Cardiovascular Diabetology

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Modulation of circulating levels of advanced glycation end products and its impact on intima-media thickness of both common carotid arteries: CORDIOPREV randomised controlled trial

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Abstract

Background Increasing evidence supports the role of advanced glycation end products (AGEs) in atherosclerosis in both diabetic and non-diabetic patients, suggesting that therapeutic strategies targeting AGEs may offer potential benefits in this population. The Mediterranean diet is associated with improved biomarkers and anthropometric measurements related with atherosclerosis in addition to its ability to modulate AGE metabolism. Our aim was to determine whether the reduction in atherosclerosis progression (measured by changes in intima-media thickness of both common carotid arteries (IMT-CC)), observed after consumption of a Mediterranean diet compared to a low-fat diet, is associated with a modulation of circulating AGE levels in patients with coronary heart disease (CHD).

Methods 1002 CHD patients were divided in: (1) Non-increased IMT-CC patients, whose IMT-CC was reduced or not changed after dietary intervention and (2) Increased IMT-CC patients, whose IMT-CC was increased after dietary intervention. Serum AGE levels (methylglyoxal–MG and Nε-Carboxymethyllysine-CML) and parameters related to AGE metabolism (*AGER1* and *Glox*I *m*RNA and sRAGE levels) and reduced glutathione (GSH) levels were measured before and after 5-years of dietary intervention.

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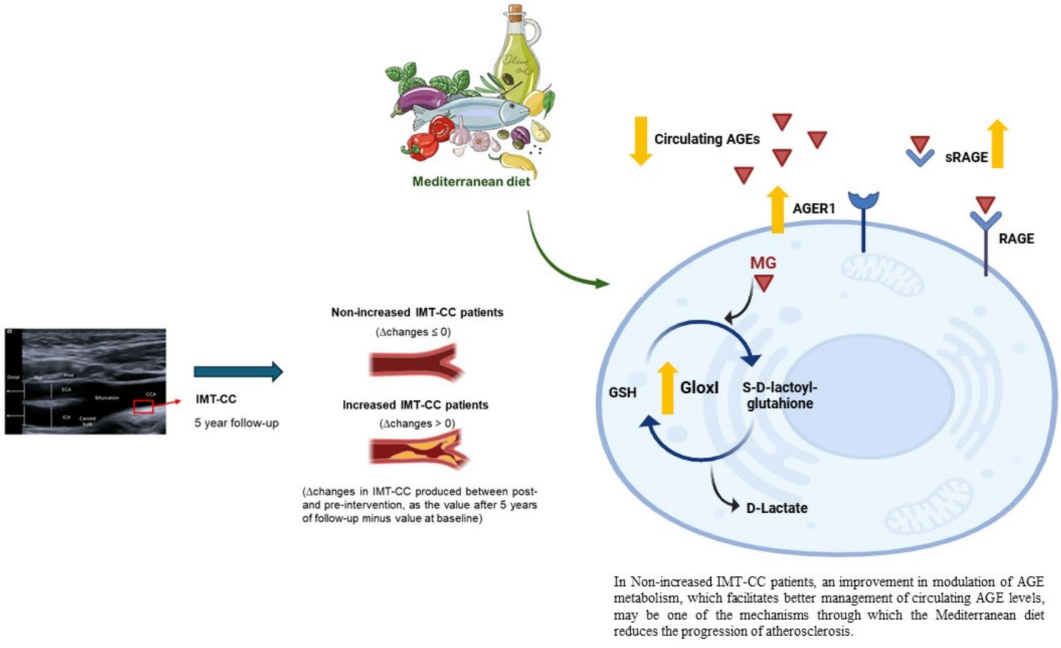
Results The Mediterranean diet did not affect MG levels, whereas the low-fat diet significantly increased them compared to baseline ($p = 0.029$), leading to lower MG levels following the Mediterranean diet than the low-fat diet ($p < 0.001$). The Mediterranean diet, but not the low-fat diet, produced an upregulation of AGE metabolism, with increased *AGER1* and *GloxI* gene expression as well as increased GSH and sRAGE levels in Non-increased IMT-CC patients (all $p < 0.05$). Although the Mediterranean diet increased MG levels in Increased IMT-CC patients, this increment was lower compared to the low-fat diet (all $p < 0.05$).

Conclusions Our results suggest that an improvement in modulation of AGE metabolism, which facilitates better management of circulating AGE levels, may be one of the mechanisms through which the Mediterranean diet, compared to a low-fat diet, reduces the progression of atherosclerosis in patients with CHD.

Trial registration **https://clinicaltrials.gov/ct2/show/NCT00924937, Clinicaltrials.gov number, NCT00924937.**

Graphical abstract



Keywords Advanced glycation end products, Atherosclerosis, Mediterranean diet, Dietary intervention

Background

Cardiovascular disease (CVD) is the leading cause of death and disability worldwide [1]. Among its manifestations, coronary heart disease (CHD) is the most widespread, imposing a significant economic burden on healthcare systems and society [2, 3]. Indeed, patients with CHD have a significantly higher risk of experiencing recurrent cardiovascular events compared to those without the disease, underscoring the need for effective strategies for the prevention and management of CHD [4, 5].

Advanced glycation end products (AGEs) are irreversible compounds formed through non-enzymatic interactions between reducing sugars and proteins, lipids, or nucleic acids. They can be generated endogenously under both physiological and pathological conditions or provided by exogenous sources, primary through diet. The formation of AGEs in food occurs spontaneously and is influenced by nutrient composition and processing methods [6, 7]. Increased intake of dietary AGEs is associated with elevated circulating levels in plasma/serum or urine [8]. This AGEs accumulation, or dysregulation of AGE metabolism, is linked to oxidative stress, elevated levels of proinflammatory cytokines, endothelial cell damage and cell adhesion molecules, all of which play key roles in the thrombotic cascade after plaque rupture and myocardial infarction [9–12]. In fact, recent findings show a significant association between accumulation of AGEs, measured by skin autofluorescence (SAF), and intima-media

thickness of the common carotid arteries (IMT-CC), a surrogate marker of subclinical atherosclerosis and predictor of future cardiovascular events [13–16]. Circulating AGE levels also correlated with the incidence and severity of CHD, irrespective of diabetic status [17]. This aligns with our previous research, in which we found elevated circulating AGE levels in patients with CHD and type 2 diabetes mellitus (T2DM) who also exhibited increased IMT-CC [18]. While SAF offers a non-invasive method for estimating skin AGEs, it may underestimate the total AGE burden by missing non-fluorescent AGEs like Nε-Carboxymethyllysine (CML), a key marker of metabolic processes and oxidative stress [19]. In contrast, circulating AGEs provide a more dynamic and accurate reflection of metabolic changes, making this approach more suitable for dietary intervention studies [20]. In either case, these findings underscore the clinical necessity of monitoring and managing AGEs, especially in the context of secondary prevention of CVD, offering a promising avenue for enhancing clinical outcomes.

Restricting AGEs through dietary modifications has been reported to reduce the availability of precursors for glycation reactions and subsequent AGE formation [19, 21]. Indeed, our previous research emphasizes the Mediterranean diet as a particularly effective approach, not only due to its low dietary AGE content compared to other dietary models but also its ability to reduce circulating AGEs, such as methylglyoxal (MG) and CML [22–24], by AGE metabolism modulation. Additionally, we have also shown that the Mediterranean diet, compared to a low-fat diet, slows atherosclerosis progression, as evidenced by a reduction in IMT-CC in patients with CHD [25].

Based on the above findings, we now investigated whether the reduction in atherosclerosis progression, observed after consumption of a Mediterranean diet compared to a low-fat diet, is associated with a modulation of circulating AGE levels in patients with CHD. This study intends to provide clinical insights into the efficacy of dietary interventions for mitigating atherosclerosis by targeting AGE-related mechanisms.

Methods

Design and study population

The current work was conducted within the framework of the CORDIOPREV study (clinicaltrials.gov number NCT00924937). Briefly, the CORDIOPREV study is a single center, prospective, randomized, single-blind and controlled dietary intervention clinical trial, based on an intention-to-treat analysis, including 1002 patients with CHD who had their last coronary event more than 6 months before enrollment. The primary objective of the CORDIOPREV study was to evaluate the efficacy of a Mediterranean diet, rich in MUFAs from olive oil, as compared with a low-fat diet to prevent clinical events and mortality in patients with previous CHD in a long-term follow-up study [26]. The present study aims to evaluate a secondary endpoint of the CORDIOPREV study, the effect of the two dietary patterns on modulation of AGE metabolism and its influence of atherosclerosis (as measured by IMT-CC).

