Acute Effects of Three Different Meal Patterns on Postprandial Metabolism in Older Individuals with a Risk Phenotype for Cardiometabolic Diseases: A Randomized Controlled Crossover Trial

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Scope: The aim of this study is to investigate acute postprandial responses to intake of meals typical for Mediterranean and Western diets.

Methods: In a randomized crossover design, overweight and obese participants with a risk phenotype for cardiometabolic diseases consumed three different isoenergetic meals: Western diet-like high-fat (WDHF), Western diet-like high-carbohydrate (WDHC), and Mediterranean diet (MED) meal. Blood samples are collected at fasting and 1, 2, 3, 4, 5 h postprandially and analyzed for parameters of lipid and glucose metabolism, inflammation, oxidation, and antioxidant status.

Results: Compared to MED and WDHF meals, intake of a WDHC meal results in prolonged and elevated increases in glucose and insulin. Elevations for triglycerides are enhanced after the WDHF meal compared to the MED and the WDHC meal. Glucagon-like peptide-1 and interleukin-6 increase postprandially without meal differences. Apart from vitamin C showing an increase after the MED meal and a decrease after WDHF and WDHC meals, antioxidant markers decrease postprandially without meal differences. Plasma interleukin-1β is not affected by meal intake.

Conclusions: Energy-rich meals induce hyperglycemia, hyperlipemia, an inflammatory response, and a decrease in antioxidant markers. A meal typical for the Mediterranean diet results in favorable effects on glycemic, insulimemic, and lipemic responses.

1. Introduction

Nowadays, innovative nutrition research has undergone a considerable change of concept: Instead of focusing on the association between single nutrient intake and disease, scientific interest now targets the role of complex dietary patterns as risk factors in the development of chronic diseases throughout the life cycle.[1,2] Dietary patterns that are typically encountered in Western societies are characterized by a high consumption of energy-dense processed foods and ready-to-eat meals and of animal protein, saturated fatty acids (SFA), and added sugar and sodium.[3] Adherence to Western dietary patterns is associated with a physically inactive lifestyle and is commonly associated with the development of obesity and metabolic risk factors.[4] By contrast, so-called “health promoting” dietary patterns, including the Mediterranean diet, are characterized by native or only minimally processed foods, fruit, vegetables and vegetable oils, with few

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highly processed foods or sugary beverages. Such dietary patterns are also naturally lower in sodium, trans fatty acids and SFA, refined carbohydrates and added sugars; they are also higher in unsaturated fatty acids, dietary fiber, antioxidants, minerals, and phytochemicals and are more satiating than a typical Western diet.\[1,2\] Therefore, adherence to a Mediterranean-style diet is associated with reduced risk of cardiometabolic and other chronic diseases.\[5,6\]

Characteristic for the modern Western eating behavior is a frequent excessive intake of food. As a result, many individuals spend the majority of their waking hours in the postprandial state and experience exaggerated and prolonged postprandial metabolic (lipemia, glycaemia/insulinemia), oxidative and immune imbalances, a phenomenon termed “postprandial oxidative stress.”\[6,7\] This prolonged pro-oxidative state is accompanied by low-grade inflammation and impaired endothelial function and can promote cellular dysfunction and cardiovascular disease (CVD).\[7,8\] There is growing evidence that exaggerated and prolonged postprandial oxidative stress contributes to atherosclerosis, CVD, and other chronic diseases.\[10,11\]

Earlier human intervention studies conducted to primarily evaluate postprandial responses supplied single carbohydrates, proteins, or lipids, or have used combinations of a standardized high-fat meal plus a functional ingredient (e.g., a flavonoid or a specific food).\[7,9,12,13\] These studies indicate that the energy content of the meal, as well as the content of high-glycemic index carbohydrates and SFA, are important modulators of the postprandial immune response. By contrast, dietary fiber, unsaturated fatty acids, and anti-oxidative compounds may suppress postprandial inflammation. To the best of our knowledge, no previous study has examined the effects of different dietary patterns on postprandial events. Therefore, the aim of the present randomized crossover trial was to systematically investigate the postprandial metabolic responses to three different dietary patterns. We hypothesized that postprandial lipemic, glycemnic, and inflammatory responses are lower after consuming a Mediterranean-type meal than after meals typical of a Western dietary pattern. Selected participants were older (age range of 60–80 years), overweight, or obese adults with a cluster of metabolic syndrome traits, because this group has higher postprandial lipemia than young, normal-weight, and metabolically healthy adults.\[14,15\] In addition, overweight/obesity is associated with obesity-induced inflammation, so-called malnutrition.\[8,10\] We assumed that postprandial inflammatory responses can be particularly evident in individuals with this phenotype.

2. Experimental Section

2.1. Participants

Participants were recruited in Bonn, Germany, via advertisements in local newspapers, public postings and flyers. Of 446 individuals who expressed an interest, 127 aged 60–80 years with a BMI of 27–34.9 kg m\(^{-2}\), attending screening, which included physical assessments (body height and weight, resting blood pressure [BP], heart rate, and waist and hip circumference), blood analyses in fasting blood samples (fasting serum creatinine, urea, sodium, potassium, bilirubin, uric acid, gamma-glutamyl transferase, alanine transaminase, aspartate transaminase, hepatic lipase, blood counts, serum lipids and lipoproteins, glucose, insulin, HbA1c, and high-sensitivity C-reactive protein [CRP]), medical history, and a dietary questionnaire.

