (0.477)	-0.898 (0.475)	-1.144 (0.461)
Triglycerides (*n=168*)	0.078 (0.111)	0.059 (0.114)	0.168 (0.112)	0.037 (0.109)	-0.074 (0.114)
HDL cholesterol (*n=168*)	0.056 (0.029)	0.059 (0.030)	0.023 (0.029)	0.042 (0.028)	0.034 (0.030)
sTNFr1 (*n=143*)	-105.92 (54.58)	-176.02 (56.03)	-136.25 (53.76)	-139.77 (55.43)	-108.35 (54.82)
GDF15 (*n=124*)	-61.03 (31.24)	-56.39 (32.14)	-40.13 (29.50)	-63.45 (31.15)	-20.61 (31.60)
Chair-Rise-Test (GLMM, *n=156*)	0.152 (0.065)	0.171 (0.070)	0.164 (0.066)	0.176 (0.064)	0.156 (0.069)

Supplementary Table 1: Effects in groups at last visit compared to first visit for outcomes without hints for group-by-visit interaction. For details see Table 2.

Supplementary Methods

Participants, more details

Respondents to the questionnaire qualified if they confirmed the following self-reported attributes at the time of their response.

- No prescription drugs (except cholesterol-lowering drugs and antihypertensive drugs; thyroid medication was allowed ad-hoc) and no severe chronic diseases.
- No known food allergy/intolerance, no food allergy/intolerance to strawberries in direct relatives.
- Strawberry salespoint of Karls Erdbeerhof easily accessible, to obtain fresh fruit regularly.
- No intake of dietary supplements or vitamin supplements exceeding standard recommendations.
- Consumption of the intervention food in the past, that is, weekly in the summer for strawberries; weekly, monthly or rarely for capers.
- Weight <= 100kg (for reasons of "dosage" of the intervention food).
- Age 50-80 years at time of responding to the questionnaire.

Intervention food, more details

Fresh strawberries were provided for free by the supporting strawberry farm Karls Erdbeerhof; participants received vouchers which they were asked to redeem at a salespoint they could reach easily and they were told that freshness of the berries is important. Freeze-dried strawberries and capers in “native olive oil extra” (Rapunzel, Legau, Germany) were provided by *Karls Erdbeerhof* and handed out at the study center. The variety of fresh strawberry consumed was not recorded. Every two weeks, strawberry samples were frozen for future analyses. Strawberry samples from August 2021 (varieties: *Milwana* and *Florentina*) as well as the capers were subjected to mass-spectrometry analysis of selected polyphenols (fisetin and quercetin), see Box 1. Participants were instructed to adhere to the consumption plan, but to not eat the food if it was causing a high degree of aversion; in this case they were instructed to inform study personnel at their next visit. Further, participants were explicitly allowed to consume other high-quality fresh strawberries according to consumption plan for at most two weeks if they traveled away from a salespoint of Karls Erdbeerhof. The instructions also included explicit statements to not change dietary habits otherwise; to not destroy the health-promoting ingredients of the intervention food e.g. by cooking or baking; to consume the olive oil together with the capers (if any); to not consume other food with a specific health-related claim (mentioning green tea or garlic as examples) regularly and in high amounts, and they were given a sheet with 5 recipes compatible with these guidelines (see Supplementary Files, in German) as well as detailed personalized consumption plans (for a sample, see Supplementary Files, in German).

Sample size and Randomization, more details.

In a 19-participant 4-group unpublished pilot trial in August 2020, we had found that one week of fresh strawberry consumption and two SIDs one week apart were associated with improvements in LDL cholesterol and CRP. Based on the results of the pilot study, sample size was planned using the R-package *WebPower* for repeated measures ANOVA, assuming a between-groups effect size of 0.4 and sphericity of at least 0.6, which gave a sample size of 180 participants for a power of 80% to detect a group-by-time interaction, reflecting the hypothesis of differences in change over time between groups. The study was planned for two primary endpoints, namely LDL and hsCRP, applying a type I error of 2.5%. The randomization list was generated with a block size of 10 to avoid imbalances of allocation within weeks, and with stratification by smoking status only.

Sequencing and bioinformatics methods, more details.

RNA quantity and purity was measured by determining the UV/VIS absorbance (NanoDrop One, Thermo Fisher Scientific) at three analytical wavelengths: *230, 260* and *280 nm*. 3'mRNA sequencing libraries were then generated using the QuantSeq 3'mRNA-Seq Library Prep Kit (Moll, Ante, Seitz, & Reda, 2014) according to manufacturer's instructions (Lexogen, Austria), and sequenced on a HiSeq4000 platform (Illumina, San Diego) using a single-end protocol with an RNA read-length of 100, at the Cologne Center for Genomics (University of Cologne, Germany). The gene expression analysis of the samples started with Salmon version 1.9.0 (Patro, Duggal, Love, Irizarry, & Kingsford, 2017), mapping the RNA reads to the human transcriptome GRCh38, as retrieved from Ensembl. For each sample, the two technical replicates were combined by summing the gene counts. DESeq2 release 3.16 (Love, Huber, & Anders, 2014) was then used to calculate differential gene expression (log fold change, p-value) between timepoints, for each group. DEseq2 first estimated the variance-mean dependence in the gene expression data, and then tested for differential expression across the timepoints, for each group, based on a model using the negative binomial distribution. A PCA of the samples was created, using the R function plotPCA from the R package BiocGenerics version 0.42.0 (Huber et al., 2015), using the BiocGenerics parameter *ntop = 500* to focus on the top 500 variable genes across the samples, which are more likely to be differentially expressed. The function *gconvert* was used to convert the gene IDs from the Ensembl format to the Entrez_gene format. An enrichment analysis was then carried out with GSEA method using the R library clusterProfiler version 4.3.1 (Subramanian et al., 2005), for the Gene Ontology (GO) and KEGG. GSEA is a canonical functional scoring enrichment method, that, differently from overrepresentation analysis, does not set a threshold for differentially expressed genes. Thus it is more suitable in the case of smaller, but biologically meaningful, gene expression changes. We only report significant enrichments (with a negative log-normalized *q-value* ≥ *1.5*). REVIGO version 2015-02-17 (Supek, Bosnjak, Skunca, & Smuc, 2011) was used to reduce the number of GO terms by half (reduction parameter set to 0.5, using *homo sapiens* as *species* parameter and *higher absolute value* as *Enrichment score* parameter). Barplots were created to show the terms featuring the at most 25 terms with highest absolute *Enrichment scores*. Heatmaps were prepared using the R package Pheatmap.

Supplementary methods: oxylipins and polyunsaturated fatty acids

Plasma samples were dissolved in 1 mL MeOH (10% v/v). 15-deoxy-delta-12,14-PGJ2-d_4, 5-HETE-d_8, 9(10)-EpOME-d_4, 9,10-DiHOME-d_4, 9-HODE-d_4 and PGE2-d_4 (Cayman Chemical) were added to each sample as internal standards in a concentration of 0.02 µg/mL (sMRM transitions see suppl. table 2). Oxylipins were isolated by SPE as described by Dumlao (Dumlao, Buczynski, Norris, Harkewicz, & Dennis, 2011). The chromatographic method described by Wiley (Wiley et al., 2021) was used with minor modifications.