Patients were recruited from November 2009 to February 2012, mostly at Reina Sofia University Hospital, Córdoba, Spain, but also from other hospitals in the neighboring provinces of Córdoba and Jaen. Details of the rationale, study methods, inclusion and exclusion criteria, cardiovascular risk factors and baseline characteristics of the patients have been recently described [27]. To summarize, eligible patients included men and women aged 20–75 years who had established CHD, were able to follow a long-term dietary intervention, and had no severe illnesses or an expected life expectancy lower than the length of the study. The upper age limit was set on the basis of the life expectancy at the conception of the trial (2007) and according to the usual practice in contemporary long-term cardiovascular studies. The protocol was written in accordance with the principles of the Declaration of Helsinki. The respective Institutional Review Board by the Human Investigation Review Committee of the Reina Sofa University Hospital (Córdoba, Spain) approved the study protocol (No. 1496/27/03/2009). All patients provided written informed consent.

Randomization and dietary intervention

Randomization was performed by the Andalusian School of Public Health, as previously described [27]. The study dietitians were the only members of the intervention team to know about each participant's dietary group. Briefly, the randomization was based on the following variables: sex (male, female), age (<60 and ≥60 years old) and previous myocardial infarction (yes, no). Each patient was randomly stratified, in addition to the conventional treatment for CHD, to one of two potentially healthy diets: (a) a Mediterranean diet, with a minimum of 35% of total calories from fat [22% MUFA, 6% polyunsaturated fatty acids (PUFA), and <10% SFA], 15% proteins, and a maximum of 50% carbohydrates and (b) a low-fat, high complex carbohydrate diet, as recommended by the National Cholesterol Education Program, with <30% of total calories from fat (12–14% MUFAs, 6–8% PUFAs, <10% SFAs), ≥ 55% from carbohydrates and 15% from protein. In both diets, the cholesterol content was adjusted to <300 mg/day. Both study diets included foods from all major food groups, but no total calorie restriction was set. Full details on dietary assessment, adherence and recommendations, as well as follow-up visits, have been published elsewhere [27–29]. No intervention to increase physical activity or lose weight was included.

Participants in both intervention groups received the same intensive dietary counselling. The present study was conducted over a follow-up period of 5 years. Details of the specific recommended diets, mean baseline values and changes in energy and nutrient intake after 5 years of intervention with both dietary patterns have been previously described [28].

Moreover, although both dietary models share common characteristics in some of the major components (i.e., high intake of vegetables, fruit, legumes, and whole grains), patients consuming the Mediterranean diet also had a high intake of oily fish, nuts, and extra virgin olive oil, together with a low intake of harmful foods such as red/processed meats and pastries/commercial bakery products. The 14-item The Mediterranean diet Adherence Screener (MEDAS) was used to measure adherence to the Mediterranean diet and a 9-item dietary screener was used to evaluate adherence to the low-fat diet. Details of dietary adherence assessment have been also published previously [28].

Laboratory tests

At 8.00 am, following a 12-h fast, the patients were admitted to the laboratory for anthropometric and biochemical tests [weight, body mass index (BMI), waist circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), HDL-cholesterol, LDL-cholesterol, triglycerides, total cholesterol, high sensitive C-reactive protein (hsCRP), fasting glucose and insulin and hemoglobin A1c (HbA1c) as previously described [22].

Carotid ultrasonography

The carotid study was ultrasonically assessed bilaterally by quantification of IMT-CC as well as carotid plaque number and height, at the beginning of the study and after 5 years of dietary intervention. Briefly, carotid arteries were examined using a Doppler ultrasound high-resolution B-mode (Envisor C Ultrasound System, Philips, Eindhoven, The Netherlands), following the recommendations of the American Society of Echocardiography Carotid Intima-Media Thickness Task Force [30]. All images were analyzed off-line using dedicated analysis tools (QLAB Advance Ultrasound Quantification Software, v5.0, Phillips, USA). Analysis was performed by technicians blinded to clinical information and previous imaging. A full description of the methodology has been recently described [25].

Out of the 1002 patients, a total of 939 completed the carotid ultrasound study at baseline ($n=459$, low-fat diet and $n=480$, Mediterranean diet; 63 patients did not complete the ultrasonography study). Of these patients, 809 completed the 5-year follow-up carotid ultrasound study ($n=377$, low-fat diet and $n=432$, Mediterranean diet; 130 patients did not complete the ultrasonography study). To sum up, data were missing for 193 patients, mainly because they did not complete the ultrasonography study (at baseline or during follow-up) due to problems related to disapproval of the technique, refusal to participate, death, or withdrawal for other reasons (Fig. 1). Baseline characteristics of those patients with complete carotid ultrasound study (during follow-up) did not differ with patients who did not complete it [25].

For the objective of this study, patients were divided into two groups depending on the change in IMT-CC produced by the dietary intervention (Δchanges in IMT-CC produced between post- and pre-intervention, as the value after five years of follow-up minus value at baseline) (Fig. 1):

1. Non-increased IMT-CC patients, ($n=408$ with 166 in the low-fat diet and 242 in the Mediterranean diet), whose IMT-CC was reduced or not changed after dietary intervention ($\Delta changes \leq 0$).
2. Increased IMT-CC patients ($n=401$ with 211 in the low-fat diet and 190 in the Mediterranean diet), whose IMT-CC was increased after dietary intervention ($\Delta changes > 0$).

Dietary AGEs intake

The assessment of dietary AGE (dAGE) content was performed using 3-day weighed food diaries completed by the participants at baseline and after every year during the dietary intervention study until 5 years of follow-up has elapsed, with a strong emphasis on cooking methods in both diets. dAGE content was estimated from a database of approximately 560 foods listing their AGE values and was expressed as AGE kilounits $g-1$ food [22].

Determination of circulating MG and CML levels

Blood samples were taken from the participants at baseline and after 5 years of follow-up of dietary intervention. Blood was separated into serum and plasma through centrifugation within an hour of collection (1500 x g for 20 min at 20 °C and 1500 x g for 15 min at 4 °C, respectively).

Circulating MG and CML levels were measured in the serum using competitive ELISA kits (OxiSelect Methylglyoxal Competitive ELISA Kit and OxiSelect N-epsilon-(Carboxymethyl) Lysine Competitive ELISA Kit, Cell Biolabs, Inc., San Diego, CA, USA), following the manufacturer's instructions [31]. These well-validated competitive ELISAs are an enzyme immunoassay developed for rapid detection and quantitation of MG-H1 (methylglyoxal-hydro-imidazolone) and CML protein adducts, respectively [32, 33]. The values indicate the accumulation of AGEs in the body and are reflective of stable protein- or peptide-associated CML and MG rather than the

