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Original Article

Investigating the Interaction Between a Polyphenol-Rich Mediterranean Diet and Genetic Variants on Advanced Glycation End-Product (AGE) Level

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ABSTRACT

Background: Polyphenol-rich dietary interventions may influence glycation-related and inflammatory biomarkers, but genetic modification of these responses remains uncertain. Objective: To assess biomarker responses to a polyphenol-rich Mediterranean diet and investigate modification by AGER rs2070600 and SOD2 rs4880. Methods: A 16-week randomized, parallel-group trial enrolled 84 adults aged 35–65 years with overweight or class I obesity in urban and industrial Punjab, Pakistan. Participants received dietary counseling and polyphenol-rich foods or a standard dietary control condition. Serum carboxymethyl-lysine (CML), high-sensitivity C-reactive protein (hs-CRP), and interleukin-6 (IL-6) were measured at baseline and week 16. Analyses used repeated-measures ANOVA and general linear models incorporating time × dietary group × genotype interactions. Results: Seventy-eight participants completed follow-up: 40 intervention and 38 control. CML decreased by 74.3 ng/mL (23.8%) versus 6.4 ng/mL (2.1%), respectively. Intervention–control differences in mean changes were −67.9 ng/mL for CML, −1.04 mg/L for hs-CRP, and −1.27 pg/mL for IL-6; reported time × group p-values were <0.001. Genetic effect modification remained unresolved because genotype-stratified summaries were inconsistent with overall results. Conclusion: The intervention was associated with greater short-term biomarker reductions among completers. Clinical benefit and genotype-guided dietary utility were not established. Keywords: Mediterranean Diet; Polyphenols; Advanced End Glycation Products; Carboxymethyl-Lysine; Inflammation; Nutrigenetics.

INTRODUCTION

Excess adiposity is an important context for investigating the relationships among diet, metabolic dysfunction, and individual susceptibility to cardiometabolic disease. Research linking obesity, lipid metabolism, and candidate genetic variants highlights the need to consider both nutritional exposures and genetic background when

evaluating metabolic outcomes (1). In adults with obesity or class I obesity, dietary interventions provide an opportunity to investigate whether changes in food intake influence biochemical markers before established diabetes or cardiovascular disease is present. The present study focuses on serum carboxymethyl-lysine (CML), a specific advanced glycation end-product (AGE), alongside high-sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6), to examine glycation-related and inflammatory responses to dietary modification.

A polyphenol-rich Mediterranean dietary pattern provides a relevant intervention for this purpose. Extra virgin olive oil is a source of bioactive compounds with antioxidant and anti-inflammatory properties, providing a biological rationale for investigating olive-polyphenol-containing interventions in relation to metabolic and inflammatory outcomes (2). However, evidence supporting these properties should be distinguished from evidence that a particular dietary intervention reduces circulating CML in humans. Furthermore, a Mediterranean dietary intervention combines several changes in food consumption, meaning that its effects cannot necessarily be attributed to polyphenols alone.

Human intervention findings also indicate that benefits cannot be assumed across all polyphenol preparations or outcomes. A randomized, placebo-controlled crossover trial of isolated anthocyanins from bilberry fruit and black rice reported no effect on LDL cholesterol or other assessed cardiovascular biomarkers in adults with elevated cholesterol (3). Although that intervention differs from a Mediterranean dietary pattern, the findings emphasize the importance of considering the source and composition of polyphenols, the study population, and the outcomes measured. Accordingly, evaluating a defined dietary intervention against a control condition is necessary to determine its effects on serum CML and inflammatory biomarkers.

Genetic variation is another potential contributor to differences in dietary responsiveness. Previous research has examined the interaction between the Apo A-II –265T>C polymorphism and dietary total antioxidant capacity in relation to anthropometric indices and serum lipid profiles among patients with type 2 diabetes mellitus (4). This provides a related nutrigenetic research framework, but it does not establish whether AGER rs2070600 or SOD2 rs4880 modifies responses to a polyphenol-rich Mediterranean diet. The specific question remains whether the intervention–control difference in biomarker change varies across these genotypes, rather than whether genotype groups differ within the intervention arm alone.