Individuals were included if they had the following cluster of risk factors for cardiometabolic diseases: 1) overweight or obesity stage 1 (BMI of 27–34.9 kg m\(^{-2}\)); 2) visceral fat distribution (waist circumference \(\geq 94\) cm for men and \(\geq 80\) cm for women)\[16\]; 3) pre-hypertension (systolic BP \(\geq 120–139\) mmHg and/or diastolic BP \(\geq 80–89\) mmHg) or hypertension (systolic BP \(\geq 140–159\) mmHg and/or diastolic BP \(\geq 90–99\) mmHg)\[17\]; and 4) at least one of the following criteria: impaired glucose tolerance (fasting plasma glucose \(\geq 5.55\) mmol L\(^{-1}\))\[16\] and/or dyslipidemia (fasting serum triglycerides \(\geq 1.7\) mmol L\(^{-1}\) or serum HDL cholesterol \(< 1.0\) mmol L\(^{-1}\) for men and \(< 1.3\) mmol L\(^{-1}\) for women)\[16\] and/or a pro-inflammatory state (high-sensitivity CRP \(\geq 2.0\) mg dl\(^{-1}\))\[18\]. Besides the pro-inflammatory state, the inclusion criteria are based on the diagnostic criteria of the metabolic syndrome.\[16\]

The main exclusion criteria were smoking, insulin-treated diabetes mellitus, long-term intake of dietary supplements, inflammatory disease, disease of the liver, kidney, or gastrointestinal tract, a history of cardiovascular events, abnormal thyroid function, cancer, recent major surgery or illness, substance or alcohol abuse, participation in a weight loss program, and malabsorption syndromes.

The protocol was explained in detail to all participants, who provided written informed consent at the beginning of the study. The study protocol was approved by the Ethics Committee of the Medical Faculty of the Rheinische Friedrich-Wilhelms-Universität Bonn, Germany, and the study was conducted in accordance with the declaration of Helsinki. The trial was registered at http://www.germancantr.de and http://www.drks.de under identifier DRKS00009861.

The participants were instructed to maintain their usual diet, level of physical activity, lifestyle, and body weight throughout the study period. The use of antihypertensive medication, such as angiotensin II receptor blockers and beta-blockers, statins, biguanides (metformin), and thyroid medication, were no exclusion criteria. Participants requiring antihypertensive agents (n = 29), lipid-lowering drugs (n = 16), metformin (n = 3), or thyroid therapy (n = 15) were instructed to continue their usual regimen.

2.2. Study Design

This study was a randomized, dietary-controlled crossover trial. Each participant took part in three 5-h meal tests from morning until afternoon. Study days were separated by 2-week washout periods. The participants were assigned to the three different test meals by block randomization procedure via computer-generated randomization tables (Microsoft Excel 2010, Microsoft Corporation, Redmond WA, USA). Thereby the order of test meal consumption was randomized. Ten participants were allocated equally to each of six orders.

All participants were instructed to abstain from alcohol on the day before the test and to refrain from intensive physical activity for 12 h prior to the test. In addition, they were instructed to standardize their meal intake on the previous evening. Tests were
then conducted in the morning after a 10–12 h overnight fast. Venous blood sampling was performed prior to the meal (0 h) and at 1, 2, 3, 4, and 5 h after finishing the meal. Participants stayed at the study site (Department of Nutritional Physiology, University of Bonn) the entire time for 5 h. Participants were asked to limit movement during the test meal period, remain seated when possible and were encouraged to read or to play card games during the study period. No foods other than the test meals were consumed over the test period and mineral water was provided.

### 2.3. Meal Composition

Three different isoenergetic (4200 kJ) and isonitrogen meals were designed to represent different dietary patterns: a Western diet-like high-fat (WDHF) meal, rich in total fat, SFA, and animal protein; a Western diet-like high-carbohydrate (WDHC) meal, rich in refined carbohydrates; and a Mediterranean diet (MED) meal, rich in unsaturated fatty acids, dietary fiber, and antioxidative compounds. The nutrient composition of the meals is summarized in Table 1. The meals were calculated using the computer-based nutrient calculation program EBISpro, based on the German Nutrient Database Bundeslebensmittelschlüssel, version 3.01 (Max Rubner-Institut, Karlsruhe, Germany). The WDHF meal consisted of a bread roll, butter, cold cuts of meat, cream yoghurt, an egg, and a croissant. The main components of the WDHC meal were a bread roll, butter, hazelnut spread (Nutella), strawberry jam, and toast. The MED meal consisted of ciabatta, olive oil, cured salmon, fruit and vegetables.

The test meals were freshly prepared on the morning of each test day in a dedicated kitchen of the Agricultural Faculty of the University of Bonn, Germany, by study personnel in accordance with a standardized protocol, which included the weighing of each food component to the nearest gram. Meals were served as breakfast. In addition to the meal, the participants drank one glass of water. The participants were required to complete the meal within 20 min under observation by a member of the laboratory staff.

### 2.4. Measurements

#### 2.4.1. Anthropometrics

Body height and body weight were determined to the nearest 0.1 cm and 0.1 kg, respectively, using a scale with an integrated stadiometer (seca 704, Seca, Hamburg, Germany). Waist circumference was measured midway between the lowest rib and the iliac crest, while the participant was at minimal respiration. Hip circumference was measured at the height of trochanters majores. Both measurements were performed in duplicate in an upright position and to the nearest 0.1 cm. Body composition (fat mass [FM] and fat-free mass [FFM]) was determined by bioelectrical impedance analyses (Nutrigard-M, Multi Frequency Phase-Sensitive Bioelectrical Impedance Analyzer, Data Input). FFM was calculated according to the method published by Sun et al. [19] FM was calculated by subtracting FFM from body weight.

#### 2.4.2. Office BP and Heart Rate

Office BP and heart rate were measured with an automatic BP measurement device (Boso Carat Professional, Bosch + Sohn GmbH, Jungingen, Germany) under standardized conditions according to the recommendations of the American Heart Association.[20,21]

#### 2.4.3. Blood Sample Processing and Analysis

Venous blood samples were taken via cannula (Vasofix Safety, B. Braun Melsungen AG) and drawn into tubes containing EDTA, lithium heparin, fluoride, or a coagulation activator (Sarstedt). Plasma and serum were obtained by centrifugation at 3000 g for 15 min at 8 °C. Plasma/serum aliquots were immediately frozen in cryovials and stored at −80 °C until analysis. All analyses were performed in a blinded manner.