A Shimadzu Prominence LC-20AD coupled to a Shimadzu LCMS8040 mass spectrometer via ESI interface was used. Solvent A was 95% H_2O, 5% acetonitrile with 0.1% acetic acid and 10 mM ammonium acetate, solvent B was 95% acetonitrile, 5% H_2O with 0.1% acetic acid 10 mM ammonium acetate. The gradient was: 0-2 min: 5% B, 14 min: 70% B, 24-29 min 95% B,29.5 min: 5% B, re-equilibration at 5% B for 10 min, with a flow of 0.3 mL/min. A Phenomenex Kinetex c18 (150mmx2.1mm, 2.6µm, 100A) column was used for the separation. The temperature for the column oven was set to 40°C and injection volume to 20 µL.

The conditions for the ESI-MS were: nebulizing gas flow: 3 L/min, drying gas flow:10 L/min, desolvation line temperature: 150°C, heating block temperature: 350°C, interface bias: -3.5 kV. CID gas was Ar with a pressure of 230 kPA. The MS was operated in sMRM mode. A list of all MRM transitions can be found in the supplementary table I and II (Chen et al., 2021) (Dumlao et al., 2011) (Lu et al., 2022) (Rund et al., 2018).

Semi-quantification of the oxylipins was done relative to the respective internal standards (see supplementary table I).

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Supplementary Table I: sMRM transitions of the oxylipins and polyunsaturated fatty acids

polarity	analyte	precursor m/z	product m/z	collision energy / eV	dwell time /ms	acquisition start /min	acquisition stop /min	internal standard used for semi-quantification
negative	12(13)-EpOME*	295.2	195.0	25	100	20	24	9(10)-EpOME-d_4
negative	12,13-DiHODE*	311.2	183.1	30	100	15	20	9,10-DiHOME-d_4
negative	12,13-DiHOME*	313.2	183.2	19	100	15	20	9,10-DiHOME-d_4
negative	12,13-DiHOME	313.1	129.1	30	100	15	20	9,10-DiHOME-d_4
negative	12oxo LTB4*	333.0	153.0	15	100	1	10	5-HETE-d_8
negative	13,14-dihydro-15-keto-PGE1*	353.3	221.2	30	100	14	20	PGE2-d_4
negative	13-HODE*	295.2	195.2	17	100	17.1	20.9	9-HODE-d_4

polarity	analyte	precursor m/z	product m/z	collision energy / eV	dwell time /ms	acquisition start /min	acquisition stop /min	internal standard used for semi-quantification
negative	13-HODE	295.2	113.1	30	100	17.1	20.9	9-HODE-d_4
negative	13-HOTrE*	293.0	113.0	19	100	17.3	24	9-HODE-d_4
negative	13-HOTrE	293.2	195.1	23	100	17.3	24	9-HODE-d_4
negative	13-oxoODE*	293.0	113.0	22	100	17.1	24	9-HODE-d_4
negative	15(16)-EpODE*	293.3	235.2	20	100	15	20	9(10)-EpOME-d_4
negative	15,16-DiHODE*	311.2	223.2	29	100	15	20	9,10-DiHOME-d_4
negative	15-keto-PGF1α*	353.0	193.0	25	100	14	20	PGE2-d_4
negative	1a,1b-dihomo-15-deoxy-delta-12,14-PGJ*	343.2	299.2	15	100	18	28	15-deoxy-delta-12,14-PGJ2-d_4
negative	1a,1b-dihomo-15-deoxy-delta-12,14-PGJ2	343.2	255.2	15	100	18	28	15-deoxy-delta-12,14-PGJ2-d_4
negative	5-oxoETE*	317.2	203.2	25	100	14	20	5-HETE-d_8
negative	9(10)-EpOME*	295.3	171.1	23	100	20	24	9(10)-EpOME-d_4
negative	9(10)-EpOME	295.3	277.0	22	100	20	24	9(10)-EpOME-d_4

polarity	analyte	precursor m/z	product m/z	collision energy / eV	dwell time /ms	acquisition start /min	acquisition stop /min	internal standard used for semi-quantification
negative	9(10)-ep-stearic acid*	297.0	170.8	28	100	17.5	24	9(10)-EpOME-d_4
negative	9,10-DiHOME*	313.2	201.2	29	100	15	20	9,10-DiHOME-d_4
negative	9,10-DiHOME	313.1	171.1	30	100	15	20	9,10-DiHOME-d_4
negative	9-HODE*	295.2	171.1	16	100	17.1	20.9	9-HODE-d_4
negative	adrenic acid (AdA)*	331.0	287.0	14	100	22	28	5-HETE-d_8
negative	α-linolenic acid (ALA)*	277.3	259.2	16	100	22	28	5-HETE-d_8
negative	arachidonic acid (ARA)*	303.0	259.0	13	100	22	28	5-HETE-d_8
negative	dh PGF2a*	355.0	337.0	20	100	21	28	PGE2-d_4
negative	docosahexaenoic acid (DHA)*	327.0	283.0	15	100	22	28	5-HETE-d_8
negative	dihomo PGF2α*	381.0	337.0	20	100	21	28	PGE2-d_4
negative	eicosapentaenoic acid (EPA)*	301.0	257.0	10	100	22	28	5-HETE-d_8
negative	linoleic acid(LA)*	279.2	261.3	18	100	22	28	5-HETE-d_8

polarity	analyte	precursor m/z	product m/z	collision energy / eV	dwell time /ms	acquisition start /min	acquisition stop /min	internal standard used for semi-quantification
negative	LXB4*	351.0	221.0	14	100	1	10	9,10-DiHOME-d_4
negative	PGE2 and PGD2*	351.2	271.2	15	100	10	16.5	PGE2-d_4
negative	PGE2 and PGD2	351.2	315.2	20	100	10	16.5	PGE2-d_4
negative	PGF1α*	355.4	293.2	22	100	21	28	PGE2-d_4

*transition used for quantification

Supplementary Table II: sMRM transitions of the internal standards

polarity	internal standard	precursor m/z	product m/z	collision energy / eV	dwell time /ms	acquisition start /min	acquisition stop /min
negative	15-deoxy-delta-12,14-PGJ2-d_4*	319.1	275.2	14	100	16.5	22
negative	15-deoxy-delta-12,14-PGJ2-d_4	319.1	203.1	14	100	16.5	22

negative	5-HETE-d_8*	327.5	116.0	14	100	18	24
negative	9(10)-EpOME-d_4*	299.2	172.2	25	100	20	24
negative	9,10-DiHOME-d_4*	317.2	203.4	18	100	15	20
negative	9-HODE-d_4*	299.2	172.3	18	100	17.1	20.9
negative	PGE2-d_4*	355.2	275.3	16	100	10	16.5
negative	PGE2-d_4	355.2	319.3	14	100	10	16.5

*transition used for quantification

Supplementary Results and Discussion, gene expression analyses.

