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Association between a Mediterranean lifestyle and growth differentiation factor 15: The seniors ENRICA-2 cohort

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ABSTRACT

Background: Growth Differentiation Factor 15 (GDF-15) is a marker of inflammation and oxidative stress that has been associated with multiple age-related chronic diseases. Since lifestyle is key for preventing these adverse health outcomes, we examined the association between a Mediterranean lifestyle and GDF-15 serum concentrations in Spanish older adults.

Methods: We used cross-sectional data from 2502 older adults participating in the Seniors ENRICA-2 cohort. Adherence to the Mediterranean lifestyle was assessed with the 27-item MEDLIFE index, divided into three blocks: 1) "Mediterranean food consumption, 2) Mediterranean dietary habits, 3) Physical activity, rest, social habits, and conviviality". Analyses of the association between the MEFLIFE index and GDF-15 concentrations were performed using multivariable linear regression models adjusting for the main potential confounders.

Results: The MEDLIFE index was inversely associated with GDF-15. Compared with participants in the lowest quartile of the MEDLIFE score, GDF-15 mean percentage differences (95% CI) were −3.0% (−8.0, 2.3) for the second quartile, −8.7% (−13.0, −4.1) for the third quartile, and −10.1% (−15.0, −4.9) for the fourth quartile (p-trend<0.001). Block 3 of MEDLIFE, and particularly doing sufficient physical activity, adequate sleep duration, and participating in collective sports, was individually linked to lower concentrations of GDF-15. Results remained similar after excluding participants with cardiovascular disease, type 2 diabetes, or obesity.

Conclusions: A Mediterranean lifestyle was associated with reduced levels of GDF-15, suggesting that a combination of multiple lifestyles may be an integral approach to reduce chronic inflammation and disease burden in older adults.

1. Introduction

Healthy aging is a process defined as the maintenance of the functional ability that enables well-being in older age [1]. However, it is frequently altered by chronic diseases, injuries, or reduced physiological reserves that lead to geriatric syndromes such as frailty. One important mechanism underlying unhealthy aging is low-grade, age-associated, systemic chronic inflammation (also known as *inflammaging*) due to an imbalance between pro- and anti-inflammatory factors [2].

Growth Differentiation Factor 15 (GDF-15) is a member of the Transforming Growth Factor-β (TGF-β) cytokine superfamily that is secreted in response to oxidative stress and inflammation, being a good biomarker of chronic disease burden [3]. Indeed, due to its pleiotropic effects, GDF-15 has been associated to age-related disorders and multiple adverse outcomes such as mitochondrial dysfunction [4], cardiovascular disease (CVD) [5], diabetes [6], metabolic syndrome [7], liver

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and kidney disease [8], or all-cause mortality [9]. Also, GDF-15 is gaining attention as a new diagnostic and prognostic biomarker of CVD and could represent a potential therapeutic target against it [10,11], although further research is needed to understand its exact biological functions and determinants.

Lifestyle is a key determinant of low-grade inflammation, oxidative stress and chronic disease [12]. Interestingly, some studies have found a mediating role of GDF-15 in the association between diet quality and all-cause mortality [13], probably due to its anti-inflammatory and anti-oxidant properties. In addition, previous research has shown individual associations between physical activity [14], alcohol consumption [15], diet quality [16] or smoking [17] and GDF-15. However, no study has yet examined the joint role of multiple lifestyle behaviors in GDF-15 concentrations. Since there is evidence of the synergistic influence of each lifestyle component on a number of health outcomes including CVD [18], diabetes [19], metabolic syndrome [20], and frailty [21] -conditions that involve chronic inflammation and oxidative stress-, an association between a set of combined lifestyles and concentrations of GDF-15 in older adults seems plausible. Therefore, this study aimed to examine if a Mediterranean lifestyle, characterized by adherence to the Mediterranean diet and other healthy food habits, appropriate physical activity, low sedentary behavior, regular napping, sociability, and conviviality is associated with lower concentrations of GDF-15 in older adults.