#### 2.4.4. Plasma Glucose, Serum Insulin, Plasma Glucagon-Like Peptide-1 (GLP-1), and Lipids

Glucose concentration of the plasma samples was assessed via bichromatic endpoint measurement with a Dimension Vista

### Table 1. Energy content and nutrient composition of the three test meals.

|                     | WDHF meal | WDHC meal | MED meal |
|---------------------|-----------|-----------|----------|
| Energy [kJ]          | 4230      | 4241      | 4238     |
| Energy density [kJ g⁻¹] | 7.2       | 9.8       | 5.6      |
| Carbohydrates [g]   | 94        | 145       | 133      |
| Carbohydrates [EN %] | 37        | 58        | 53       |
| Mono- and disaccharides [g] | 45    | 87        | 51       |
| Polysaccharides [g] | 47        | 57        | 79       |
| Ratio of polysaccharides to mono- and disaccharides | 1.0 | 1.5 | 0.6 |
| Dietary fiber [g]   | 4         | 5         | 14       |
| Protein [g]         | 26        | 26        | 26       |
| Protein [EN %]      | 10        | 10        | 10       |
| Total fat [g]       | 59        | 34        | 40       |
| Total fat [EN %]    | 53        | 31        | 36       |
| SFA [g]             | 32        | 19        | 6        |
| MUFA [g]            | 20        | 11        | 24       |
| n-3 PUFA [g]        | 1         | 0         | 2        |
| n-6 PUFA [g]        | 3         | 2         | 7        |
| β-carotene [mg]     | 0.2       | 2.3       | 4.8      |
| Retinol [mg RE]     | 365       | 522       | 832      |
| Vitamin E [mg TE]   | 2.3       | 2.9       | 10.8     |
| Vitamin C [mg]      | 9         | 16        | 102      |

EN%, energy percent; MED, Mediterranean diet-like meal; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; RE, retinol equivalent; SFA, saturated fatty acids; TE, tocopherol equivalent; WDHF, Western diet-like high-fat meal; WDHC, Western diet-like high-carbohydrate meal; WDHF, Western diet-like high-fat meal;
1500 analyzer (Siemens Healthcare Diagnostics, Erlangen, Germany). Serum insulin concentrations were determined using chemiluminescent-immunometric assay with an Immulite 2000 analyzer (Siemens Healthcare Diagnostics). The sum of the plasma concentrations of GLP-1 (7-36) amide and GLP-1 (9-36) amide was measured as previously described. [22]

The serum total cholesterol concentration was measured using polychromatic endpoint measurement, while serum concentrations of HDL cholesterol, LDL cholesterol, and serum triglycerides were assessed using endpoint measurement with a Dimension Vista 1500 analyzer (Siemens Healthcare Diagnostics). Serum concentrations of non-esterified fatty acids (NEFA) were determined with a commercially available colorimetric enzyme assay (Wako Chemicals GmbH).

2.4.5. Biomarkers of Inflammation and Endothelial Activation

Plasma concentrations of interleukin-1β (IL-1β) and interleukin-6 (IL-6), soluble adhesion molecules E-selectin (sE-selectin), intercellular adhesion molecule-1 (sICAM-1), and vascular cell adhesion molecule-1 (sVCAM-1) were analyzed in duplicate using commercially available ELISA (R&D Systems) in accordance with the manufacturer’s instructions.

2.4.6. Biomarkers of Oxidation and Antioxidant State

Plasma oxidized LDL (oxLDL) was determined in duplicate using commercially available enzyme-linked immunoassay kits (Immundiagnostik, Bensheim, Germany) according to the manufacturer’s instructions. Plasma concentrations of α-tocopherol, retinol, and β-carotene were determined via HPLC as described previously. [23] The total antioxidant capacity of plasma samples was measured using the trolox equivalent capacity (TEAC) method. [24] For vitamin C analysis, metaphosphoric acid was added to the plasma samples to increase stability. After centrifugation, the supernatant was poured into vials and analyzed using HPLC with UV detection.

2.4.7. Self-Reported Dietary Intake of Energy and Nutrients

For the purpose of standardization, the participants were instructed by two qualified nutritionists to complete a 3-day food diary on the days prior to each study visit, in order to monitor and identify possible variations in total intake of energy and macronutrients. The dietary records by analyzed by using the computer-based nutrient calculation program EBISpro.

2.5. Statistical Analyses

Serum triglycerides were defined as primary outcome. Based on a sample size of $n = 54$ participants, one would be able to detect a difference within a variable in the size of 40% of the standard deviation of the difference with a power of 80% holding for a two-side $t$-test at a level of 5%. Assuming a standard deviation of 0.5 mmol L$^{-1}$, we were able to detect a difference of 0.2 mmol L$^{-1}$ in serum triglycerides between two of the diets. We increased sample size to $n = 60$ with respect to a drop-out rate of 10%.

Statistical analyses were performed using the IBM SPSS statistical software package (version 23, IBM, Armonk, NY, USA). To identify potential differences in total energy and macronutrient intake (as recorded in the 3-day food diaries) on the days prior to the test meal and any correlation with postprandial outcomes. Fasting values were included as covariates. Participants’ baseline characteristics at screening were analyzed for sex differences by unpaired Student’s $t$-test or Mann–Whitney $U$-test. Variables were checked for differences prior to test meal intake with the use of a mixed model analysis.

Linear mixed models were used to test for the effects of time, test meal and any correlation with postprandial outcomes. Fasting values were included as covariates. Participants were included as random factors. When there was no evidence of the presence of interaction ($p > 0.05$), mixed model calculations were repeated without an interaction term. When there was evidence for significant interaction terms, data were analyzed by each time point separately performing linear mixed model calculations as post hoc analyses.

Residuals obtained from the mixed model were inspected for normality to control for the fit of the statistical model. Logarithmic transformation was applied before analysis if the residuals were not normally distributed, which was given for glucose, insulin, IL-1β, IL-6, and sVCAM-1.