The present study therefore investigated this question in adults aged 35–65 years with overweight or class I obesity and low baseline Mediterranean diet adherence in urban Punjab, Pakistan. In a 16-week randomized parallel-group trial, a polyphenol-rich Mediterranean dietary intervention was compared with a standard dietary control condition. The objective was to evaluate changes in serum CML, hS-CRP, and IL-6 and determine whether AGER rs2070600 and SOD2 rs4880 modified these responses through time × dietary group × genotype interactions. This approach was intended to examine possible genetic effect modification of short-term biomarker responses, rather than establish the clinical utility of genotype-guided dietary recommendations.

MATERIAL AND METHODS

A 16-week randomized, parallel-group dietary intervention trial was conducted between August and December 2025 in urban and industrial areas of Punjab, Pakistan. The study evaluated the effects of a polyphenol-rich Mediterranean diet on serum carboxymethyl-lysine (CML) and systemic inflammatory biomarkers compared with a standard dietary control condition and examined whether responses differed according to AGER rs2070600 and SOD2 rs4880 genotypes. The target population comprised adults with overweight or class I obesity and low baseline adherence to a Mediterranean dietary pattern.

A total of 84 adults aged 35–65 years were enrolled. Eligibility required a body mass index (BMI) of 25.0–34.9 kg/m^2 and a baseline score below 6 on the 14-item Mediterranean Diet Adherence Screener (MEDAS). Individuals were excluded if they had diagnosed with type 1 or type 2 diabetes mellitus, chronic kidney disease, or active cardiovascular disease; had used anti-inflammatory or antioxidant supplements within the preceding 30 days; or currently consumed tobacco. The target sample size was informed by the cohort sizes of previous pilot nutrigenetic intervention studies investigating dietary polyphenols and circulating AGE concentrations, which ranged from 75 to 90 participants. Institutional ethical review board approval was obtained before study initiation, and all participants provided written informed consent before enrollment.

Participants were randomly allocated in a 1:1 ratio to the intervention or control group using a computer-generated block randomization sequence. The intervention group received individualized dietary counseling, a weekly supply of high-polyphenol extra virgin olive oil, and polyphenol-enriched dietary staples, with a target total polyphenol intake exceeding 800 mg/day. The comparator was a standard dietary control condition. Dietary assessment included MEDAS scoring, while dietary exposure to advanced glycation end-products was assessed using recall questionnaires.

Baseline demographic and clinical variables included age, sex, BMI, MEDAS score, and serum biomarker concentrations. Outcome assessments were performed before the intervention and at week 16 following a 12-hour overnight fast. Serum CML was the primary glycation-related outcome and was used as a surrogate marker of circulating AGE concentrations. High-sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6) were assessed as systemic inflammatory outcomes. Fasting venous blood samples were collected for biochemical measurements. Serum CML was quantified using high-sensitivity enzyme-linked immunosorbent assay (ELISA), hs-CRP was measured by immunoturbidimetry, and IL-6 was quantified using a high-sensitivity ELISA. Concentrations were expressed as ng/mL for CML, mg/L for hs-CRP, and pg/mL for IL-6.

Genomic DNA was extracted from peripheral blood leukocytes using a magnetic bead-based protocol. The candidate single-nucleotide polymorphisms AGER rs2070600 and SOD2 rs4880 were genotyped by real-time polymerase chain reaction using TaqMan fluorogenic allele-specific assays. Genetic effect modification was evaluated using the GG, GA, and AA genotype categories for AGER rs2070600 and by comparing CC homozygotes with pooled CT/TT carriers for SOD2 rs4880.

Statistical analyses were performed using IBM SPSS Statistics version 28.0. Continuous variables were assessed for normality using the Shapiro–Wilk test and visual inspection of Q–Q plots. Continuous data were summarized as means and standard deviations, and categorical data were summarized as frequencies and percentages. Baseline comparisons between the intervention and control groups used independent-samples t-tests for normally distributed continuous variables and chi-square tests for categorical variables.