2. Methods

2.1. Study design and participants

We used data from the baseline wave of the Seniors-ENRICA-2 cohort, whose methods are similar to those used in the Seniors ENRICA-1 study [22]. Briefly, 3273 individuals were selected between 2015 and 2017 by stratified random sampling of community-dwelling individuals aged 65 or older holding a national healthcare card and living in the metropolitan area of Madrid, Spain. Information on sociodemographic data, morbidity and lifestyle was obtained through a telephone interview. Two home visits were subsequently conducted to collect biological samples, to perform a physical examination and to assess food consumption through a diet history. All participants provided written informed consent. The study was approved by the Clinical Research Ethics Committee of [blinded for review].

2.2. Study variables

2.2.1. Mediterranean lifestyle assessment

Food consumption was obtained through a computer-assisted face-to-face validated diet history, developed from the diet history used in the EPIC Spanish cohort [23]. Data from physical activity and sedentary behavior were collected, respectively, with the Spanish-validated versions of the EPIC-cohort study [24] and the Nurses' Health Study [25] questionnaires. Participants also reported data about sleep, napping, conviviality, and meal habits.

The MEDLIFE index was built according to a published modified version [20] of the original score [26]. The modified MEDLIFE index comprised 27 items arranged in three thematic blocks: a) "Mediterranean food consumption", with 15 items referring to food groups consumption (i.e., vegetables, fruits, legumes, olive oil, processed meat); b) "Mediterranean dietary habits", with 6 items referring to habits and practices around meals (i.e., limiting sweetened beverages, minimal snacking, limiting salt with meals); c) "Physical activity, rest, social habits and conviviality", with 6 items referring to resting and collective activities (i.e., regular naps, watching TV, sports, socializing). Each item scores as 0 (non-adherent) or 1 point (adherent) (Supplementary Table S1); thus, the final index ranged from 0 to 27. We modelled MEDLIFE index and blocks as quartiles and as continuous variables (per 2-point increment); additionally, each MEDLIFE item was modelled as a categorical variable according to their description (Supplementary Table S1) in order to evaluate its independent association.

2.2.2. GDF-15 assessment

Data on serum GDF-15 concentrations were obtained from fasting blood samples collected from each participant at the first home visit. Samples were collected in rapid serum tubes with thrombin-based clot activator and polymer gel (Becton Dickinson). One hour after the collection, tubes were centrifuged at 2520 g and room temperature (20–23 °C) for 10 min. Serum was then aliquoted and frozen at −80 °C and stored up to 3.6 years at the Department of [blinded for review] at the [blinded for review].

Serum GDF-15 was measured between July 2019 and June 2020 at the Department of [blinded for review] of [blinded for review] by electrochemiluminescence Elecsys® immunoassay method using a Cobas® 6000 analyzer (Roche Diagnostics). For a mean concentration of 7343 pg/mL, the interassay CV was 5.4%; and for a mean concentration of 1428 pg/mL, it was 7.7%.

2.2.3. Potential confounders

Participants reported sociodemographic and other lifestyle data including sex, age, educational level (primary, secondary, university) and tobacco status (current, former, never). Body mass index (BMI) was calculated as weight (kg) divided by square height (m), both measured in standardized conditions [22]. Energy intake was estimated from the diet history [23]. CVD was assessed by self-reporting a medical diagnosis of myocardial infarction, stroke, or congestive heart failure; type 2 diabetes by self-reporting a medical diagnosis of diabetes, being under antidiabetic medication or having a fasting blood glucose of $\geq$126 mg/dL. Systolic blood pressure was measured under standardized conditions at least 3 times [27]; the average of the second and third measurements were used for analyses. Blood glucose, HDL-cholesterol and tryglycerides were measured with colorimetric enzymatic methods with Atellica® solution (Siemens Healthineers). LDL-cholesterol levels were calculated with the Friedewald formula (LDL = total cholesterol – tryglycerides/5 – HDL) except for those with tryglycerides >250 that the measure of LDL-cholesterol was assessed directed from blood samples [28]. Only LDL-cholesterol was used for analyses.