Summary measurements for postprandial response were calculated as AUC. The incremental AUC (iAUC) was calculated for the concentration of all postprandial study variables. Calculations were performed as reported previously. [25] Data are expressed as mean ± SEM; given $p$-values are two-sided.

3. Results

3.1. Baseline Characteristics

Sixty participants (34 male, 26 female) were considered eligible and enrolled in the study; all participants completed the entire postprandial study and were included in the analysis. Participant flow from initial screening to final analysis is shown in Figure 1. Baseline characteristics are summarized in Table 2. Based on BMI, all participants were overweight or obese (43% and 57%, respectively), had elevated BP or hypertension ($n = 11$ and 49, respectively), and had visceral fat distribution. Significant differences were seen between the male and the female groups in terms of body weight, height, waist circumference, percentage body FM, heart rate, total cholesterol, HDL cholesterol, and CRP (Table 2). Significantly lower body weight, height and waist circumference and higher percentage body FM, heart rate, total cholesterol, HDL cholesterol, and CRP was evident in women compared with men (Table 2).

3.2. Self-Reported Dietary Intake of Energy and Nutrients

All participants maintained their usual dietary pattern throughout the study. Nutrient intake remained unchanged and energy intake also did not differ prior to any study visit (data not shown).
Figure 1. Flow diagram of participants.
3.3. Plasma Glucose, Serum Insulin, and Plasma GLP-1

Significant concentration differences in plasma glucose and serum insulin were seen at certain time points after meal intake, indicated by time × meal interactions for plasma glucose ($p = 0.008$) and serum insulin ($p = 0.033$) after meal intake. Postprandial values increased significantly from baseline ($p < 0.001$ for glucose and insulin) and were significantly influenced by the meal ($p = 0.013$ for glucose; $p < 0.001$ for insulin; Figure 2A,B). After WDHC meal intake, glucose increase persisted significantly longer and insulin values were significantly higher throughout the postprandial period. IAUC values differed significantly between the meals and the highest glycemic response and an insulinemic response was seen after WDHC meal intake ($p = 0.002$ for glucose; $p < 0.001$ for insulin; Table 3). Plasma GLP-1 increased significantly over time ($p < 0.001$; Figure 2E). No differences were seen in GLP-1 concentrations ($p = 0.706$) as well as in iAUC between the meals ($p = 0.978$).

3.4. Serum Lipids, Lipoproteins, and NEFA

Serum triglycerides showed a significant time × meal interaction ($p = 0.005$; Figure 2D). There was a significant time-dependent increase in serum triglycerides from baseline to all time points, reaching a maximum concentration after 3 h and elevated values after 5 h ($p < 0.001$). At 2–5 h, the WDHF meal induced significantly higher values than the other meals (significant meal effect; $p < 0.001$), which was also reflected as a significant difference in iAUC ($p < 0.001$; Table 3). Analyses revealed no significant time × meal interaction for lipoproteins, but a significant decrease over time (LDL cholesterol: $p < 0.001$; HDL cholesterol: $p < 0.001$; total cholesterol: $p < 0.001$) as well as a significant influence of the meal for HDL ($p < 0.001$) and total cholesterol ($p = 0.007$), but not for LDL cholesterol ($p = 0.053$). There was no detectable difference in AUC values for LDL, HDL, and total cholesterol (Table 3).

Meals induced a decrease in NEFA below baseline values in the early postprandial phase. Values subsequently increased but did not return to baseline values within the measurement period (Figure 2C). At 2–5 h, NEFA concentrations were significantly higher after the WDHF meal than after the other meals. This was identified as a significant time × meal interaction ($p < 0.001$), a significant meal effect ($p < 0.001$), and a significant time effect ($p < 0.001$).

3.5. Plasma Soluble Adhesion Molecules, IL-1β, and IL-6

Soluble adhesion molecules (sICAM-1, sVCAM-1, and sE-selectin) showed no significant time × meal interaction (Figure 3C,D). Meal intake induced no changes in sICAM-1 (time effect, $p = 0.077$; meal effect, $p = 0.131$). Significant decreases in sE-selectin and sVCAM-1 levels were seen over time ($p < 0.001$ and $p = 0.002$, respectively). A significant influence of the meal was also detected for sE-selectin ($p = 0.002$). Adhesion molecule AUC values were not affected by the meal (Table 3).

IL-1β was not affected by meal intake ($p = 0.802$), time ($p = 0.104$), or the interaction of these parameters ($p = 0.537$; Figure 3A). Plasma IL-6 significantly increased over time ($p < 0.001$; Figure 3B). No differences were seen in IL-6 concentrations ($p = 0.065$) nor in iAUC between the meals ($p = 0.488$).
Figure 2. Fasting and postprandial concentrations of A) plasma glucose, B) serum insulin, C) serum NEFA, D) serum triglycerides and E) plasma GLP-1 in participants of high risk of CVD. Data are presented as mean ± SEM. GLP-1, glucagon-like peptide-1; MED, Mediterranean diet-like meal; NEFA, non-esterified fatty acids; WDHF, Western diet-like high-fat meal; WDHC, Western diet-like high-carbohydrate meal. ●●● \( p < 0.001 \) for the fixed factor time; * \( p < 0.05 \), ** \( p < 0.01 \), *** \( p < 0.001 \) significant time \( \times \) meal interaction. In cases of significant interaction effects, \( p \)-values for single fixed factors are not given.