Presentation of the group 1 gene expression data. We also obtained gene expression data of 5 participants of the control group 1 for the first and the last time-point. As expected, longitudinally without an intervention, the signal in the data for group 1 is weaker than for the intervention groups; maximum q-values (on the negative log scale) of up to 0.35 are observed, compared to q-values up to 0.45 (in group 2; 5 participants profiled), up to 5 (in group 4; 5 participants profiled) and up to 14 (in group 5; 21 participants profiled). Nevertheless, similarly to the intervention groups, we find GOBP terms “enriched-up” related to mitochondria, immunity, cell-killing, metabolism and stress response (leukocyte mediated cytotoxicity/ cell killing/ positive regulation of NF−kappaB/ ATP metabolic process) (Fig. A). Similarly, the KEGG “enriched-up” pathways feature mitochondria, immunity and metabolism (Natural killer cell mediated cytotoxicity/ Fc gamma R−mediated phagocytosis/ Oxidative phosphorylation/ Thermogenesis) (Fig. B). The most likely explanation for these weak enrichments is a seasonal effect, given that participants were in the study during 10 weeks from mid-June to 1 October. In particular, immune challenges surface from the end of August onwards, as was also seen in seasonal patterns (Dopico et al., 2015) (their fig. 5a,c,d), reflecting in particular the seasonal upregulation of immune terms (incl. phagocytosis) and of genes (incl. CRP) from August onwards.

We refrain from interpreting the cross-sectional comparison between groups (e.g. of group 5 to group 1 at the (first or) last time point). Of note, a few of the 21 participants of group 5 contribute to high heterogeneity in gene expression patterns of group 5, in stark contrast to the cross-participant homogeneity of the 5 participants selected from group 1 (see, e.g., the heatmap for the transcripts underlying the enrichment of the GO term *phagocytosis* in the Suppl. Data); this heterogeneity does not cancel out in a cross-sectional comparison as it does in any longitudinal comparison where all participants serve as their own controls. See the Suppl. Data for the tables of DEGs, the enrichment data (tables), and more heatmaps.

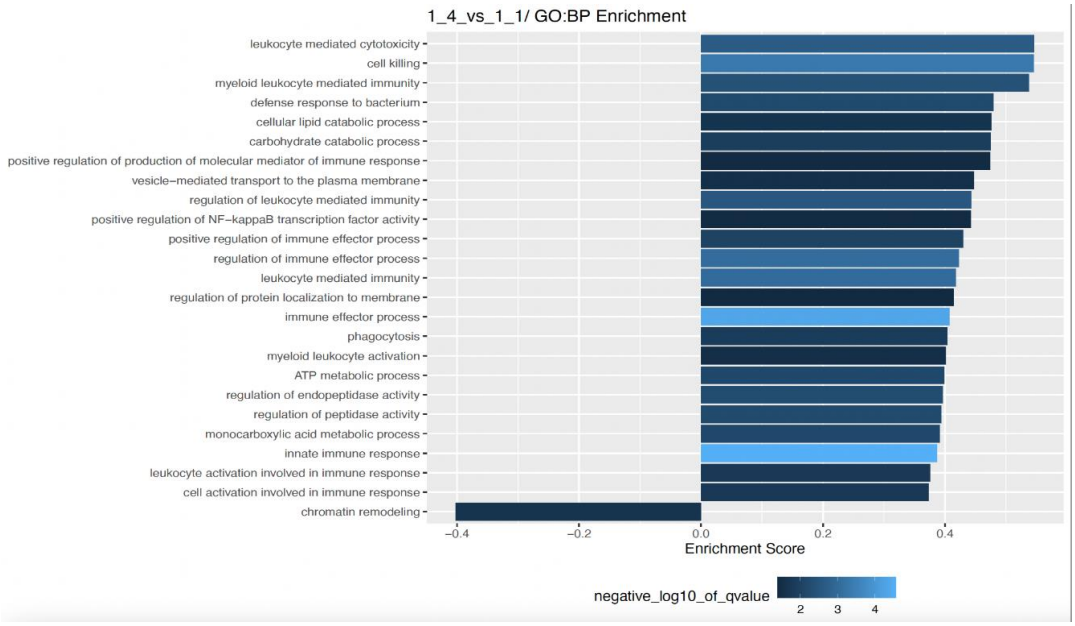

Fig. A. Differential Gene Expression enrichment results for group 1, and associated heatmap. Group 1 longitudinal differences t1 versus t4, ***enriched GOBP terms***. The

size of the bar represents the *Enrichment score* and the color represents the negative log10 of the *qvalue* measuring the significance of the enrichment.

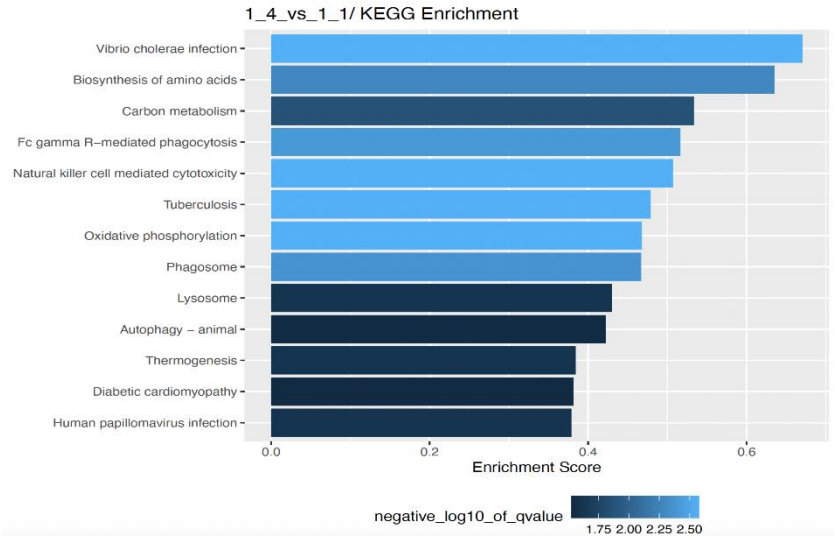

Fig. B. Differential Gene Expression enrichment results for group 1, and associated heatmap. Group 1 longitudinal differences t1 versus t4, enriched ***KEGG pathways***. The size of the bar represents the *Enrichment score* and the color represents the negative log10 of the *qvalue* measuring the significance of the enrichment.

Supplementary Discussion, previous findings.

Guidelines for the Discussion and previous findings. Placing our results into context, in the following we focus on human studies, preferably systematic reviews and meta-analyses or summaries thereof, and only for hypotheses about mechanisms we refer to animal or in-vitro studies considering their limited translatability. For (straw-)berries, we will refer to our own recent summary [1]. For quercetin, we will refer to recent systematic reviews and meta-analyses with a focus on lipid profiles or inflammation, which reported some specific yet conflicting evidence for effects. Summarizing these papers, quercetin effects on lipid profiles were found to be limited to triglycerides [2], to HDL and triglycerides following interventions of more than 8 weeks [3] and to LDL and TC in case of metabolic syndrome and related disorders [4]. Anti-inflammatory effects of quercetin in terms of CRP reductions were found [5], sometimes limited to subgroups such as subjects with diagnosed diseases or metabolic syndrome [4], also for IL6 (but not for TNFα) [6].