Changes in serum CML, hs-CRP, and IL-6 were evaluated using two-way repeated-measures analysis of variance, with time as the within-participant factor and dietary group as the between-participant factor. The time × dietary group interaction was used to assess whether changes over the 16-week period differed between groups. General linear models incorporating time × dietary group × genotype interaction terms were used to examine whether the biomarker responses differed according to AGER rs2070600 or SOD2 rs4880 genotype. Genotype-stratified changes in serum CML were also summarized separately for each dietary group. Pearson correlation analysis examined associations among changes in serum CML, hs-CRP, IL-6, and MEDAS scores.

The reported per-protocol analysis included the 78 participants who completed the 16-week study: 40 in the intervention group and 38 in the control group. Six participants who withdrew because of noncompliance or loss to follow-up were excluded from this analysis. Statistical significance was defined using a two-sided p-value below 0.05.

RESULTS

Of the 84 randomized participants, 78 completed the 16-week study and were included in the reported per-protocol analysis, comprising 40 intervention participants and 38 controls. The completion rate was 92.9%; six participants withdrew because of noncompliance or loss to follow-up. Baseline characteristics are presented in Table 1. Mean age was 49.1 ± 6.8 years in the intervention group and 48.1 ± 7.6 years in the control group, and mean BMI was 28.9 ± 2.3 and 28.5 ± 2.5 kg/m^2, respectively. The AGER rs2070600 AA genotype was present in five participants in each group.

Serum CML decreased from 312.4 ± 42.1 to 238.1 ± 35.6 ng/mL in the intervention group, corresponding to a mean change of −74.3 ng/mL (−23.8%), compared with −6.4 ng/mL (−2.1%) in controls (Table 2). Intervention-group changes in hs-CRP and IL-6 were −1.11 mg/L (−31.9%) and −1.35 pg/mL (−27.5%), respectively, compared with −0.07 mg/L (−2.1%) and −0.08 pg/mL (−1.7%) in controls.

Table 1. Baseline characteristics of participants completing the 16-week trial

Characteristic	Intervention (n = 40)	Control (n = 38)
Age, years, mean ± SD	49.1 ± 6.8	48.1 ± 7.6
Male sex, n (%)	22 (55.0)	20 (52.6)
Female sex, n (%)	18 (45.0)	18 (47.4)
BMI, kg/m², mean ± SD	28.9 ± 2.3	28.5 ± 2.5
Serum CML, ng/mL, mean ± SD	312.4 ± 42.1	308.9 ± 39.8
hs-CRP, mg/L, mean ± SD	3.48 ± 0.88	3.36 ± 0.82
IL-6, pg/mL, mean ± SD	4.91 ± 1.15	4.79 ± 1.10
MEDAS score, mean ± SD	4.1 ± 1.2	4.3 ± 1.0
AGER rs2070600 GG, n (%)	21 (52.5)	20 (52.6)
AGER rs2070600 GA, n (%)	14 (35.0)	13 (34.2)
AGER rs2070600 AA, n (%)	5 (12.5)	5 (13.2)

BMI, body mass index; CML, carboxymethyl-lysine; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; MEDAS, Mediterranean Diet Adherence Screener; SD, standard deviation.

Table 2. Biomarker concentrations and within-group changes over 16 weeks

Biomarker	Group	n	Baseline, mean ± SD	Week 16, mean ± SD	Mean change	Change, %
CML, ng/mL	Intervention	40	312.4 ± 42.1	238.1 ± 35.6	−74.3	−23.8
CML, ng/mL	Control	38	308.9 ± 39.8	302.5 ± 41.2	−6.4	−2.1
hs-CRP, mg/L	Intervention	40	3.48 ± 0.88	2.37 ± 0.64	−1.11	−31.9
hs-CRP, mg/L	Control	38	3.36 ± 0.82	3.29 ± 0.79	−0.07	−2.1
IL-6, pg/mL	Intervention	40	4.91 ± 1.15	3.56 ± 0.89	−1.35	−27.5
IL-6, pg/mL	Control	38	4.79 ± 1.10	4.71 ± 1.05	−0.08	−1.7

CML, carboxymethyl-lysine; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; SD, standard deviation.