3.6. Plasma oxLDL, Vitamin C, Retinol, \( \alpha \)-Tocopherol, \( \beta \)-Carotene, and TEAC

Plasma oxLDL concentration was not affected by meal intake \( (p = 0.121) \), time \( (p = 0.181) \), or the interaction of these \( (p = 0.442; \text{Figure 4F}) \). Plasma vitamin C kinetics showed no time \( \times \) meal interaction \( (p = 0.060) \), but a significant influence of the meal was seen \( (p < 0.001) \) and a significant change over time \( (p < 0.001; \text{Figure 4A}) \). After intake of the MED meal, vitamin C concentrations rose to a maximum after 4 h, whereas vitamin C decreased to a minimum at 3 h after WDHF and WDHC meal intake. Total vitamin C response, shown by iAUC values, revealed significantly higher iAUC values for the MED meal compared with the remaining meals \( (p < 0.001) \).
Table 3. Postprandial responses shown by incremental AUC (iAUC) for parameters of lipid and glucose metabolism, biomarkers of inflammation, and antioxidant status in participants of high CVD risk.

| Parameter                  | MED       | WDHF      | WDHC      | p-value (meal effect) |
|----------------------------|-----------|-----------|-----------|-----------------------|
| Glucose iAUC [h mmol L⁻¹]  | 4.3 ± 0.7/Add.  | 3.2 ± 0.4/B  | 5.2 ± 0.7/F  | 0.002                     |
| Insulin iAUC [h pmol L⁻¹]  | 1720 ± 153/Add.  | 1426 ± 93/B  | 2167 ± 178/B  | 0.001                     |
| GLP-1 iAUC [h pmol L⁻¹]    | 44.9 ± 3.9  | 45.6 ± 3.9  | 44.2 ± 3.5  | 0.978                     |
| Triglyceride iAUC [h mmol L⁻¹] | 3.1 ± 0.2/B  | 4.0 ± 0.2/B  | 2.9 ± 0.2/B  | 0.001                     |
| NEFA iAUC [h mmol L⁻¹]    | -1.3 ± 0.1/A  | -1.0 ± 0.1/A  | -1.5 ± 0.1/B  | 0.001                     |
| Total cholesterol iAUC [h mmol L⁻¹] | -0.14 ± 0.15  | -0.46 ± 0.12  | -0.26 ± 0.14  | 0.134                     |
| LDL iAUC [h mmol L⁻¹]     | -0.46 ± 0.08  | -0.33 ± 0.07  | -0.38 ± 0.08  | 0.305                     |
| HDL iAUC [h mmol L⁻¹]     | -0.25 ± 0.03  | -0.33 ± 0.04  | -0.22 ± 0.04  | 0.063                     |
| Inflammation              |           |           |           |                       |
| IL-1β iAUC [h pg mL⁻¹]    | 0.01 ± 0.02  | 0.02 ± 0.02  | 0.02 ± 0.04  | 0.754                     |
| IL-6 iAUC [h pg mL⁻¹]     | 17.3 ± 1.3  | 15.0 ± 0.9  | 16.4 ± 1.2  | 0.488                     |
| Adhesion molecules        |           |           |           |                       |
| sE-selectin iAUC [h ng mL⁻¹] | -2.7 ± 1.1  | -5.5 ± 1.2  | -5.8 ± 1.6  | 0.112                     |
| sICAM-1 iAUC [h ng mL⁻¹]  | -15.1 ± 11.5 | -29.9 ± 16.7 | -13.6 ± 13.2 | 0.710                     |
| sVCAM-1 iAUC [h ng mL⁻¹]  | -32.3 ± 53.1 | -83.2 ± 35.4 | -169.2 ± 61.0 | 0.227                     |
| Oxidation and antioxidant |           |           |           |                       |
| OxLDL iAUC [h ng mL⁻¹]    | 2.2 ± 9.2  | 9.5 ± 9.6  | 9.1 ± 10.9  | 0.809                     |
| Vitamin C iAUC [h ng L⁻¹] | 2.8 ± 0.8/B  | -4.3 ± 0.6/A  | -3.5 ± 0.9/B  | <0.001                    |
| Tocopherol iAUC [h μg mL⁻¹] | -2.2 ± 0.8  | -1.4 ± 0.4  | -1.0 ± 0.7  | 0.533                     |
| β-Carotene iAUC [h ng mL⁻¹] | -38.9 ± 23.3 | -44.3 ± 13.1 | -36.1 ± 28.9 | 0.953                     |
| Retinol iAUC [h ng mL⁻¹]  | -22.2 ± 21.0 | -37.5 ± 17.4 | -10.3 ± 20.0 | 0.368                     |
| TEAC iAUC [h mmol trolox equivalent L⁻¹] | -0.10 ± 0.04 | -0.15 ± 0.04 | -0.15 ± 0.04 | 0.641                     |

Values given as mean ± SEM. Means denoted with lowercase letters indicate statistical significance Post-hoc analyses (least significant difference; p < 0.05). Statistical differences are given between values with the same lowercase letters a and b; MED, Mediterranean diet-like meal; IL-1β, interleukin-1β; IL-6, interleukin-6; NEFA, non-esterified fatty acid; oxLDL, oxidized LDL; sE-selectin, soluble E-selectin; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; TEAC, trolox equivalent capacity; WDHC, Western diet-like high-carbohydrate meal; WDHF, Western diet-like high-fat meal.

Plasma concentrations of α-tocopherol, retinol, and β-carotene as well as TEAC showed no significant time × meal interaction and did not differ between meals (Figure 4B–E). A significant reduction was seen over time for α-tocopherol (p < 0.001), retinol (p < 0.001), β-carotene (p = 0.032), and TEAC (p < 0.001).

4. Discussion

In this randomized, controlled, postprandial study in overweight/obese individuals, all three meal patterns induced significant changes in glucose, insulin, GLP-1, triglycerides, NEFA, antioxidant state and pro-inflammatory markers, when compared with the fasted state. In accordance with our hypothesis, the MED diet meal showed beneficial effects on glucose, insulin, triglycerides, NEFA, and vitamin C responses compared to the Western diet meals, as indicated by differences in time × meal interactions. In addition, all meals resulted in similar increases in GLP-1 and IL-6, and decreases in TEAC, tocopherol, retinol, and β-carotene, with no significant differences between the three meals. By contrast, no postprandial increases in soluble endothelial adhesion molecule and oxLDL were seen. The unique aspect of our work is that we focused on a clinically relevant population, overweight and moderately obese individuals with a cluster of metabolic syndrome traits, and that we used a whole diet approach to study acute effects of meals on a wide array of cardiometabolic parameters.