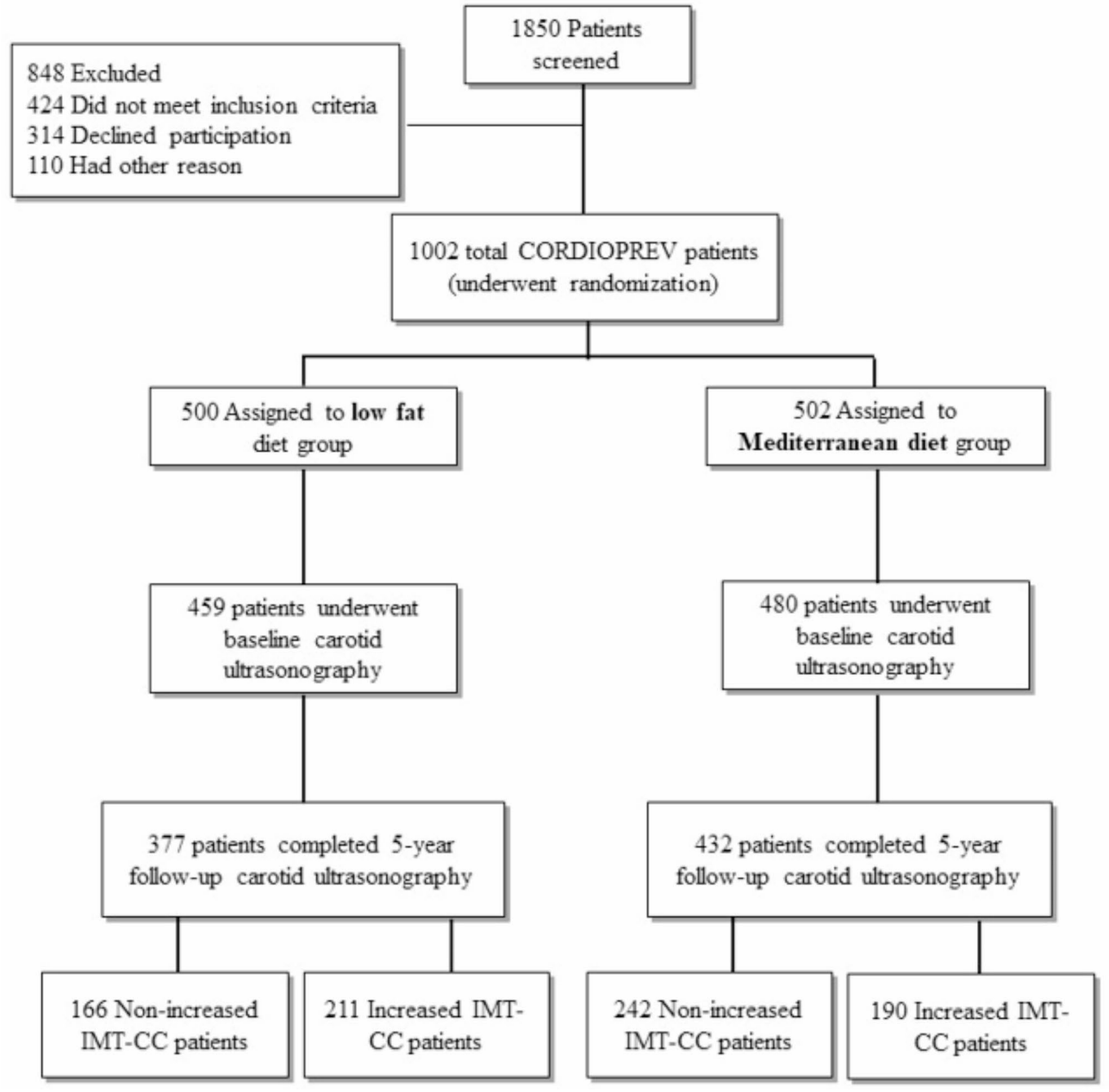


Fig. 1 Screening and randomization flow-chart of the study

free compounds. The inter-assay coefficients of variation for CML and MG were 4.3% and 5.8%, respectively, while the intra-assay coefficients of variation were 5.6% and 5.1% for CML and MG, respectively.

Quantification of the gene expression related to AGEs metabolism by real-time PCR

In response to high AGEs levels, the host defense system employs different mechanisms to restrict their toxicity. Glyoxalase system, particularly GloxI, is an important regulator of the control of intracellular AGEs, mainly, MG [34]. AGER1 binds extracellular AGEs and delivers them to the lysosomes where they are detoxified, thus reducing AGE-related oxidative stress and inflammation [35].

Peripheral blood mononuclear cells (PBMCs) were isolated from blood, at baseline and after 5 years of follow-up of dietary intervention [36]. RNA from the PBMCs was extracted using RNeasy® Kit by Qiagen and digested with DNAse I (AMPD-1 KT, Sigma) before being quantified using a Nanodrop ND-1000 v3.5.2 spectrophotometer (Nanodrop Technology®, Cambridge, UK). RNA quality was checked by agarose gel electrophoresis and stored at −80ºC. The first strand cDNA was synthesized

using the RevertAid First Strand CDNA Synthesis kit (Thermo scientific) from 1 µg of total RNA following manufacturer's instructions. Real-time PCR reactions were performed on the Bio-Rad PCR platform following the manufacturer's instructions. cDNA was diluted 1:5 (v/v) and 5 µl of the dilution was used for each reaction. Primer pairs for the genes were selected from the Bio-Rad primePCR database (Bio-Rad Laboratories; https://www.bio-rad.com/en-in/product/primepcr-pcr-primers-assays-arrays?%20ID=M0HROA15) and included receptor for AGEs (AGE receptor-1 (AGER1; DDOST, qHsaCED0019839) and glyoxalase I (GloxI; GLO, qHsaCID0011227). The expression for each analysed gene was determined relative to two housekeeping genes: glyceraldehyde-3-phosphate dehydrogenase (GAPDH; qHsaCID0015464) and β-actin (ACTB; qHsaCED0036269). The Bio-Rad CFX Maestro Software (Bio-Rad Laboratories) was used to analyze the dataset.

Determination of circulating parameters related to AGE metabolism

sRAGE, soluble form of receptor for AGEs (RAGE), acts as a protective anti-inflammatory agent by serving as a decoy receptor, binding RAGE ligands (as AGEs), and preventing their interaction with membrane-bound RAGE. sRAGE levels were measured in plasma samples, at baseline and after 5 years of follow-up of dietary intervention, using an ELISA commercial kit (R&D Systems, Minneapolis, MN), following the manufacturer's protocol [37]. Monoclonal antibodies against the NH2 terminus of the extracellular domain of RAGE are used.

Reduced glutathione (GSH) levels were measured in plasma samples, at baseline and after 5 years of follow-up of dietary intervention, using BIOXYTECH® GSH-400 kit (OxisResearch®, Portland, USA) [38].

Statistical analysis

The statistical analyses were carried out using IBM SPSS version 25.0 for Windows (IBM Corp, Armonk, NY, USA). The Kolmogorov–Smirnov normality test was performed to evaluate the distribution of the quantitative variables, and continuous variables that deviated significantly from the assumption of normality were transformed. Continuous variables were presented as mean ± standard error of the mean (SE), while categorical variables were presented as proportions. Categorical variables were compared using Chi-Square tests. Within- and between-group changes were assessed with a paired *t* test and unpaired *t* test, respectively. To evaluate the data variation according to diet, group and time (baseline to 5 years), repeated-measures ANOVA analyses were used, as well as post hoc multiple comparisons analysis using the Bonferroni correction, adjusted for potential cofounders or potential effect modifiers (age, sex, BMI, hypertension, energy, smoking habits (non-, past- and current smokers), pharmacological treatments–lipid-lowering therapy, use of antidiabetic drugs and anti-hypertensive drugs). Δchanges were calculated as changes produced between post- and pre-intervention (the value after five years of dietary intervention minus value at baseline).

Multiple logistic analysis was carried out to estimate the independent contribution of the modulation of AGE metabolism by the diet (changes in serum levels of MG and sRAGE, and gene expression of *GloxI* and *AGER1* after dietary intervention) as well as other potential variables as baseline IMT-CC, age and sex, fasting glucose, HbA1c, HDL-cholesterol, triglycerides and hsCRP levels, smoking habits, and medications–lipid-lowering therapy, use of antidiabetic drugs and anti-hypertensive drugs) to changes in IMT-CC (Non-increased/Increased IMT-CC patients). The differences were considered significant when $p<0.05$.

Results

Baseline characteristics of the study population

Baseline clinical and metabolic characteristics, lipid profiles and treatment regimens of patients who completed baseline and the follow-up carotid ultrasound study ($n=809$) are presented in Table 1. No differences were found between Non-increased and Increased IMT-CC patients in terms of baseline clinical and metabolic characteristics.

Baseline IMT-CC values and other parameters related to carotid ultrasonography study are showed in Fig. 2A and Supplementary Fig. 1, respectively. Non-increased IMT-CC patients had higher baseline IMT-CC compared to Increased IMT-CC patients (0.811 ± 0.086 mm vs. 0.689 ± 0.066 mm, respectively, $p<0.001$) (Fig. 2A). No significant differences were observed in baseline carotid plaque height and number between Non-increased and Increased IMT-CC patients (Supplementary Fig. 1).

Dietary intervention modulates parameters related to carotid ultrasonography

Increased IMT-CC patients showed an increase in IMT-CC (0.076 ± 0.006 mm, $p<0.001$) and in carotid plaque number, but not in carotid plaque height, after dietary intervention compared to baseline (Δchange 0.246 ± 0.011 n, $p=0.033$). Non-increased IMT-CC patients showed a decrease in IMT-CC (-0.091 ± 0.006 mm, $p<0.001$) but without differences in carotid plaque height and number after dietary intervention compared to baseline (Fig. 2A and Supplementary Fig. 1, respectively).