Cholesterol. In detail, past meta-studies reported cholesterol effects for (straw-)berries [1], and average LDL reductions were between −0.21 mmol/L [7] and −0.23 mmol/L (−8.86 mg/dL) [8], while average TC reductions were −0.17 mmol/L (−6.49 mg/dL) [9]. For quercetin in particular, one meta-analysis found LDL and TC reductions in people with metabolic syndrome or related disorders [4]. Thus, the reduction of LDL (up to –0.148 mmol/L) and of TC (up to –0.25 mmol/L) in our study is comparable to what was reported in the literature.

Inflammation. In detail, in the past, anti-inflammatory effects by (straw-)berry interventions [1] were reported to average −0.33 mg/L [10], −0.47 mg/L [9], and −0.63 mg/L [11] for CRP, and −0.41 pg/ml for IL6 [10]. Reductions by quercetin interventions averaged −0.33 mg/L [5] and –0.11 mg/L for CRP [6], and –0.58 pg/mL for IL6 [6]. Thus, the anti-inflammatory effects we report for CRP (up to –0.7 mg/L) are comparable to the literature. We also witnessed comparable reductions of IL6 between −0.5 to −1 pg/mL, yet we were surprised that these were also found in the control group. We have no explanation for this finding, although we can explain the only moderate reduction of IL6 in the highest-intervention group, see main text.

Interactions of Cholesterol and Inflammation. There are numerous interactions between the negative (atherogenic) changes in lipid metabolism and inflammatory processes that reinforce each other. Thus, the changes shown here are likely physiologically more positive than any changes in the individual factors per se [12] [13] [14]. CRP colocalizes with and binds to LDL phospholipids, particularly when the structure of CRP is subtly altered by oxidation. Moreover, CRP is a ligand of lectin-like oxidized LDL receptor-1 (LOX-1) [15] and increases LOX-1 expression in human aortic endothelial cells [16] and thus enhances the uptake of oxidized LDL. Such interplay with LOX-1 and atherogenic LDL may enhance endothelial dysfunction and additionally promote atherogenesis [17].

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Box 1. Quercetin (and fisetin) contents in strawberries, capers and controls.

Summary

We employed mass spectrometry to measure the amounts of quercetin and fisetin in strawberries of two of the varieties consumed by the participants, that is, *Malwina* and *Florentina* (the latter measured twice in August 2021, two weeks apart), see Methods (below). We found approximately 0.35-1.04 µg/g of quercetin, but β-glucuronidase pretreatment resulted in amounts of up to 40 times higher, of approximately 3.91-14.07 µg/g. Since β-glucuronidase is expressed by human cells (Gervasi et al., 2022), (Barreca et al., 2021) we assume that these higher amounts of quercetin are bioavailable in principle. In capers, we found 46.1 µg/g before, and 108.4 µg/g after β-glucuronidase pretreatment; we also found quercetin in lovage samples (positive control). We could not measure any of the quercetin from the capers that may have been dissolved in the olive oil during storage. We failed to detect fisetin in strawberries, yet we found it in smoketree samples (positive control). The amount of quercetin (canonically measured without β-glucuronidase pre-treatment) reported in the

literature varies widely, for strawberries as for other berries, and also for capers (see, e.g., (Basu, Nguyen, Betts, & Lyons, 2014), (Azzini et al., 2010)); measurement variability is certainly an issue here. Natural variation is an issue as well, however.

We expect that polyphenol amounts in general, and quercetin amounts in particular, thus varied widely in the food consumed by our participants, depending, e.g., on the exact variety being consumed, on the day the fruits were harvested, and for how long and how the fruits were stored before eating. 100g freeze-dried strawberries were derived from approx. 1.0-1.2 kg of fresh fruit; we did not measure quercetin in the dried fruit, but strawberry powder based on freeze-drying was used in past trials and is known to still contain high polyphenol contents (Basu, Nguyen, et al., 2014). Moreover, we only measured the quercetin aglycone and glucuronidated quercetin, while there are a lot more quercetin derivatives found in food (Dabeek & Marra, 2019). Given all the unknowns mentioned, we refrain from any attempts to estimate the amount of quercetin consumed by the various groups in our study; total polyphenol content would be even more difficult to estimate.

Finally, in the plasma of participants of the pilot trial, we could not detect quercetin (similar to (Azzini et al., 2010)), nor fisetin, see below. Given the half-life of quercetin, we expect that the quercetin available in the food was mostly modified or degraded by metabolism by the time the blood samples were taken (Dabeek & Marra, 2019), (Robbins et al., 2021), (Egert et al., 2008).

Detailed Results.

Quercetin was detected in all strawberry varieties studied here. In particular, after treatment of the crushed strawberries with β-glucuronidase, a significant increase in free quercetin was detected after extraction of the samples, indicating that quercetin was previously strongly bound to glucuronides (Table A). Exemplary ion chromatograms for the MRM transitions of quercetin of a β-glucuronidase treated and an untreated strawberry sample are shown in Figure C. The increase was greatest in the second Florentina sample with approximately 55-fold increase, with the first Florentina sample showing the least increase with approximately 4-fold increase. As a positive control, samples of lovage or capers were included in each experiment, which showed a clear quercetin signal in the mass spectrometer. Here, significantly more quercetin was detected in lovage samples without β-glucuronidase treatment (148.4 µg/g) than with treatment (39.7 µg/g). Capers, on the other hand, showed a significant increase in concentration under β-glucuronidase treatment (108.4 µg/g vs. 46.1 µg/g in a freshly processed sample). Exemplary ion chromatograms for the MRM transitions of quercetin and acridine orange, as internal standard, of a β-glucuronidase treated and an untreated caper sample are shown in Figure D.

Table A: Measured amount of quercetin in strawberries. *n = 2*.

Strawberry type	fresh extracted on the day of measurement (µg/g)	Without β-glucuronidase pretreatment, overnight incubation, extraction (µg/g)	β-glucuronidase pretreatment, overnight incubation, extraction (µg/g)
Malwina	0.35 ± 0.18	0.43 ± 0.27	14.07 ± 4.20
Florentina early August 2021	1.04 ± 0.45	0.43 ± 0.13	3.91 ± 0.64
Florentina late August 2021	0.52 ± 0.06	0.26 ± 0.15	14.23 ± 4.74

In contrast to quercetin, fisetin could not be detected in any of the strawberry samples either freshly processed or after treatment with β-glucuronidase. In the positive control of smoketree samples included here, fisetin was detected both freshly worked up and after treatment with β-glucuronidase. Exemplary ion chromatograms for the MRM transitions of fisetin and acridine orange of a β-glucuronidase treated and an untreated smoketree sample are shown in Figure E.

Neither fisetin nor quercetin was detected in pilot-study-based serum samples tested at various time points (0;4;8;24 hours and 8 days). Treatment of the serum samples with β-glucuronidase also did not lead to any positive result.

Detailed Discussion.