Table 3. Between-group differences in biomarker changes and week-16 concentrations

Biomarker	Difference in mean changes, intervention − control	Reported time × group p-value	Week-16 mean difference, intervention − control	Week-16 difference, 95% CI	Week-16 Welch p-value
CML, ng/mL	−67.9	<0.001	−64.4	−81.8 to −47.0	<0.001
hs-CRP, mg/L	−1.04	<0.001	−0.92	−1.25 to −0.59	<0.001
IL-6, pg/mL	−1.27	<0.001	−1.15	−1.59 to −0.71	<0.001

CI, confidence interval; CML, carboxymethyl-lysine; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6. Time × group p-values correspond to the reported repeated-measures analysis of variance. Week-16 confidence intervals and p-values use independent-samples Welch t-tests without covariate adjustment.

Table 4. Reported Pearson correlations between changes in biomarkers and MEDAS score

Variable pair	Pearson r
CML and hs-CRP	0.620
CML and IL-6	0.584
CML and MEDAS score	−0.712
hs-CRP and IL-6	0.695
hs-CRP and MEDAS score	−0.548
IL-6 and MEDAS score	−0.512

CML, carboxymethyl-lysine; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; MEDAS, Mediterranean Diet Adherence Screener. Associations were assessed using Pearson correlation.

The intervention–control differences in mean changes were –67.9 ng/mL for CML, –1.04 mg/L for hs-CRP, and –1.27 pg/mL for IL-6; the reported time × group interaction p-values were <0.001 for all three outcomes (Table 3). In supplementary unadjusted comparisons of week-16 concentrations, the between-group mean differences were –64.4 ng/mL for CML (95% CI, –81.8 to –47.0), –0.92 mg/L for hs-CRP (95% CI, –1.25 to –0.59), and –1.15 pg/mL for IL-6 (95% CI, –1.59 to –0.71).

Changes in CML were positively correlated with changes in hs-CRP (r = 0.620) and IL-6 (r = 0.584), while changes in hs-CRP and IL-6 were also positively correlated (r = 0.695). Changes in MEDAS score were negatively correlated with changes in CML (r = –0.712), hs-CRP (r = –0.548), and IL-6 (r = –0.512) (Table 4).

DISCUSSION

The primary findings of this investigation demonstrated that a 16-week high-polyphenol Mediterranean diet intervention significantly attenuated circulating advanced glycation end-product concentrations, specifically serum carboxymethyl-lysine, alongside systemic inflammatory biomarkers including high-sensitivity C-reactive protein and interleukin-6 in an overweight adult cohort from Urban Core Punjab (9). Crucially, the magnitude of these favorable metabolic and anti-inflammatory adaptations was strongly modulated by candidate genomic variations within the AGER (rs2070600) and SOD2 (rs4880) loci. Participants carrying the wild-type GG genotype at AGER rs2070600 achieved pronounced reductions in serum carboxymethyl-lysine compared to those harboring the homozygous mutant AA allele. Similarly, SOD2 rs4880 CC homozygotes exhibited superior attenuation of systemic inflammatory markers relative to T allele carriers, confirming that background genetic architecture dictates individual physiological responsiveness to targeted bioactive polyphenolic interventions (10).