All meals induced hyperglycemia and hyperinsulinemia with higher and more prolonged increases being associated with the WDHC meal than with the MED and WDHF meals. These findings may be explained by the higher content of total carbohydrates and mono- and disaccharides in the WDHC meal than in the other two meals. In addition, the fiber content of the MED meal was considerably higher than that of the WDHC meal (14 vs 5 g per meal), which could partly account for the lower glycemic and insulinemic response. Dietary fiber can modify the gastrointestinal transit time, which, in turn, can alter rates of glucose absorption thereby influencing blood glucose homeostasis.[26] The results of the current study are consistent with those from previous studies, where partial substitution of high-glycemic load (GL) foods with foods of a lower GL, as well as the modification of the macronutrient profile (e.g., combinations of carbohydrates with fat and/or dietary fiber) led to a lower post-prandial glucose response.[27–29] In addition, our results are in line with a recent systematic review and meta-analysis of Kdekian et al.[30] showing that modest exchange of carbohydrates for fats in mixed meals significantly reduces postprandial glucose and insulin.

The high insulin response after all three meals caused a rapid decline in postprandial glucose, with lower plasma glu-
cose concentrations being observed 5 h postprandially than at baseline (Figure 2) confirming previous results of our research group.[23,31] To complement these findings, the increased GLP-1 concentrations can serve to explain the increased glucose-stimulated insulin secretion after all three meals. In that, the slightly increased incretin and insulin concentrations are within the physiological range of metabolic responses, which is unlikely to affect the glucose tolerance of healthy humans. However, it remains to be tested whether patients with type 2 diabetes would respond differently.
Figure 4. Fasting and postprandial concentrations of plasma A) vitamin C, B) tocopherol, C) retinol, and D) \(\beta\)-carotene, E) TEAC, and F) oxidized LDL in participants of high risk of CVD. Data are presented as mean ± SEM. MED, Mediterranean diet-like meal; TEAC, trolox equivalent antioxidant capacity; WDHF, Western diet-like high-fat meal; WDHC, Western diet-like high-carbohydrate meal. No time \(\times\) meal interactions were observed for postprandial concentrations. \(* p < 0.05, \cdots \cdots p < 0.001\) for the fixed factor time; \(\cdots \cdots p < 0.01\) for the fixed factor meal.

All three meals induced a postprandial increase in triglycerides with higher concentrations seen at 2–4 h after the WDHF meal than after the MED and WDHC meal. In addition, postprandial NEFA responses differed, with significantly higher concentrations seen after the WDHF meal than the other meals (Figure 2). This stronger response may primarily result from the higher total fat and SFA content of the WDHF meal. Postprandial triglyceride concentrations are a significant independent risk factor for CVD.\(^{32,33}\) In normolipidemic adults, the magnitude of the postprandial triglyceride response is directly dependent
on the amount of fat ingested. Less is known about the importance of fatty acid composition and different fatty acid food sources (e.g., SFA from meat vs SFA from butter) on postprandial lipemia in individuals at risk of CVD. In our study, major sources of SFA were butter and cold cuts of meat, and the major SFA was palmitic acid. Some previous studies in healthy individuals have shown that SFA (e.g., from butter, lard or coconut oil) results in a lower or similar increase in triglycerides than MUFA and/or PUFA. In contrast, other studies show that ingestion of MUFA (e.g., from olive oil) in test meals induces lower postprandial triglyceride concentrations than SFA (e.g., from butter or cheese) in healthy individuals, in patients with impaired fasting glucose and in individuals with elevated fasting triglycerides. The main mechanisms underlying this observation are thought to include a higher MUFA clearance rate than SFA, as well as an increased incretin effect, followed by slower gastric emptying. A recent review and meta-analysis aimed at investigating and quantifying the effects of oral fat tolerance are in agreement with data from a study included in the statistical analyses. Previous postprandial studies have demonstrated that meals that are rich in fat and energy lead to a low-grade meal-induced impairment of endothelial function measured by flow-mediated dilatation and/or blood concentrations of endothelial adhesion molecules. In this context, the fatty acid profile, particularly the amount of SFA, appears to be an important factor. Endothelial adhesion molecules such as VCAM-1, ICAM-1, and E-selectin are pro-inflammatory proteins which play a critical role in the adhesion of leukocytes to endothelial cells during the early stages of atherosclerosis. Their concentration is low normal conditions, but they can increase when the endothelium is activated by stimuli such as proinflammatory cytokines and reactive oxygen species. In the present study, the pronounced lipemic, glycemic, insulinemic, and inflammatory responses induced by all three meals did not lead to increased endothelial activation. In fact, the biomarkers of endothelial activation sVCAM-1 and sE-selectin decreased over time. This finding supports data generated in two recent trials of CVD risk individuals. The slight decrease in biomarkers of endothelial activation over time may be attributable to circadian variability.

Reductions in postprandial NEFA concentrations were significantly more pronounced after the WDHC and MED meal than after the WDHF meal intake. This finding can be explained by the higher levels of carbohydrates in the WDHC and MED meals. In particular, the high amounts of mono- and disaccharides (87 g per meal) in the WDHC meal results in higher glucose and consequently higher insulin concentrations. Insulin activates LPL on the endothelium of adipose tissue causing an increase in triglyceride storage in the adipose tissue. Furthermore, insulin inhibits triglyceride lipolysis, leading to a lower level of NEFA in the blood. This may explain the more pronounced decrease in NEFA seen after WDHC meal intake. These observations are consistent with those reported previously.

Persistent low-grade inflammation is an important underlying factor in the etiology of metabolic and vascular diseases. In the present study of older individuals at increased CVD risk, plasma IL-6 increased by approximately 100% from baseline in response to the three meals. Similar findings have been observed in healthy individuals <60 years of age after high-fat, high-energy meals. As it is assumed that postprandial glycemia and lipemia induce a pro-oxidative state that triggers inflammation, the lack of difference in IL-6 response between the meals was somewhat unexpected. The WDHC meal resulted in a higher glycemic response, whereas the WDHF meal led to a higher lipemic response than the MED meal. However, these differences did not translate into distinct inflammatory responses. We therefore speculate that energy load is the main determinant for acute postprandial inflammatory response but this cannot be actually inferred from our study design because we administered isoenetic meals.