The strawberry cultivars examined in this study contained varying amounts of quercetin, regardless of cultivar. Most likely, quercetin concentration in strawberries is influenced by external factors such as the weather (sunlight or rain), or challenges such as insects or microbes. Quercetin was shown to protect the plants as an antioxidant and additionally strengthens various processes such as photosynthesis, growth and development (Singh, Arif, Bajguz, & Hayat, 2021). Most of the quercetin we could detect was accessible after treatment with β-glucuronidase. This is not surprising, since glucuronidation makes the substances more hydrophilic and they are then present in a dissolved form in the aqueous environment of the cell. Furthermore, it has already been found by other authors that quercetin is present as glucuronides in food (Boots, Haenen, & Bast, 2008). In particular, quercetin-3-O-glucuronide is known from wine (Satue-Gracia, Andres-Lacueva, Lamuela-Raventos, & Frankel, 1999), medicinal plants like *Hypericum hirsutum* or *Nelumbo nucifera* (Kitanov, 1988) (Kashiwada et al., 2005) and showed antimicrobial as well as antioxidant behaviour (Razavi, Zahri, Zarrini, Nazemiyeh, & Mohammadi, 2009).

On the other hand, fisetin was not detected in the strawberry cultivars studied here. Potentially, the failure to detect fisetin in our samples and the amounts found in the study of (Arai et al., 2000) can be explained by the different technology used at that time. Using our approach, employing various tests with different solvents and extraction variants, fisetin was not detected, using up to 50 g as input. In serum, fisetin was also not detected, though it was shown that fisetin is rapidly metabolised and thus may have eluded our analysis even if it was present in the food (Grynkiewicz & Demchuk, 2019).

Quercetin was not detected *in serum* above our detection limit (LOD 0.12 µM and LOQ 0.3 µM) even after treatment with β-glucuronidase. We cannot exclude that it was present especially in the first hours after strawberry ingestion. In a study with frozen onions, maximum concentrations were reached after about 3 hours and after 48 hours concentrations were very low (at 10 ng/ml) (Hollman et al., 1996). Furthermore, it was shown that in addition to quercetin, two monomethylated derivatives, isorhamnetin (3'-O-methyl quercetin), and tamarixetin (4'-O-methyl quercetin) can also predominate (Burak et al., 2017), but these were not considered in our study.

Methods.

Several workup methods were used for the identification and quantification of fisetin and quercetin from strawberries, capers, and human plasma. For the former, the focus was on different varieties of strawberries (Malwina as well as Florentina from two different harvest dates) and capers in olive oil. As positive controls, samples of lovage (leaves, positive control quercetin) and smoketree (leaves and branches, positive control fisetin) were also examined. For analysis, 0.1-0.5 g of frozen mortared sample material was used. For the nearly anhydrous samples (capers, lovage, smoketree), 500 µl of water was added. This step was not necessary for the strawberries and was thus omitted. Subsequently, 50 µl of a 5 µM acridine orange solution was added to the samples as an internal standard. Ethyl

acetate was chosen as the extraction solvent. The samples were then extracted twice with 1 ml ethyl acetate. After an extraction time of one hour, the samples were centrifuged at 14,000 rpm (26.342 g) for 5 min. The organic supernatant was concentrated to dryness in a vacuum concentrator (SpeedVac SPD130DLX, Thermo Fisher Scientific, Asheville, NC, USA) and then reconstituted in 100 µl of acetonitrile. This workup only captures free fisetin and quercetin and would not properly capture glucuronide-bound fisetin and quercetin. Therefore, additional samples to those described above were prepared one day before measurement to which β-glucuronidase was added. For this, 1 ml of 1 mM KH2PO4 buffer pH 4.5 and 25 µl of β-glucuronidase (≥ 1250 U/ml) from helix pomatia (Sigma Aldrich, Taufkirchen, Germany) were added to the sample material and incubated overnight at 37 °C with gentle shaking. Sample material without β-glucuronidase was also analyzed. Furthermore, 1 mM KH2PO4 buffer with and without the addition of β-glucuronidase was included as negative control. Before extraction on the following day, the internal standard was added as for the other samples. Subsequently, due to the larger initial volume of the samples with β-glucuronidase, 2 ml ethyl acetate was chosen twice for the extraction. After concentration to dryness, these samples were also reconstituted in 100 µl acetonitrile.

For analysis from human plasma, 500 µl of plasma was used for each of the three sample workups. Different time points (0;4;8;24 hours and 8 days) after strawberry and caper ingestion from five pilot study donors were analysed.

To assess the amount of substance in the sample material, a common serial standard row was prepared from stock solutions of fisetin (50 mM) and quercetin (60 mM), both dissolved in DMSO, in acetonitrile. A range of 0.05-0.5 mM was chosen for fisetin and 0.03-0.6 mM for quercetin. From these concentrations in acetonitrile, 10 µl was added to 990 µl of water followed by 50 µl of 5 µM acridine orange solution. The final concentration range was thus 0.5-5 µM for fisetin and 0.3-6 µM for quercetin. For the evaluation of plasma samples, the common serial dilution was performed directly in human plasma. Therefore, the final concentration range for fisetin was set to 0.25-5 µM and for quercetin 0.3-3 µM. Further sample processing is identical to the one described above.

5 µl from all described samples were injected for analysis by LC-MS/MS. Separation was achieved using a Shimadzu LC-20AD HPLC with a Multospher 120 C18 AQ column 125 × 2 mm, 5 µm particle size (CS-Chromatographie Service GmbH, Langerwehe, Germany) coupled to a guard column (20 mm × 3 mm , 5 µm particle size). Water was chosen as mobile phase A and acetonitrile as mobile phase B, both containing 0.2 % formic acid. The flow rate was set to 0.3 ml/min. First, the initial conditions of 15% mobile phase B were maintained for 3 min. Subsequently, a linear gradient up to 50 % B was selected for the next 7 min and then increased to 100 B within the next 2 min. Thereafter, the initial conditions were restored within 3 min and the column was re-equilibrated for another 5 min. The total run time for one sample measurement was 20 min. The oven temperature was set to 40 °C. Mass spectrometric analysis was carried out on a Shimadzu LCMS-8050 triple quadrupole mass spectrometer. Fisetin and quercetin were measured in positive and negative mode, while the internal standard acridine orange was measured only in the positive mode. The dwell time for all ions was set to 100 msec. For the assessment of the presence of fisetin and quercetin in the analysed samples all fragment ions and their relative intensity were used. The respective transitions and associated mass spectrometric settings as well as the general settings of the triple quadrupole are shown in Tables B/C.

Table B: Parameters of the triple quadrupole interface.

Interface ESI parameter	**Value**
Nebulizing gas flow	3 L/min
Heating gas flow	10 L/min
Interface temperature	300 °C
Desolvation temperature	526 °C
DL temperature	250 °C
Heat block temperature	400 °C
Dry gas flow	10 L/min

Table C: Mass spectrometric parameters of fisetin, quercetin and the internal standard acridine orange.