2.3. Statistical analyses

From the initial 3273 participants, we excluded 684 individuals with missing data on GDF-15 and 85 with missing information on the covariates. Therefore, our final analytical sample comprised 2504 participants (Supplementary Fig. S1). Participant's characteristics were summarized with mean and standard deviation for continuous variables and proportions for categorical variables.

The association between the MEDLIFE index and GDF-15 was assessed with multivariable linear regression models where the dependent variable was the log-transformed GDF-15 and used as continuous variable. Associations were summarized using mean percentage differences, with 95% confidence intervals (CI). Mean percentages differences were calculated by subtracting 1 from the exponentiated β-coefficients resulting from the regression models and multiplying the result by 100. We built several models with incremental adjustment for potential confounders: Model 1, adjusted for sex, age, and educational level; Model 2, further adjusted for tobacco status, BMI, energy intake, CVD, and diabetes; and Model 3, further adjusted for systolic blood pressure, glucose, and LDL levels. We replicated the analyses for each MEDLIFE block (with further adjustment for the remaining block) and each MEDLIFE item (with further adjustment for the MEDLIFE index excluding the corresponding item). *P* values for linear trend were calculated using quartiles of MEDLIFE as continuous variable. Deviation from linearity was evaluated using a restricted cubic spline regression model, adjusted as in Model 3.

Robustness of the results was checked with several sensitivity

analyses: 1) stratifying the analyses by the main covariates (sex, age groups, educational level, BMI, CVD, and type 2 diabetes) to assess whether there were differences between groups. Interaction terms, defined as the product of MEDLIFE categories and each variable, were tested. 2) Given that GDF-15 is a biomarker of inflammation and chronic disease burden [4] we replicated the main analysis excluding participants with CVD, diabetes, or obesity and with all of the three conditions. 3) We excluded consumption of legumes and low-fat dairy items from the MEDLIFE score, due to unexpected results, to check the magnitude of the association without those items. And finally, 4) we conducted the main analysis using quintiles of the MEDLIFE to account for the expected irregular distribution of the discrete nature of the MEDLIFE index where the mode and the mean are rather close.

Analyses were performed using Stata version 16.0 (StataCorp LLC, College Station, TX). Statistical significance was established at $p < 0.05$ level, two-sided.

3. Results

Participants had a mean (SD) age of 71.56 (4.38) and 52.96% were women. The mean (SD) score for the MEDLIFE index was 13.16 (2.63) points. Participants with higher MEDLIFE score were more likely to have higher educational level, higher energy intake, lower frequency of chronic diseases and to smoke less (Table 1). Compared to the analytical sample, excluded participants were more likely to have lower education, higher frequency of BMI, more chronic diseases, and lower MEDLIFE score (Supplementary Table S2).

3.1. MEDLIFE and GDF-15

Higher score in the MEDLIFE index was associated with lower levels of GDF-15, in a dose-response manner (Table 2). Compared to the first quartile of the MEDLIFE index, the mean percentage differences (95% CI) in the most adjusted model were −3.0% (−8.0, 2.3) for the second quartile, −8.7% (−13.0, −4.1) for the third quartile, and −10.1% (−15.0, −4.9) for the fourth quartile (*p*-trend<0.001). The mean

Table 1
Baseline characteristics of older adults participating in Seniors ENRICA-2 cohort by quartiles of adherence to the MEDLIFE index.