Considering the effect of each dietary model (shown as Δchanges produced between post- and pre-intervention), the Mediterranean diet produced a higher decrease in IMT-CC compared to the low-fat diet in

Table 1 Baseline clinical and metabolic characteristics, lipid profiles and treatment regimens of the CHD patients who completed baseline and the follow-up carotid ultrasound study

	Total population (*n* = 809)	Non-increased IMT-CC patients* (*n* = 408)	Increased IMT-CC patients* (*n* = 401)	*p* Value
Age (years)	59.5 ± 0.3	59.8 ± 0.4	58.9 ± 0.5	0.072
Men/Women (n)	672/137	365/67	307/70	0.260
Weight (kg)	85.1 ± 0.5	84.8 ± 0.7	85.4 ± 1.0	0.889
BMI (kg/m^2)†	31.1 ± 0.1	31.0 ± 0.2	31.1 ± 0.3	0.606
Waist circumference (mm)	105.1 ± 0.4	105.2 ± 0.5	104.4 ± 0.6	0.274
Diastolic blood pressure (mmHg)	77.3 ± 0.3	77.5 ± 0.5	77.4 ± 0.6	0.876
Systolic blood pressure (mmHg)	138.7 ± 0.6	140.0 ± 1.0	138.2 ± 0.9	0.115
Hypertension (%)§	67.5	64.2	70.4	0.076
LDL-cholesterol (mg/dL)	88.6 ± 0.8	89.4 ± 1.2	87.2 ± 1.3	0.207
HDL-cholesterol (mg/dL)	42.2 ± 0.2	42.1 ± 0.4	42.5 ± 0.6	0.612
Total cholesterol (mg/dL)	155.5 ± 2.0	159.5 ± 1.4	156.4 ± 1.6	0.144
Triglycerides (mg/dL)	132.5 ± 2.1	132.2 ± 3.1	129.5 ± 3.3	0.548
hsCRP (mg/mL)	2.45 ± 0.01	2.35 ± 0.09	2.29 ± 0.10	0.700
Fasting glucose (mg/dL)	113.8 ± 1.2	114.2 ± 1.8	110.8 ± 1.8	0.188
Fasting insulin (mU/L)	11.1 ± 0.7	11.3 ± 0.6	10.1 ± 0.6	0.141
HbA1c (%)	6.65 ± 0.04	6.59 ± 0.05	6.62 ± 0.06	0.757
T2DM, %‡	52.8	51.2	54.2	0.436
Carotid plaque presence (%)	79.3	77.3	81.8	0.102
Smoking (%)				
Non-smokers	27.7	13.2	14.5	0.432
Past-smokers	63.5	33.1	30.4	0.105
Current smokers	8.8	4.1	4.7	0.287
Use of antihypertensive drugs (%)				
ACEIs or ARB	82.1	83.5	81.4	0.162
Calcium channel blockers	17.4	17.8	17.3	0.109
Beta-blockers	81.7	82.3	78.2	0.430
Nitrates	10.0	9.8	10.1	0.845
Diuretics	40.7	40.5	38.5	0.309
Use of lipid lowering drugs (%)				
Statins	85.7	86.1	84.0	0.723
Fibrates	3.6	3.6	3.7	0.901
Use of oral anti-diabetics, %	34.6	35.5	33.8	0.605

Values are represented as the mean ± standard error or percentage of participants, unless otherwise stated. We used unpaired *t* tests for quantitative variables and Chi-squared tests for categorical variables ($p < 0.05$, Non-increased IMT-CC vs. Increased IMT-CC patients)

*Non-increased IMT-CC patients, whose intima-media thickness of both common carotid arteries (IMT-CC) was reduced or not changed after dietary intervention and Increased IMT-CC patients, whose IMT-CC was increased after dietary intervention

†Body mass index (BMI) was calculated as weight in kg divided by the square of height in m (kg/m^2)

§Hypertension was defined as a systolic blood pressure ≥ 140 mm Hg, a diastolic blood pressure ≥ 90 mm Hg, or the use of antihypertensive therapy

‡Diabetes was defined as being diagnosed as diabetic before the start of the study and those diagnosed by a fasting blood glucose level ≥ 126 mg/dL on two occasions, or a 2-h plasma glucose level ≥ 200 mg/dL during a 75-g oral glucose tolerance test, during the first procedures of the study

‡Serum creatinine (sCr)-based estimated glomerular filtration rate (eGFR) was evaluated using the CKD-Epi (CKD Epidemiology Collaboration) equation

CHD, coronary heart disease; *T2DM*, type 2 diabetes mellitus; *LDL*, low-density lipoprotein; *HDL*, high-density lipoprotein; *hsCRP*, high sensitive C-reactive protein; *HbA1c*, glycated haemoglobin; *ACEIs*, angiotensin converting enzyme inhibitors; *ARB*, angiotensin-receptor blockers

Non-increased IMT-CC patients (-0.101 ± 0.011 mm vs. -0.056 ± 0.002 mm, respectively, $p = 0.009$). Both diets determined an increase in IMT-CC without differences between them, in Increased IMT-CC patients (Fig. 2B). However, we found a higher percentage of Non-increased IMT-CC patients (56.02% vs. 44.03%) and a lower percentage of Increased IMT-CC patients (43.98% vs. 55.97%) in the Mediterranean diet group compared to the low-fat diet group ($p < 0.001$) (Supplementary Table 1).

Regarding to other parameters related to carotid ultrasonography study, the Mediterranean diet produced lower carotid plaque height (-0.012 ± 0.094 mm vs. 0.226 ± 0.106 mm, $p = 0.031$) and number (0.055 ± 0.102 n vs. 0.312 ± 0.115 n, $p = 0.026$) compared to the low-fat diet in Non-increased IMT-CC patients. No differences were found in carotid plaque height and number in Increased IMT-CC patients (Supplementary Table 2).

Dietary intervention modulates circulating AGE levels and AGE metabolism

As we previously reported, both diets, the Mediterranean and the low-fat diet, equally provided lower amount of dAGEs after dietary intervention compared to baseline (all $p < 0.05$) [22].

Baseline levels and changes after dietary intervention (Δchanges produced between post and pre-intervention) on circulating levels of AGEs and parameters related