Substance	**Precursor (*m/z*)**	**Product (*m/z*)**	**Q1 Pre Bias (V)**	**CE (V)**	**Q3 Pre Bias (V)**	**Measuring mode**
Fisetin	287.2	137.0	-11	-32	-27	positive
Fisetin	287.2	213.0	-10	-29	-22	positive
Fisetin	287.2	128.1	-11	-48	-14	positive
Fisetin	285.0	135.0	20	22	13	negative
Fisetin	285.0	121.0	20	27	20	negative
Fisetin	285.0	163.15	20	17	16	negative
Quercetin	303.0	229.05	-18	-30	-24	positive
Quercetin	303.0	152.9	-12	-33	-30	positive
Quercetin	303.0	257.0	-13	-25	-27	positive
Quercetin	301.0	151.2	21	21	14	negative
Quercetin	301.0	179.2	21	18	18	negative
Quercetin	301.0	106.95	21	28	22	negative
Acridine Orange	266.3	250.1	-14	-35	-28	positive

Acridine Orange	266.3	234.0	-14	-52	-26	positive
Acridine Orange	266.3	222.1	-14	-34	-24	positive

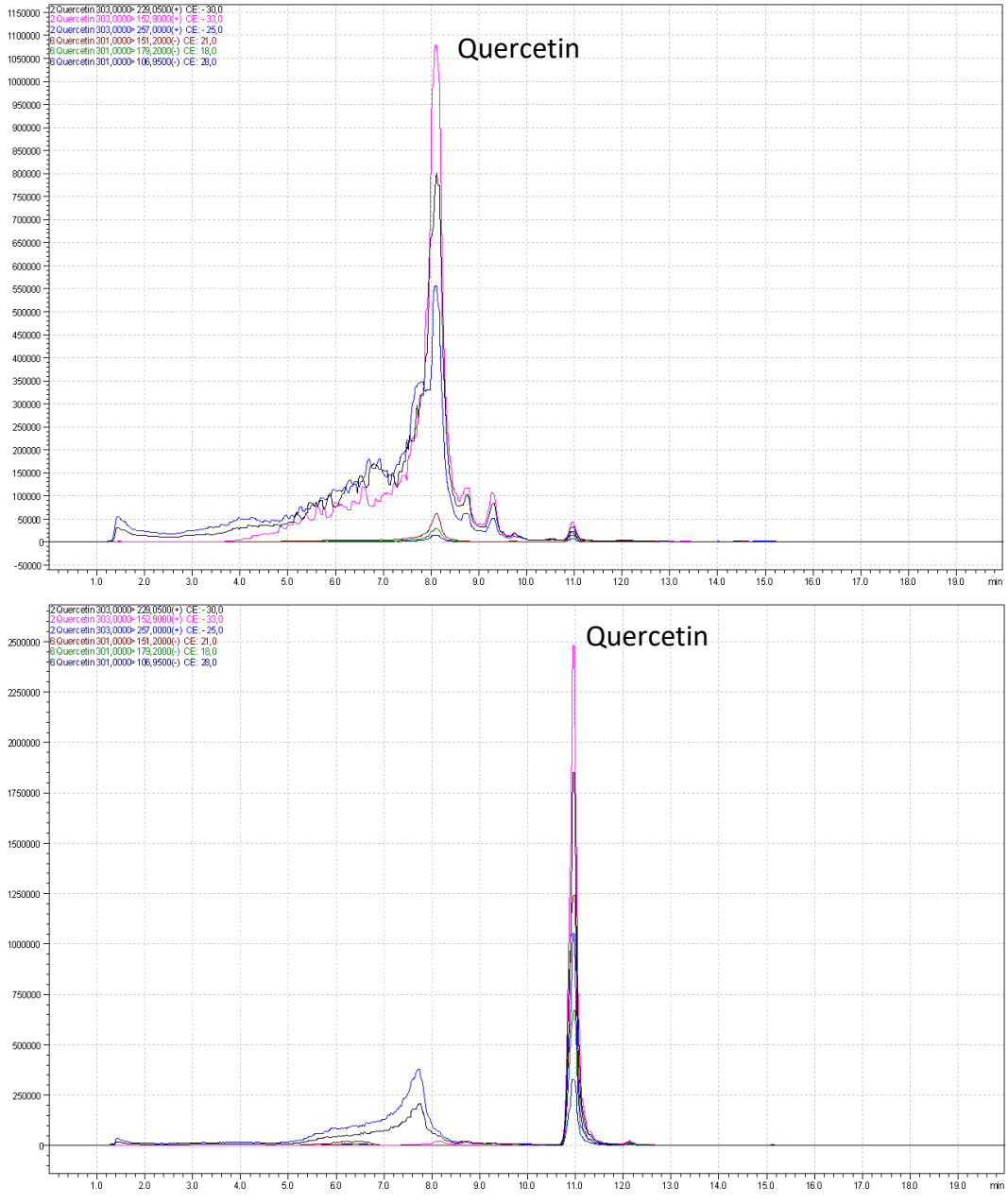

Figure C: Measured MRM transitions for quercetin of strawberry samples (Malwina). Shown are the mass traces from a freshly processed sample of Malwina (top) and a Malwina sample treated with β-glucuronidase (bottom).

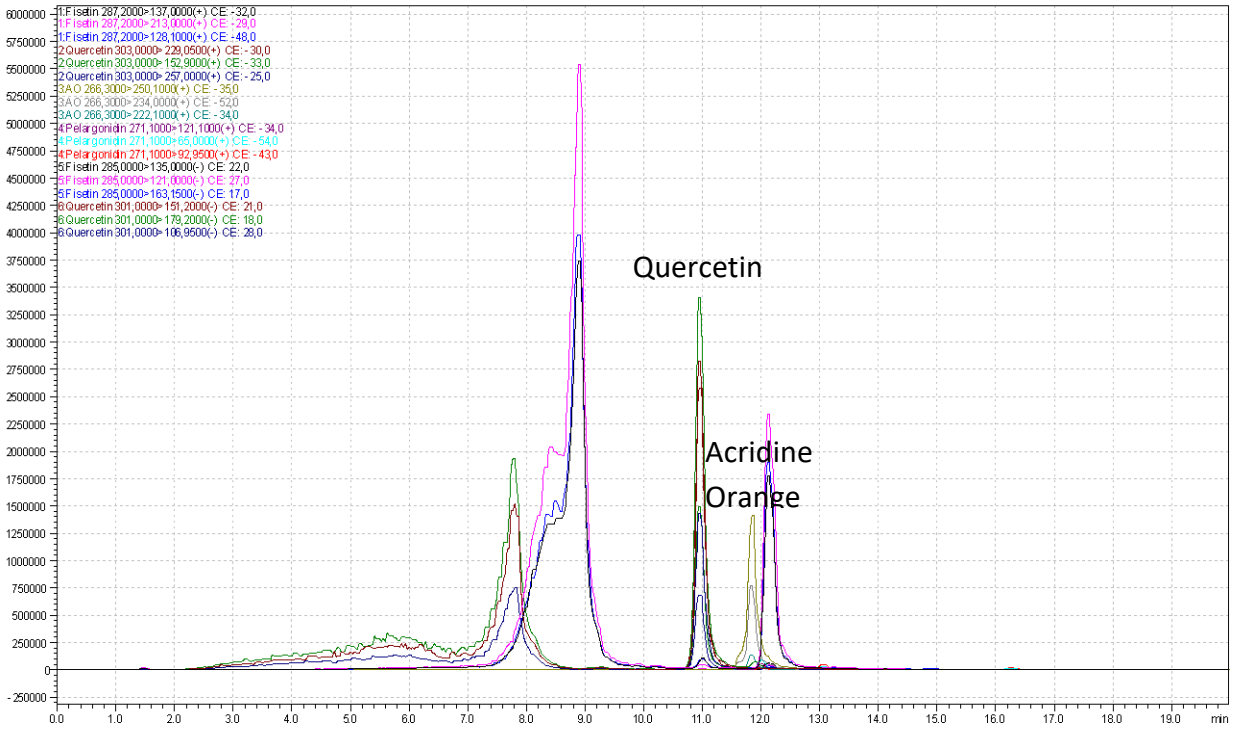

Figure D: Measured MRM transitions of a freshly processed caper sample.