These results align with and expand upon existing nutrigenetic literature establishing that dietary phytochemicals suppress endogenously formed glycation products through multiple metabolic mechanisms. Previous dietary trials reported marked declines in serum advanced glycation end-products following high-polyphenol supplementation, attributing these effects to the direct chemical scavenging of reactive dicarbonyl intermediates like methylglyoxal and the downregulation of intracellular reactive oxygen species (11). The present study reinforces these observations while providing critical nuance regarding inter-individual heterogeneity. The blunted response observed among AGER rs2070600 AA homozygotes suggests that sequence variations altering soluble receptor for advanced glycation end-products (sRAGE) expression or receptor binding affinity may impair the physiological clearance of circulating dicarbonyl species, thereby diminishing the protective capacity of dietary polyphenols. Likewise, the differential response observed across SOD2 rs4880 genotypes highlights the requirement for optimal endogenous enzymatic antioxidant machinery to fully synergize with exogenous polyphenolic antioxidants in mitigating systemic inflammatory tone (12).

The clinical and public health implications of these gene-diet interactions are particularly relevant for urbanizing populations experiencing rapid metabolic transitions. Elevated advanced glycation end-product accumulation, driven by modern thermally processed diets combined with sedentary lifestyle habits, accelerates vascular stiffening, endothelial dysfunction, and subclinical cardiometabolic disease (13). Demonstrating that targeted dietary modifications can significantly suppress serum carboxymethyl-lysine and systemic inflammation validates high-polyphenol Mediterranean dietary patterns as a robust non-pharmacological strategy for cardiometabolic risk reduction. Furthermore, establishing that specific single-nucleotide polymorphisms modulate intervention efficacy underscores the inadequacy of one-size-fits-all nutritional recommendations, providing empirical support for the integration of nutrigenetic profiling into personalized preventative medicine.

Several key methodological strengths enhanced the validity of these empirical findings. The randomized, controlled parallel-group design minimized confounding variables, while the mandatory provision of high-polyphenol extra virgin olive oil and structured dietary counseling ensured high protocol adherence among intervention participants. The geographical focusing on Urban Core Punjab targeted a demographic disproportionately burdened by cardiometabolic disease and high dietary exposure to thermal glycation products (14). Additionally, evaluating specific serum biomarker panels alongside candidate genotyped loci permitted a rigorous, mechanistically grounded assessment of gene-nutrient interactions rather than relying solely on subjective self-reported dietary metrics.

Despite these strengths, certain limitations warrant consideration when interpreting the outcomes. The study duration of 16 weeks, while sufficient to detect significant biomarker shifts, was insufficient to evaluate long-term clinical endpoints such as incident cardiovascular events or structural vascular remodelling. The sample size, although adequately powered based on prior pilot nutrigenetic trials, yielded relatively small sub-cohorts when stratified across specific homozygous mutant genotypes, potentially limiting the statistical sensitivity to detect smaller interaction effects. Furthermore, dietary advanced glycation end-product intake was monitored through recall questionnaires rather than direct chemical quantification of duplicate meal portions, introducing potential recall bias regarding baseline exogenous glycation exposure (15).

Future research should focus on multi-center prospective cohorts with extended follow-up periods to determine whether the observed biomarker reductions translate into sustained clinical risk reduction over time (16).

Expanding nutrigenetic panels beyond candidate single-nucleotide polymorphisms to incorporate genome-wide polygenic risk scores and comprehensive transcriptomic profiling will provide a more holistic understanding of gene-diet crosstalk. Additionally, investigating the mediating role of the gut microbiome in metabolizing parent polyphenols into active circulating metabolites will further clarify the complex biochemical pathways governing individual responsiveness to therapeutic nutritional interventions.

CONCLUSION

This 16-week intervention demonstrates that a high-polyphenol Mediterranean diet significantly reduces circulating advanced glycation end-products and systemic inflammation in adults from Urban Core Punjab. Crucially, the therapeutic magnitude of these anti-glycation and anti-inflammatory benefits is heavily governed by single-nucleotide variations within the AGER (rs2070600) and SOD2 (rs4880) loci. By establishing that background genetic architecture modulates individual metabolic responsiveness to dietary bioactive compounds, these empirical findings highlight the limitations of broad nutritional guidelines and provide a mechanistic foundation for integrating nutrigenetic profiling into personalized precision strategies for cardiometabolic disease prevention.

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