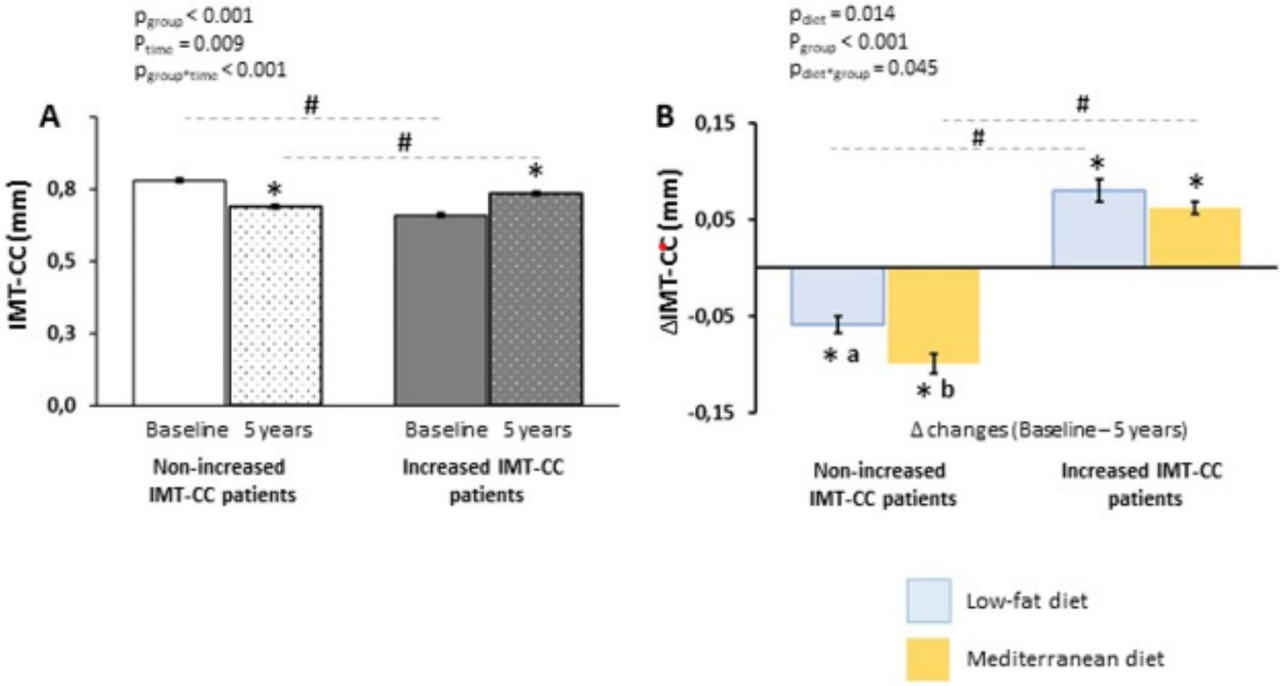


Fig. 2 Effect of dietary intervention on IMT-CC in CHD patients classified according to IMT-CC progression. (A) IMT-CC at baseline and after 5-year of dietary intervention; (B) Changes produced between post- and preintervention in IMT-CC after each dietary model. Values are represented as the mean ± standard error. Variables were compared using the analysis of variance (univariate ANOVA) adjusted by age, sex, BMI, hypertension, energy, smoking habits (non-, past- and current smokers), and pharmacological treatments–lipid-lowering therapy, use of antidiabetic drugs and anti-hypertensive drugs. Differences were significant when $p < 0.05$. *Significant differences between post- and preintervention. #Significant differences between Non-increased IMT-CC and Increased IMT-CC patients. Different common letter superscripts denote significant differences. Non-increased IMT-CC patients, whose IMT-CC was reduced or not changed after dietary intervention ($n = 408$, 166 in the low-fat diet and 242 in the Mediterranean diet). Increased IMT-CC patients, whose IMT-CC was increased after dietary intervention ($n = 401$, 211 in the low-fat diet and 190 in the Mediterranean diet). CHD, Coronary heart disease; IMT-CC, intima-media thickness of both common carotid arteries

to AGE metabolism according to randomized dietary groups are shown in Table 2. We did not observe differences in baseline circulating AGE levels (MG and CML) or of *AGER1* and *GloxI* gene expression and sRAGE levels comparing both randomized dietary groups. After dietary intervention, the Mediterranean diet did not alter MG levels, whereas the low-fat diet increased them compared to baseline (Δchange 0.44±0.04 μg/mL, $p = 0.029$), resulting that the consumption of the Mediterranean diet determined lower MG levels than the low-fat diet ($p < 0.001$). Regarding to parameters related to AGE metabolism, the Mediterranean diet produced higher levels of sRAGE (187.3±22 pg/mL vs. -5,44±12 pg/mL) and a higher expression of *AGER1* (0.22±0.04 AU vs. -0.19±0.09 AU) and *GloxI* (0.08±0.07 AU vs. -0.10±0.07 AU) compared to the low-fat diet (all $p < 0.05$) (Table 2).

According to the studied group, Increased IMT-CC patients showed an increment in circulating MG levels and a reduction in *GloxI* gene expression compared to baseline (Δchange 0.53±0.03 μg/mL, $p = 0.033$ and Δchange −0.14±0.01 AU, $p = 0.013$, respectively) (Fig. 3A and C). Non-increased IMT-CC patients showed no changes in MG levels (Fig. 3A) but exhibited an increase in *AGER1* gene expression compared to baseline (Δchange 0.04±0.01 AU, $p = 0.009$) (Fig. 4A). Both groups of patients increased sRAGE levels compared to baseline (Δchange 71.23±3.51 pg/mL, $p = 0.022$ and Δchange 101.7±6.31 pg/mL, $p = 0.031$, respectively) (Fig. 4C).

Considering the effect of each dietary model (shown as Δchanges produced between post- and pre-intervention), the Mediterranean diet did not modify circulating MG levels but produced higher *GloxI* (0.14±0.06 AU vs. -0,04±0.01 AU) and *AGER1* gene expression (0.64±0.11 AU vs. -0,43±0.09 AU) as well as sRAGE levels (209.1±32 pg/mL vs. -9.28±0.81 pg/mL) compared to the low-fat diet in Non-increased IMT-CC patients (all $p < 0.05$) (Figs. 3D and 4B and D, respectively). However, in these patients, the low-fat diet increased the levels of MG, compared to baseline (Δchange 0.38±0.03 μg/mL $p = 0.012$). In Increased IMT-CC patients, although both diets increased circulating MG levels, this increment was lower after the Mediterranean diet compared to the low-fat diet (0.18 μg/mL vs. 0.71 μg/mL, all $p < 0.05$) (Fig. 3B). The low-fat diet reduced *GloxI* gene expression compared to baseline (-0.10±0.03 AU, $p = 0.011$) (Fig. 3D)

Table 2 Baseline and changes in AGE metabolism related parameters after dietary intervention

	Low-fat diet group (*n* = 377)	Mediterranean diet group (*n* = 432)	*p* Value*
Baseline serum MG levels, µg/mL	3.05 ± 0.08	3.18 ± 0.07	0.380
ΔMG, µg/mL	0.44 ± 0.04#	-0.03 ± 0.05	< 0.001
Baseline serum CML levels, ng/mL	0.65 ± 0.02	0.63 ± 0.02	0.847
ΔsCML, ng/mL	0.01 ± 0.08	-0.05 ± 0.06	0.741
Baseline s*RAGE* levels, pg/mL	1369 ± 31	1291 ± 29	0.651
Δs*RAGE*, pg/mL	-5.44 ± 12	187.3 ± 22#	0.004
Baseline *AGER1* gene expression, AU	0.46 ± 0.05	0.48 ± 0.10	0.601
Δ*AGER1*, AU	-0.19 ± 0.09#	0.22 ± 0.04#	0.023
Baseline *GloxI* gene expression, AU	0.92 ± 0.06	0.98 ± 0.05	0.145
Δ*GloxI*, AU	-0.10 ± 0.06#	0.08 ± 0.07	0.039
Baseline GSH levels, nmol	10.4 ± 0.06	10.6 ± 0.06	0.871
ΔGSH, nmol	-0.58 ± 0.09#	0.75 ± 0.08#	0.003

Values are represented as the mean ± standard error. Total population *n* = 809

Variables were compared using the analysis of variance (univariate ANOVA) adjusted by age, sex, BMI, hypertension, energy, smoking habits (non-, past- and current smokers), and pharmacological treatments–lipid-lowering therapy, use of antidiabetic drugs and anti-hypertensive drugs

#Significant difference ($p < 0.05$) between baseline and after intervention (5 years) of follow-up in each variable

*Significant difference ($p < 0.05$) between the Mediterranean diet and the low-fat diet groups

AGE, advanced glycation end products; sMG, serum levels of Methylglyoxal; sCML, serum levels of N-carboxymethyllysine; sRAGE, soluble receptor for AGEs; GloxI, glyoxalase; GSH, reduced glutathione

and determined lower sRAGE levels than the Mediterranean diet (-11.21 pg/mL vs. -130.25 pg/mL, $p = 0.017$) (Fig. 4D).

The Mediterranean diet also reduced circulating CML levels but only in Non-increased IMT-CC patients compared to baseline (Δchange −0.13 ± 0.02 ng/mL, $p = 0.033$) (Supplementary Fig. 2A). The low-fat diet did not exert changes in the levels of CML in any group of patients (Supplementary Fig. 2B).

Differential effect of dietary intervention on GSH levels

GSH is a fundamental player for the scavenging of reactive oxygen species as well as of MG. We found that the Mediterranean diet increased GSH levels, compared to baseline, in Non-increased IMT-CC patients (Δchange 3.54 ± 0.10 ng/mL, $p = 0.002$). Conversely, the low-fat diet produced a decrease in GSH levels, compared to baseline, in Increased IMT-CC patients (Δchange −2.97 ± 0.08 ng/m, $p = 0.013$) (Supplementary Fig. 3).