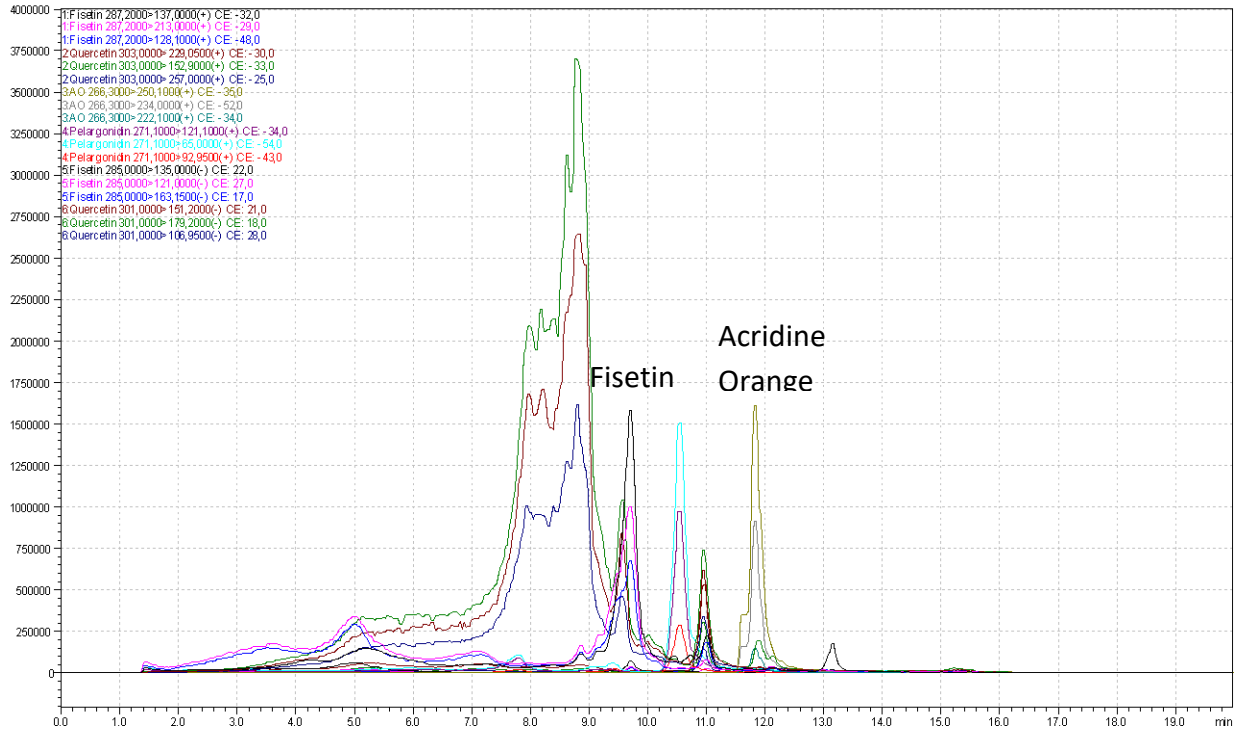

Figure E: Measured MRM transitions of a freshly processed smoketree sample.

Box 2: Side effects and toxicity.

Apart from potential allergies to components of the dietary intervention that we investigated (see main text), polyphenol toxicity and the comparatively high amount of sugar in strawberries were considered as potentially detrimental effects of the intervention. For both, it may theoretically be the case that the natural safety-net of the digestive system (loss of appetite, aversion to the food in question, vomiting or diarrhea) do not perform sufficiently well in certain people. In particular, polyphenol toxicity is occasionally mentioned in the literature, but mostly based on in vitro or animal work. A comprehensive review from 2012 noted concerns regarding iron absorption, pro-oxidant effects and competition for enzymes that may trigger drug interaction effects (Hooper & Frazier, 2012); green tea polyphenols were specifically mentioned as lookalikes of folic acid, and soy polyphenols of hormones. However, iron absorption is supposedly a problem if polyphenols are not consumed together with vitamin C, which is not the case here. Enzyme inhibition of, e.g. the cytochrome P450 enzymes also needed for drug metabolism, was reported for grapefruit and cranberries, but was not reported yet for strawberries, capers or olive oil. However, it was reported that quercetin and digoxin may compete for the P-gp (P-glycoprotein) transporter, causing lethal events in pigs (Wang, Chao, Hsiu, Wen, & Hou, 2004). Also, cyclosporin, vinblastine, vincristine and nephrotoxic drugs are contraindicated, as is warfarin (Lopes, Coimbra, Costa, & Ramos, 2021), (Lopez-Yerena, Perez, Vallverdu-Queralt, & Escribano-Ferrer, 2020). Of note, use of these drugs was incompatible with inclusion into our study.

Quercetin is approved by the US Food and Drug Administration as GRAS (Generally Recognized As Safe) for use as an ingredient at levels up to 500 mg per serving (Barreca et al., 2021) (Pawar et al., 2022). In fact, with no reported side-effects, doses of 500 mg or 1000 mg were consumed daily over 12 weeks by ~600 adults (Knab et al., 2011), 1000 mg of quercetin daily were consumed over 6 weeks by ~20 healthy adults (athletes) (McAnulty et al., 2008), and doses from 250 mg to 5000 mg of quercetin daily over 4 weeks, by 30 chronic hepatitis C patients (Lu et al., 2016). Moreover, quercetin (up to 600 mg daily) was suggested by some research groups to be a supplement of choice for COVID-19 and associated comorbid conditions (Pawar et al., 2022). Specific toxicity incidents related to anthocyanins are lacking, probably due to their very low bioavailability (Wallace & Giusti, 2015).