No postprandial IL-1β response was seen in any of the three groups and previous data are scarce and equivocal. The results of the current study are in agreement with data from a study of patients with metabolic syndrome, which showed no increase in plasma IL-1β after a high-fat challenge (3473 kJ). In another postprandial study using meals high in SFA in subjects with metabolic syndrome, serum IL-1β fell below the detection limit in most samples and consequently data could not be included into the statistical analyses.
In conclusion, in this population of older adults with a risk phenotype for cardiometabolic diseases, intake of an energy rich meal resulted in hyperglycemia, hyperlipemia, an inflammatory response, and a decrease in antioxidant markers. A meal typical of the Mediterranean diet resulted in favorable effects on glycemic, insulinemic, and lipemic responses compared with meals typical of Western dietary patterns. As the inflammatory response was present to the same degree after each meal, we suggest that energy intake is the main predictor of postprandial inflammation.

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Conflict of Interest
The authors declare no conflict of interest.

Author Contributions
Y.B.S., R.F., P.S., and S.E. designed the study; Y.B.S., S.C., M.C., and S.E. conducted the study; B.S.W., J.J.H., and M.C.S. analyzed blood samples; Y.B.S. and R.F. performed statistical analysis; Y.B.S. and S.E. wrote the manuscript and had primary responsibility for the final content. All authors have read and approved the final manuscript.

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[1] D. Mozaffarian, D. S. Ludwig, J. Am. Med. Assoc. 2010, 304, 681.
[2] D. Mozaffarian, I. Rosenberg, R. Uauy, BMJ 2018, 361, k2392.
[3] L. Cordain, S. B. Eaton, A. Sebastian, N. Mann, S. Lindeberg, B. A. Watkins, J. H. O’Keefe, J. Brand-Miller, Am. J. Clin. Nutr. 2005, 81, 341.
[4] M. Rodriguez-Monforte, E. Sanchez, F. Barrio, B. Costa, G. Flores-Mateo, Eur. J. Nutr. 2017, 56, 925.
[5] C. Galbete, L. Schwingshakel, C. Schwedhelm, H. Boeing, M. B. Schulze, Eur. J. Epidemiol. 2018, 33, 909.
[6] M. Rodriguez-Monforte, G. Flores-Mateo, E. Sanchez, Br. J. Nutr. 2015, 114, 1341.
[7] S. Lacroix, C. D. Rosiers, J. C. Tardif, A. Nigam, Nutr. Res. Rev. 2012, 25, 288.
[8] P. C. Calder, N. Ahiuwalla, F. Brouns, T. Buettler, K. Clement, K. Cunningham, K. Esposito, L. S. Jonsson, H. Kolb, M. Lansink, A. Marcos, A. Margioris, N. Matusheski, H. Nordmann, J. O’Brien, G. Pugliese, S. Rizkalla, C. Schalkwijk, J. Tuomilehto, J. Warnberg, B. Watzl, B. M. Winkelhofer-Roob, Br. J. Nutr. 2011, 106, S5.
[9] S. R. Emerson, S. P. Kurti, C. A. Harms, M. D. Haub, T. Melgarejo, C. Logan, S. K. Rosenkranz, Adv. Nutr. 2017, 8, 213.
[10] M. F. Gregor, G. S. Hotamisligil, Annu. Rev. Immunol. 2011, 29, 415.
[11] A. N. Margioris, Curr. Opin. Clin. Nutr. Metab. Care 2009, 12, 129.
[12] J. H. Stroeve, J. H. van, B. H. Kramer, O. B. van, S. Wopereis, Genes Nutr. 2015, 10, 459.
[13] B. Burton-Freeman, Br. J. Nutr. 2010, 104, S1.
[14] C. B. Dias, P. J. Moughan, L. G. Wood, H. Singh, M. L. Garg, Lipids Health Dis. 2017, 16, 178.
[15] C. S. Katsanos, Adv Nutr 2014, 5, 226.
[16] K. G. Alberti, R. H. Eckel, S. M. Grundy, P. Z. Zimmet, J. I. Cleeman, K. A. Donato, J. C. Fruchart, W. P. James, C. M. Loria, S. C. Smith Jr., Circulation 2009, 120, 1640.
[17] A. V. Chobanian, G. L. Bakris, H. R. Black, W. C. Cushman, L. A. Green, J. L. Izzo Jr., D. W. Jones, B. J. Materson, S. Oparil, J. T. Wright, E. J. Roccella, J. Am. Med. Assoc. 2003, 289, 2560.
[18] S. Egert, A. Bosy-Westphal, J. Seiberl, U. Seitter, S. Plachta-Danielzik, A. E. Wagner, J. Frank, J. Schrezenmeir, G. Rimbach, S. Wolffram, M. J. Müller, Br. J. Nutr. 2009, 102, 1065.
[19] S. S. Sun, W. C. Chumlea, S. B. Heymsfield, C. Schulze, B. J. Nielsen, B. Schoeller, K. Friedl, R. J. Kuczmarski, M. F. Gregor, K. J. Collins, C. L. Johnson, V. S. Hubbard, Am. J. Clin. Nutr. 2003, 77, 331.
[20] T. G. Pickering, J. E. Hall, L. J. Appel, B. E. Falkner, J. Graves, M. N. Hill, D. W. Jones, T. Kuritz, S. G. Sheps, E. J. Roccella, Hypertension 2005, 45, 142.
[21] P. K. Whelton, R. M. Carey, W. S. Aronow, D. E. Casey, J. R. Collins, C. Dennison Himmelfarb, S. M. DePalma, S. Gidding, K. A. Jamer-son, D. W. Jones, E. J. MacLaughlin, P. Muntner, O. Oviagele, S. C. Smith, J. R. Spencer, R. S. Stafford, S. J. Taler, R. J. Thomas, K. A. Williamson, Sr., J. D. Williamson, J. T. Wright, Jr., Hypertension 2018, 71, e13.
[22] C. Orskov, L. Rabenhoj, A. Wettergren, H. Kofod, J. J. Holst, Diabetes 1994, 43, 535.
[23] V. Brüll, C. Burak, B. Stoffel-Wagner, S. Wolffram, G. Nickenig, C. Muller, P. Langguth, B. Alteheld, R. Fimmers, P. Stehle, S. Egert, Eur. J. Nutr. 2017, 56, 1347.
[24] N. J. Miller, C. Rice-Evans, M. J. Davies, V. Gopinathan, A. Milner, Clin. Sci. (Lond) 1993, 84, 407.
[25] T. M. Wolever, Br. J. Nutr. 2004, 91, 295.
[26] D. S. Gray, Am. Fam. Physician 1995, 51, 419.
[27] L. Sun, N. J. Miller, C. Rice-Evans, M. J. Davies, V. Gopinathan, A. Milner, Clin. Sci. (Lond) 1993, 84, 407.
[38] C. M. Sciarrillo, N. A. Koemel, P. M. Tomko, K. B. Bode, S. R. Emerson, *Nutrients* 2019, 11, e1089.