Multiple logistic regression model for predicting changes in IMT-CC regarding modulation of AGEs metabolism

To accurately evaluate the effectiveness of each dietary intervention on IMT-CC changes through the management of AGEs, we conducted two separate multiple logistic regression analysis, one for each dietary model (Fig. 5). In our model, after consumption of the Mediterranean diet, but not after the low-fat diet, an increase of one SD of Δchanges in circulating levels of MG determined an increase of 1.334-fold (95% CI, 1.080–1.647) the probability of increasing or maintaining stable IMT-CC after dietary intervention while an increase of one SD of Δchanges in *GloxI* gene expression determined an increase of 1.472-fold (95% CI, 1.062–2.001) the probability of decreasing IMT-CC after dietary intervention. After both diets, baseline IMT-CC and BMI and smoking habit (past smokers) also contributed to changes IMT-CC.

Discussion

This study unveils novel insights into the effect of dietary interventions in modulating circulating AGE levels and their impact on the molecular mechanisms driving atherosclerosis. Thus, long-term consumption of a Mediterranean diet, compared to a low-fat diet, in a large sample of patients with CHD, exerted a differential effect on the metabolism of AGEs and, consequently, on circulating AGEs (MG and CML). The Mediterranean diet increased gene expression of *AGER1* and *GloxI* and produced higher levels of sRAGE, all of which related to AGE detoxification mechanisms, which in turn resulted in a maintenance of stable levels of MG. These findings were notably observed in patients who, after dietary intervention, did not exhibit atherosclerosis progression (indicated by reduced or unchanged IMT-CC). In these patients, the Mediterranean diet also decreased circulating levels of CML hypothesizing that an optimal management of AGEs could be implicated in slowing the progression of atherosclerosis. By the contrast, although this diet increased MG levels in patients who presented atherosclerosis progression (those who increased their IMT-CC), this increment was lower compared to the low-fat diet.

The role of AGEs in the pathogenesis of CVD is strongly supported, mainly, through cross-sectional associations both in diabetic and non-diabetic populations [39, 40]. AGEs increase in the presence of hyperglycemia, oxidative stress, and inflammation, and serve as crucial mediators of atherosclerosis development [17, 41]. In fact, specific AGEs as well as certain metabolic oxidation products were associated with future severity of subclinical measures of atherosclerosis in patients with T2DM and atherosclerosis [20]. Among AGEs and their precursors, it is noteworthy MG, an extremely

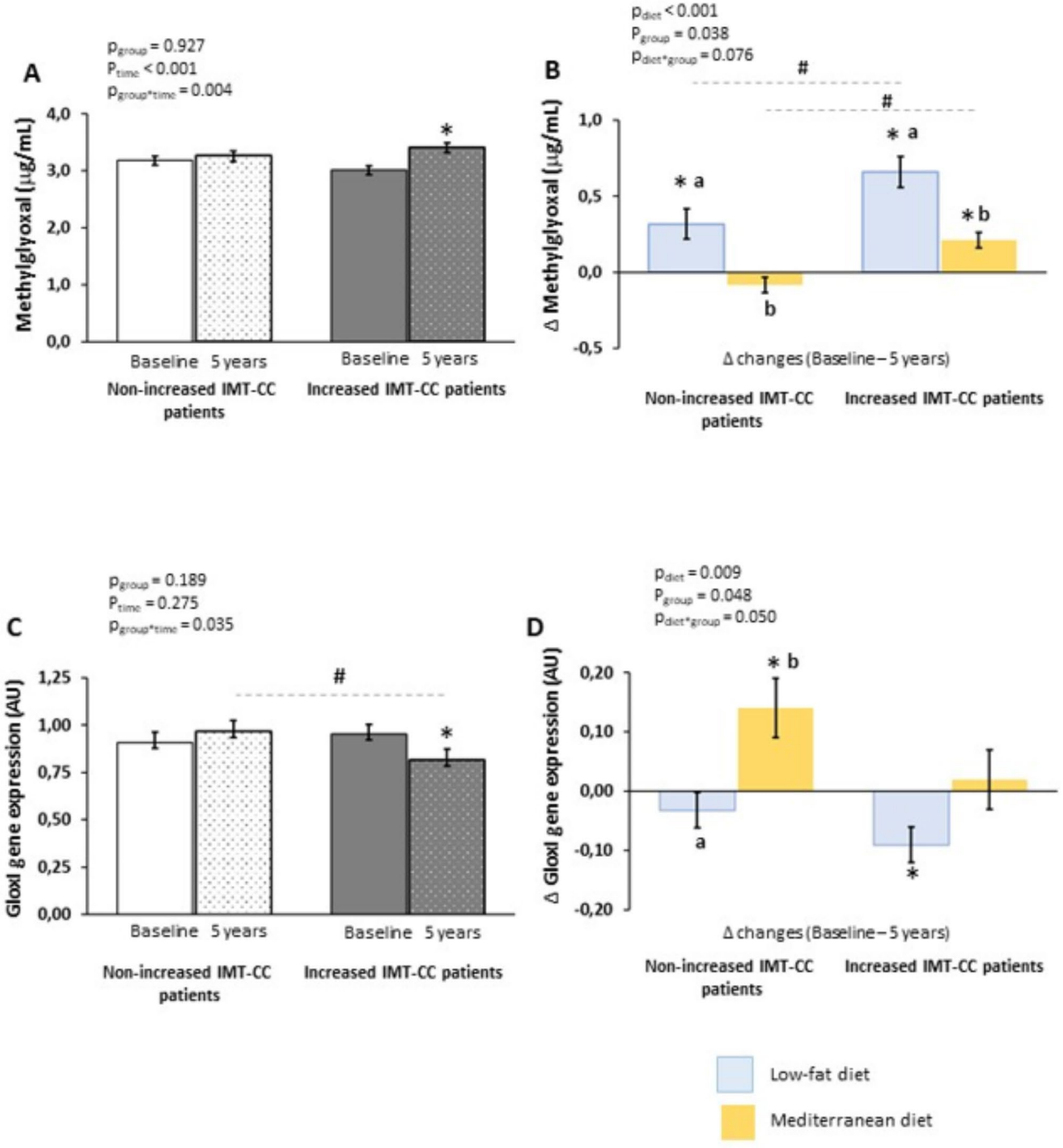


Fig. 3 Effect of dietary intervention on circulating MG levels and *Gloxl* gene expression in CHD patients classified according to IMT-CC progression. (A) Serum levels of MG at baseline and after 5-year of dietary intervention; (B) Changes produced between post- and preintervention in serum levels of MG after each dietary model; (C) *Gloxl* gene expression at baseline and after 5-year of dietary intervention; B) Changes produced between post- and preintervention in *Gloxl* gene expression after each dietary model. Values are represented as the mean ± standard error. Variables were compared using the analysis of variance (univariate ANOVA) adjusted by age, sex, BMI, hypertension, energy, smoking habits (non-, past- and current smokers), and pharmacological treatments–lipid-lowering therapy, use of antidiabetic drugs and anti-hypertensive drugs. Differences were significant when $p < 0.05$. *Significant differences between post- and preintervention. #Significant differences between Non-increased IMT-CC and Increased IMT-CC patients. Different common letter superscripts denote significant differences. Non-increased IMT-CC patients, whose IMT-CC was reduced or not changed after dietary intervention ($n = 408$, 166 in the low-fat diet and 242 in the Mediterranean diet). Increased IMT-CC patients, whose IMT-CC was increased after dietary intervention ($n = 401$, 211 in the low-fat diet and 190 in the Mediterranean diet). CHD, Coronary heart disease; IMT-CC, intima-media thickness of both common carotid arteries; MG, methylglyoxal; Gloxl, glyoxalase I