Further, pro-oxidant effects may be triggered by polyphenols, and for these we argue that such effects, if any, should be considered to be welcome because these are then a hormetic cause for improvements. We thus hypothesize, as was done for exercise, caloric restriction and many other healthy interventions (Demirovic & Rattan, 2013), that a high-polyphenol intervention may actually trigger a minor yet notable challenge to the participants, and it is the hormetic repair response which results in health improvements. More specifically, the high dose in particular in group 5 may have caused inflammation or apoptosis of e.g. senescent cells. In fact, in self-experiments

(N=4) in early 2020, we did close-meshed blood draws (every 4 hours and next morning) and found some cytokine levels to increase in the 8 hours after the intervention, returning to baseline level next day, but these observations were not significant, and were hard to interpret given that cytokines are also subject to circadian rhythms that are not well-studied. A more recent review reiterated some of these aspects (Cory, Passarelli, Szeto, Tamez, & Mattei, 2018) and suggested to favor diet over supplements. Finally, (Hernández & Gil, 2021) [Chapter 69 therein] do not add further concerns, but stress the need to go beyond in vitro and animal experiments, noting the lack of clinical trials. These authors also noted that "No beneficial effects have been observed for antioxidant supplementation in well-nourished people with a balanced diet. However, a high intake of polyphenols, even from dietary sources, can result in toxic effects (Das et al., 2012)", yet the review by (Das, Bhaumik, Raychaudhuri, & Chakraborty, 2012) is based on preclinical data, and our results suggest that healthy people can profit, at least in terms of reducing risk factors.

Regarding the sugar content of strawberries, we note first of all that overt diabetes (requiring prescription of insulin) was an exclusion criterion per se. Furthermore, we considered early on that strawberries have been tested successfully multiple times as the basis of *treating* people with metabolic syndrome and obesity (Basu, Betts, et al., 2014), and quercetin as well as anthocyanins were reported to be useful for diabetes management (Cory et al., 2018), (Barreca et al., 2021); presumably the polyphenol or fiber content of strawberries more than outweigh any problems due to the sugar content of the fruit. Regarding the capers in olive oil, we noticed the high content of salt in the food we provided to the participants, and instructed them to reduce the consumption of salty food on the days on which capers were consumed. Further, elevated values of triglycerides may be the result of a higher consumption of olive oil (Jabbarzadeh-Ganjeh, Jayedi, & Shab-Bidar, 2022). Finally, the hypothesis of synergistic positive effects of our food intervention may refer to synergistic toxic effects as well, and the combination of strawberries, capers and olive oil may not be considered a time-tested combination even though it matches the mediterranean diet scheme (Esposito et al., 2022). We thus successfully tested the combination before the trial, in *N=1* and *N=4* self experiments and in the pilot trial of 19 participants in Summer 2020, further minimizing the chance that our food combination may be synergistically toxic and yet no knowledge about toxicity exists because the intervention was not done frequently in the past.

Box 3. In-vitro testing of strawberry extract in hepatocytes expressing CRP

Summary

Stimulating cultured hepatocytes with the recombinant interleukins 1β and 6 (IL1β/IL6) resulted in a 4.2-fold increase of *CRP* mRNA level compared to untreated cells (see Figure). This CRP induction was significantly impeded by the presence of a water-soluble strawberry extract in a dose-dependent manner. The highest concentration tested (5 mg/ml) also showed the highest inhibitory activity (90 % inhibition of IL1b/IL6-dependent *CRP* mRNA induction). The lowest concentration of the strawberry extract (5 µg/ml) still exhibited a non-significant inhibitory activity of 33 % (see Figure); see Methods, below.

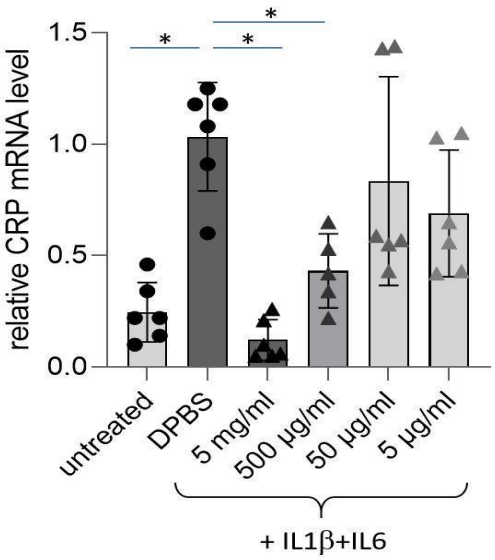

Figure. Interleukin-dependent CRP induction in human cultured hepatocytes is reduced in presence of a water-soluble strawberry extract. The *CRP* mRNA level was assessed with quantitative RT-PCR and related to the housekeeping gene expression of *GAPDH*. The mean of the solvent control group (DPBS) was set to be one. Cell culture experiments were performed in duplicates, for three times. Individual data, means and standard error of the mean are displayed.

Methods. Induction of CRP in cultured human liver cells (Hep 3B) and measurement of *CRP* mRNA level were conducted as previously described (Lüersen et al., 2023) in accordance to others (Yoshida et al., 2006; Zhang, Jiang, Rzewnicki, Samols, & Kushner, 1995). Hep 3B cells were kindly gifted by Claudia Geismann (Laboratory of Molecular Gastroenterology & Hepatology, Department of Internal Medicine I, UKSH-Campus Kiel, 24105 Kiel, Germany). Cells were cultivated in MEM with Earle's balanced salt solution (EBSS), L-glutamine (PAN Biotech, Aidenbach, Germany) and 2.2 g/L NaHCO3, supplemented with 10% (vol/vol) heat inactivated fetal bovine serum (Gibco™ by Thermo Fisher Scientific GmbH, Life Technologies™, Darmstadt, Germany) as well as 1% penicillin/streptomycin (PAN Biotech, Aidenbach, Germany) for 5 days. For CRP induction, Hep 3B cells were incubated with a commercially available freeze-dried strawberry powder (myfruits ® Janzen Ventures GmbH, Kehl, Germany) resolved at 10 mg/ml in DPBS (PAN Biotech, Aidenbach, Germany). The supernatant obtained by short centrifugation of the resolved strawberry powder was subjected to sterile filtration. The cells were then incubated with the strawberry extract at indicated concentrations (from 5 µg/ml to 5 mg/ml) in serum-free media containing 1 µM of dexamethasone (Dex) and stimulated with interleukin-1ß (IL1ß, 400 U/ml) and interleukin-6 (IL6, 200 U/ml) (both from ImmunoTools GmbH, Friesoythe, Germany) for 18 h. DPBS served as solvent control at a final dilution of 50%. RNA isolation and quantitative RT-PCR was done as previously described (Lüersen et al., 2023), (Kuhn, Pallauf, Schulz, & Rimbach, 2018). In brief, cells were harvested and RNA isolated with peqGOLD TriFast (VWR International, Radnor, PA). Gene expression was analyzed via quantitative RT-PCR with the Sensi-FAST™

SYBR® No-ROX One-Step Kit (Bioline, Luckenwalde, Germany) using a Rotorgene 6000 cycler (Corbett Life Science, Sydney, Australia). Gene expression levels were determined using a standard curve and normalized to the expression level of *GAPDH*. Primers were as follows: *CRP* forward primer: 5'-CCCTGAACTTTCAGCCGAATACA-3'; *CRP* reverse primer 5'-CGTCCTGCTGCCAGTGATACA-3'; *GAPDH* forward primer: 5'-CAATGACCCCTTCATTGACC-3'; *GAPDH* reverse primer: 5'-GATCTCGCTCCTGGAAGATG-3'. Statistical analysis was performed with the software GraphPad Prism (Ver. 7.05) comparing the different strawberry extract concentrations with the solvent control DPBS in a Kruskal-Wallis ANOVA with an uncorrected Dunn's test. A p-value <0.05 was considered significant.

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