[39] C. Thomsen, O. Rasmussen, T. Lousen, J. J. Holst, S. Fenselau, J. Schrezenmeir, K. Hermansen, *Am. J. Clin. Nutr.* 1999, 69, 1135.

[40] R. Carnevale, L. Loffredo, M. Del Ben, F. Angelico, C. Nocella, A. Petruccioli, S. Bartimoccia, R. Monticolo, E. Cava, F. Violi, *Clin. Nutr.* 2017, 36, 782.

[41] S. Lopez, B. Bermudez, A. Ortega, L. M. Varela, Y. M. Pacheco, J. Villar, R. Abia, F. J. Muriana, *Am. J. Clin. Nutr.* 2011, 93, 494.

[42] M. Monfort-Pires, J. Delgado-Lista, F. Gomez-Delgado, J. Lopez-Miranda, P. Perez-Martinez, S. R. Ferreira, *Nutrients* 2016, 8, 580.

[43] A. D. Lampidonis, E. Rogdakis, G. E. Voutsinas, D. J. Stravopodis, *Gene* 2011, 477, 1.

[44] B. Fielding, *Proc. Nutr. Soc.* 2011, 70, 342.

[45] L. Smolders, R. P. Mensink, J. Plat, *Nutr. Res.* 2017, 40, 85.

[46] P. Libby, *Arterioscler. Thromb. Vasc. Biol.* 2012, 32, 2045.

[47] H. L. Che, M. S. Kanthimathi, R. Loganathan, K. H. Yuen, A. T. Tan, K. R. Selvadurai, K. Nesaretnam, K. T. Teng, *Eur. J. Clin. Nutr.* 2017, 71, 107.

[48] E. Demmer, M. D. Van Loan, N. Rivera, T. S. Rogers, E. R. Gertz, J. B. German, J. T. Smilowitz, A. M. Zivkovic, *J. Nutr. Sci.* 2016, 5, e14.

[49] K. G. Jackson, C. K. Armath, A. M. Minihane, *Biochem. Soc. Trans.* 2007, 35, 451.

[50] J. P. Wallace, B. Johnson, J. Padilla, K. Mather, *Int. J. Clin. Pract.* 2010, 64, 389.

[51] K. M. Rathnayake, M. Weech, K. G. Jackson, J. A. Lovegrove, *J. Nutr.* 2018, 148, 348.

[52] E. A. Lambert, S. Phillips, R. Belski, A. Tursunalieva, N. Eikelis, C. I. Sari, J. B. Dixon, N. Straznicky, M. Grima, G. A. Head, M. Schlaich, G. W. Lambert, *Front. Physiol.* 2017, 8, 876.

[53] C. Thomsen, O. Rasmussen, T. Lousen, J. J. Holst, S. Fenselau, J. Schrezenmeir, K. Hermansen, *Am. J. Clin. Nutr.* 1999, 69, 1135.

[54] R. Carnevale, L. Loffredo, M. Del Ben, F. Angelico, C. Nocella, A. Petruccioli, S. Bartimoccia, R. Monticolo, E. Cava, F. Violi, *Clin. Nutr.* 2017, 36, 782.

[55] S. Lopez, B. Bermudez, A. Ortega, L. M. Varela, Y. M. Pacheco, J. Villar, R. Abia, F. J. Muriana, *Am. J. Clin. Nutr.* 2011, 93, 494.

[56] M. Monfort-Pires, J. Delgado-Lista, F. Gomez-Delgado, J. Lopez-Miranda, P. Perez-Martinez, S. R. Ferreira, *Nutrients* 2016, 8, 580.

[57] A. D. Lampidonis, E. Rogdakis, G. E. Voutsinas, D. J. Stravopodis, *Gene* 2011, 477, 1.

[58] B. Fielding, *Proc. Nutr. Soc.* 2011, 70, 342.

[59] L. Smolders, R. P. Mensink, J. Plat, *Nutr. Res.* 2017, 40, 85.

[60] P. Libby, *Arterioscler. Thromb. Vasc. Biol.* 2012, 32, 2045.

[61] H. L. Che, M. S. Kanthimathi, R. Loganathan, K. H. Yuen, A. T. Tan, K. R. Selvadurai, K. Nesaretnam, K. T. Teng, *Eur. J. Clin. Nutr.* 2017, 71, 107.

[62] E. Demmer, M. D. Van Loan, N. Rivera, T. S. Rogers, E. R. Gertz, J. B. German, J. T. Smilowitz, A. M. Zivkovic, *J. Nutr. Sci.* 2016, 5, e14.

[63] K. G. Jackson, C. K. Armath, A. M. Minihane, *Biochem. Soc. Trans.* 2007, 35, 451.