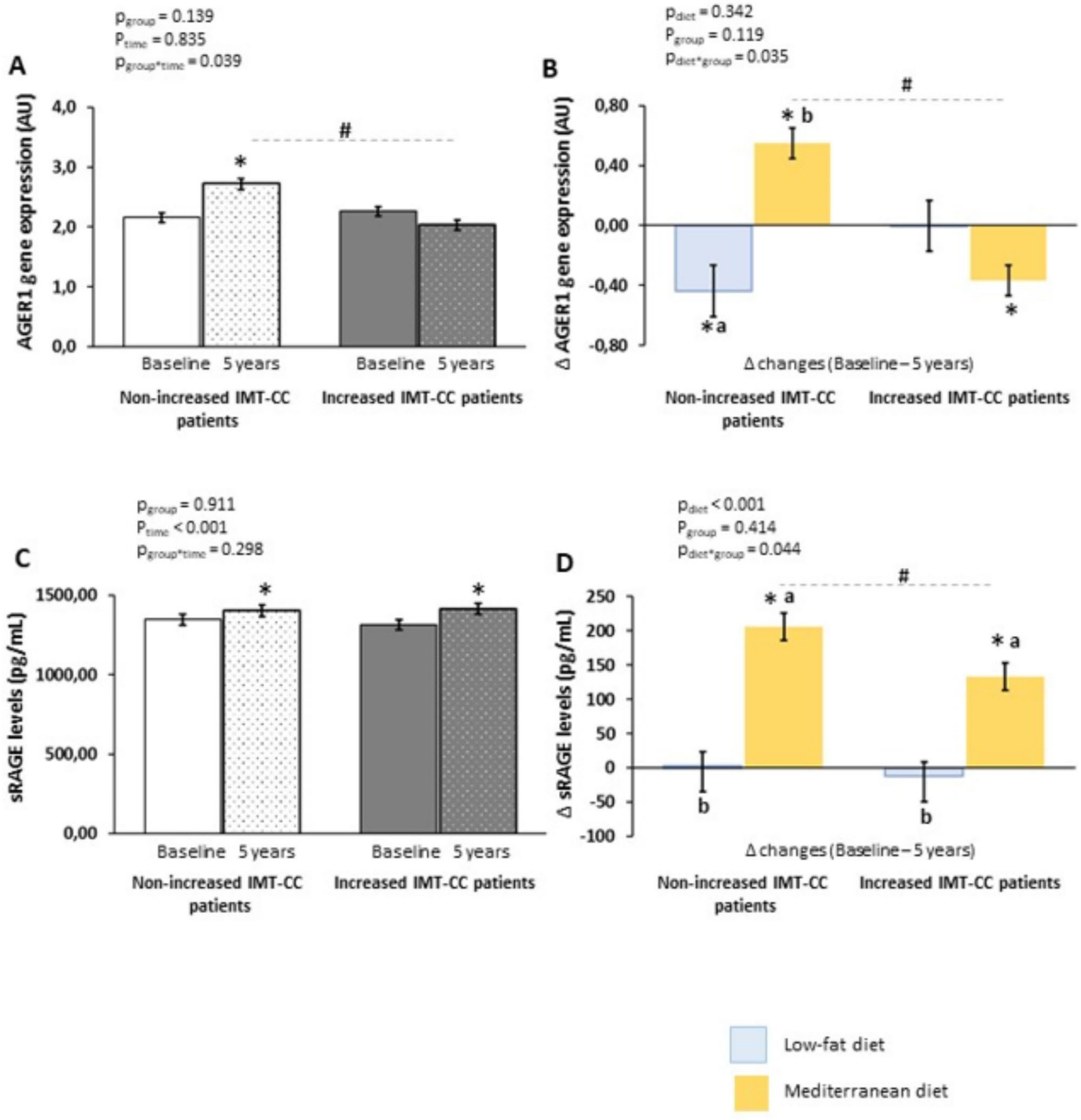


Fig. 4 Effect of dietary intervention on *AGER1* gene expression and sRAGE levels in CHD patients classified according to IMT-CC progression. (A) *AGER1* gene expression at baseline and after 5-year of dietary intervention; (B) Changes produced between post- and preintervention in *AGER1* gene expression after each dietary model; (C) *sRAGE* levels at baseline and after 5-year of dietary intervention; (D) Changes produced between post- and preintervention in *sRAGE* levels after each dietary model. Values are represented as the mean ± standard error. Variables were compared using the analysis of variance (univariate ANOVA) adjusted by age, sex, BMI, hypertension, energy, smoking habits (non-, past- and current smokers), and pharmacological treatments–lipid-lowering therapy, use of antidiabetic drugs and anti-hypertensive drugs. Differences were significant when $p < 0.05$. *Significant differences between post- and preintervention. #Significant differences between Non-increased IMT-CC and Increased IMT-CC patients. Different common letter superscripts denote significant differences. Non-increased IMT-CC patients, whose IMT-CC was reduced or not changed after dietary intervention ($n = 408$, 166 in the low-fat diet and 242 in the Mediterranean diet). Increased IMT-CC patients, whose IMT-CC was increased after dietary intervention ($n = 401$, 211 in the low-fat diet and 190 in the Mediterranean diet). CHD, Coronary heart disease; IMT-CC, intima-media thickness of both common carotid arteries; AGER1, AGE receptor-1; sRAGE: soluble form of receptor for AGEs

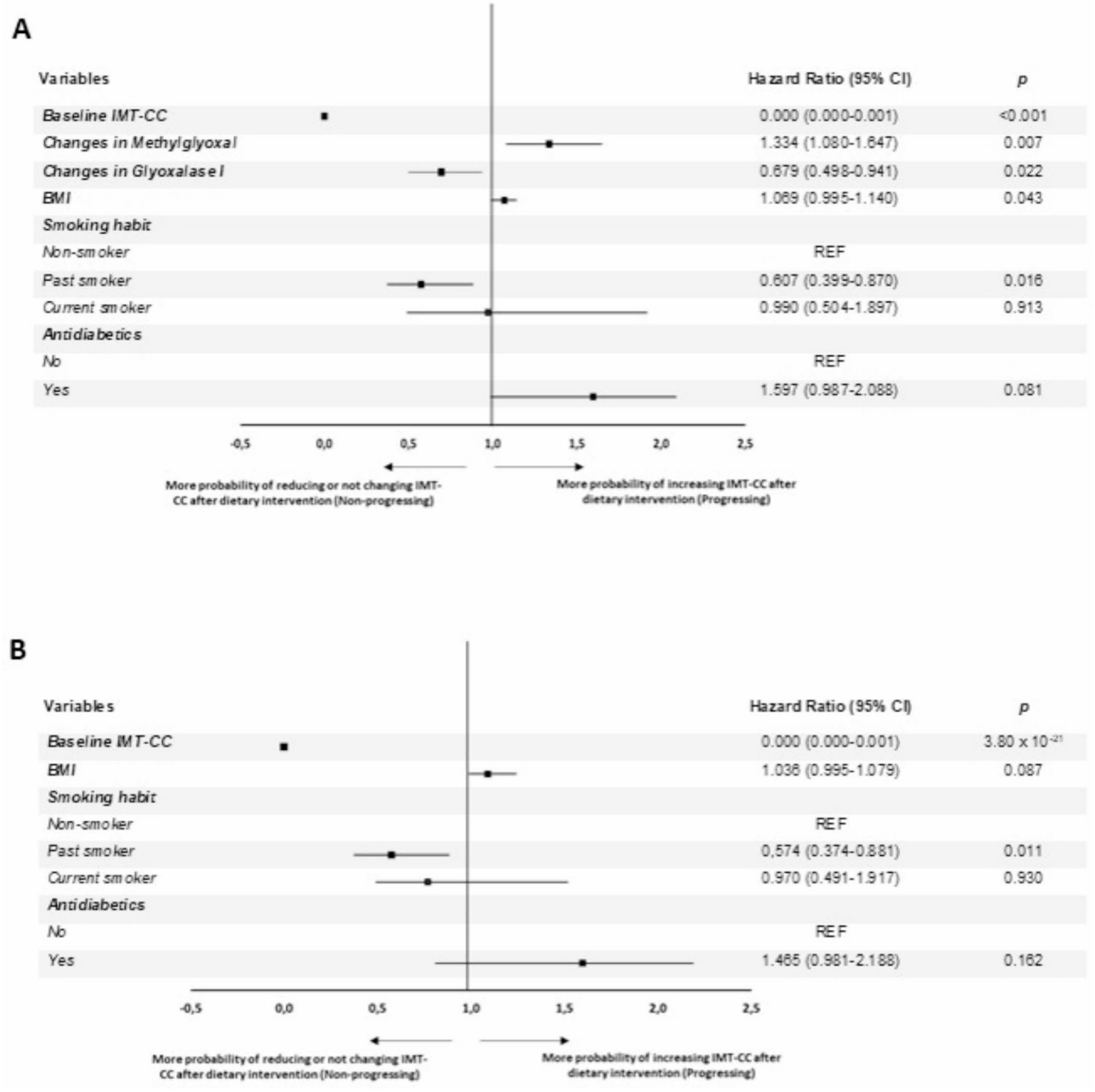


Fig. 5 Multiple logistic regression model for predicting changes in IMT-CC regarding modulation of AGEs metabolism by each dietary model. (A) Mediterranean diet ($R^2 = 0.337$, constant = 2.552 ($p = 0.001$)); (B) Low-fat diet ($R^2 = 0.249$, constant = 3.270 ($p = 0.007$)). Squares denote hazard ratios; horizontal lines represent 95% confidence intervals. Predictive variables tested by backward (conditional) method: Age (years), Sex (men and women), Baseline IMT-CC (mm), Changes in Methylglyoxal (mg/mL, Δ post-intervention minus pre-intervention), Changes in *Gloxl* expression (AU, Δ post-intervention minus pre-intervention), Changes in *AGER1* expression (AU, Δ post-intervention minus pre-intervention), fasting glucose (mg/dL), HbA1c (%), HDL-cholesterol (mg/dL), triglycerides (mg/dL) and hsCRP levels (mg/mL), smoking habits (non-, past- and current smokers) and medications–lipid-lowering therapy, use of antidiabetic drugs and anti-hypertensive drugs)

reactive α-oxoaldehyde and potent glycating agent, that rapidly forms glycation adducts on proteins and predicts cardiovascular events [42–45]. This observation is supported by recent studies in T1DM patients, where among various AGEs analyzed, only MG was found to correlate with IMT-CC progression even after adjusting for HbA1c. This suggests that glycation and MG-mediated crosslinking may play a significant role in matrix accumulation within coronary arteries [46]. MG also creates a pro-thrombotic microenvironment by directly increasing platelet aggregability and decreasing thrombus stability, further emphasizing its critical role in atherosclerosis

progression [47]. However, despite these data, there is a lack of studies focused on exploring strategies to modulate AGE levels as effective therapies for reducing cardiovascular outcomes.

In a recent longitudinal observational study, T2DM patients who maintained persistently low AGE levels had a lower risk of combined cardiovascular events, underscoring the importance of managing AGE levels [48]. This finding partially aligns with our study, in which patients who demonstrated reduced atherosclerosis progression (evidenced by a decrease in IMT-CC) maintained stable MG levels following a controlled dietary intervention. Notably, this effect was observed specifically in patients consuming Mediterranean diet, but not in those following a low-fat diet. To date, those studies focused on modulating levels of AGEs in the body have been performed with certain molecules that act as inhibitors of AGE formation, such as pyridoxamine [49] or with plant-derived substances with antioxidant properties [50, 51]. However, to our knowledge, there are no other studies evaluating the long-term impact of a specific dietary pattern aimed at reducing AGEs levels. The Mediterranean diet is widely recognized as a one of the most beneficial dietary patterns for cardiovascular health [52–54], attributed to its richness in healthful foods such as vegetables, fruits, cereals, legumes and EVOO, which provides a unique profile of fatty acids and certain minor components with anti-inflammatory and antioxidant properties [55]. Additionally, the Mediterranean diet uses cooking methods such as steaming, grilling, and boiling, which not only preserve the nutritional content of foods but may also contribute to reducing AGE production, as we observed in our study when we considered the total population. This is also supported by recent research showing that polyphenol-rich foods can capture carbonyl compounds and decrease AGE formation during cooking [56].

GloxI (and GloxII) are the main enzymes responsible for the detoxification of MG, which use GSH as a catalytic agent to convert MG into D-lactate, thereby preventing the accumulation of MG and MG-derived AGEs [57]. As we observed in our study, the Mediterranean diet increased both *GloxI* expression and GSH levels maintaining stable MG levels. Given that this effect was observed only in patients who did not exhibit atherosclerosis progression and considering that changes in MG and *GloxI* expression were significant predictors of changes in IMT-CC, these could explain the effect of the Mediterranean diet in AGE metabolism and its contribution to the reduction of atherosclerosis. Another potential mechanism to elucidate the association between the modulation of AGE levels and IMT-CC after consumption of the Mediterranean diet involved its ability to enhance *AGER1* expression. Cell-bound receptor AGER1 is found to suppress AGEs (as CML)-related oxidative stress/inflammation response [58]. However, there is some controversy on the role of the soluble form of RAGE (sRAGE). Although some studies associate sRAGE with atheroprotective properties, possibly acting as a decoy receptor for its ligands [59, 60], as we observed in our study, other authors associate elevated sRAGE levels with inflammation, but not with arterial stiffness or wall thickness [61] probably due significant heterogeneity among the analyzed populations.

Our study has several strengths. Firstly, assessing circulating AGEs and their metabolism provides approaches to implement dietary strategies to modulate and reduce their levels, establishing a starting point for prospective longitudinal studies aimed at preventing or delaying the onset of CVD and its associated comorbidities. Secondly, the study employed a randomized design, involved a substantial number of patients and two different dietary patterns that are equally healthy and have demonstrated excellent dietary adherence [26–28]. Finally, most of the clinical studies have assessed AGEs by skin autofluorescence (SAF) and their relationship with cardiovascular outcomes which is limited to detect only those AGEs with fluorescent properties [62]. In our study, while the ELISA technique may have limitations in detecting specific AGE isoforms compared to UPLC-MS/MS, which is considered the gold standard, it remains a widely used and validated method for assessing relative changes in AGE levels in biological samples [63]. Our study has several limitations. This research is based on a long-term, well-controlled dietary intervention, which ensures the quality of the study, but may not reflect the level of compliance in a free-living population. Moreover, the study observed a higher dropout rate in the low-fat diet group compared to the Mediterranean diet group, which may be attributed to the study being conducted in a Mediterranean region where there is naturally a higher acceptance and preference for the Mediterranean lifestyle. Thirdly, the results are limited to patients with CHD and may not be generalizable to other populations. Finally, AGE modulation was not the primary endpoint of the CORDIOPREV trial, but a secondary objective of this study, what make no possible to link causality from our observations.

Conclusion

Our findings suggest that improved modulation of AGE metabolism, which facilitates better management of circulating AGE levels, could be one of the potential mechanisms by which the Mediterranean diet, compared to a low-fat diet rich in complex carbohydrates, potentially contributes to slowing the progression of atherosclerosis in patients with CHD. Specific food composition and cooking techniques of this diet could be determinant for a more effective management of circulating AGE levels. Further research is needed to confirm the potential

of dietary interventions targeting AGE metabolism as a complementary approach for managing atherosclerosis progression in secondary prevention of CVD.

Abbreviations

AGEs	Advanced glycation end products
AGER1	AGE receptor-1
BMI	Body mass index
CHD	Coronary heart disease
CORDIOPREV study	CORonary Diet Intervention with Olive oil and cardiovascular PREVention study
CML	Nε-Carboxymethyllysine
CVD	Cardiovascular disease
DBP	diastolic blood pressure
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GloxI	Glyoxalase I
GSH	Reduced glutathione
HbA1c	Hemoglobin A1c
hsCRP	High sensitive C-reactive protein
IMT-CC	Intima-media thickness of both common carotid arteries
MG	Methylglyoxal
MEDAS	Mediterranean diet Adherence Screener
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
T2DM	Type 2 diabetes mellitus
SBP	systolic blood pressure
SFA	Saturated fatty acid
sRAGE	Soluble form of receptor for AGEs

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12933-024-02451-4.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

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Author contributions
The authors' responsibilities were as follows—FMG.M, AL.M, JDT.P and PG.L: collected the data; EMY.S, JL.M, FMG.M, JL.RC: designed and conducted the research and provided materials or participants; APA.D, FMG.M: analyzed the data; FMG.M and AL.M: wrote the manuscript; JD.L, RM.L, J.U, PP.M: provided significant advice and support in reviewing the drafting of the manuscript; EMY.S and JL.M: had the main responsibility for the final content.

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Data availability
Collaborations with the CORDIOPREV study are open to Biomedical Institutions, always after an accepted proposal for a scientific work. Depending on the nature of the collaboration, electronic data, hard copy data, or biological samples should be provided. All collaborations will be made after a collaboration agreement. Terms of the collaboration agreement will be specific for each collaboration, and the extent of the shared documentation (i.e., deidentified participant data, data dictionary, biological samples, hard copy, or other specified data sets) will be also specifically set on the light of each work.

Declarations

Ethics approval and consent to participate
This information is included in the manuscript body.

Consent for publication
Not applicable.

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

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