	Quartile 1 4–11 p	Quartile 2 12–13 p	Quartile 3 14–15 p	Quartile 4 16–21 p	Total
n	405	605	1004	490	2504
Sex, women, *n* (%)	237 (58.52)	326 (53.88)	515 (51.29)	248 (50.46)	1326 (52.96)
Age, years, mean (SD)	72.63 (5.15)	71.91 (4.11)	71.34 (4.30)	70.68 (3.95)	71.56 (4.38)
Education, *n* (%)					
Primary or lower	281 (69.38)	385 (63.64)	616 (61.35)	308 (62.86)	1590 (63.50)
Secondary	69 (17.04)	108 (17.85)	207 (20.62)	85 (17.35)	469 (18.73)
University	55 (13.58)	112 (18.51)	181 (18.03)	97 (19.80)	445 (17.77)
Smoking status, *n* (%)					
Current	56 (13.83)	67 (11.07)	82 (7.17)	28 (5.71)	233 (9.31)
Former	139 (34.32)	191 (31.57)	413 (41.14)	208 (42.45)	951 (37.98)
Never	210 (51.85)	347 (57.36)	509 (50.70)	254 (51.84)	1320 (52.72)
BMI, kg/m^2, mean (SD)	27.91 (4.96)	27.97 (4.54)	27.63 (4.32)	27.64 (4.25)	27.76 (4.47)
Energy intake, kcal/day, mean (SD)	1882.66 (392.15)	1915.90 (344.07)	1964.88 (342.74)	2009.76 (329.72)	1948.53 (351.43)
CVD[a], *n* (%)	21 (5.19)	22 (3.64)	27 (2.69)	15 (3.06)	85 (3.39)
Type 2 diabetes, *n* (%)	83 (20.49)	121 (20.00)	177 (17.63)	77 (15.71)	458 (18.29)
SBe (mmHg), mean (SD)	135.88 (19.98)	134.86 (17.92)	133.87 (17.60)	134.44 (17.61)	134.54 (18.09)
Serum biomarkers, mean (SD)					
Glucose, mg/dl	101.72 (24.79)	100.88 (28.17)	98.58 (21.22)	99.59 (21.44)	99.84 (23.72)
LDL-cholesterol, mg/dl	113.43 (30.04)	113.43 (28.55)	113.00 (29.18)	115.82 (29.00)	113.73 (29.04)
GDF-15, pg/ml	1684.13 (1465.13)	1540.84 (1040.07)	1373.38 (941.42)	1280.95 (792.29)	1446.02 (1050.76)
MEDLIFE index, 0–27 p, mean (SD)	9.14 (1.08)	11.57 (0.50)	13.90 (0.79)	16.90 (1.07)	13.16 (2.63)
Block 1: Mediterranean food consumption, 0–15 p, mean (SD)	3.89 (1.12)	5.07 (1.08)	6.34 (1.23)	7.99 (1.22)	5.96 (1.77)
Block 2: Mediterranean eating habits, 0–6 p, mean (SD)	3.21 (0.76)	3.65 (0.72)	3.87 (0.72)	4.24 (0.73)	3.78 (0.80)
Block 3: Physical activity, rest, social habits, and conviviality, 0–6 p, mean (SD)	2.04 (1.05)	2.84 (1.11)	3.70 (1.14)	4.68 (0.96)	3.42 (1.38)

Abbreviations: p, points; SD, standard deviation; BMI, body mass index; CVD, cardiovascular disease; SBP, systolic blood pressure; GDF-15, Growth Differentiation Factor 15.
a Including myocardial infarction, stroke, and congestive heart failure.

Table 2
Mean (95% CI) percentage differences for the association of MEDLIFE index quartiles and GDF-15 ($N = 2504$).

	Quartile 1 4–11 p	Quartile 2 12–13 p	Quartile 3 14–15 p	Quartile 4 16–21 p	*p*-trend	Per 2-point increase
n	405	605	1004	490		
Model 1[a]	Ref.	−4.1 (−9.6, 1.7)	−11.1 (−15.8, −6.1)***	−14.1 (−19.2, −8.5)***	<0.001	−3.9 (−5.3, −2.6)***
Model 2[b]	Ref.	−2.9 (−8.0, 2.5)	−8.4 (−12.9, −3.7)**	−10.2 (−15.2, −4.9)***	<0.001	−2.7 (−4.0, −1.5)***
Model 3[c]	Ref.	−3.0 (−8.0, 2.3)	−8.7 (−13.0, −4.1)***	−10.1 (−15.0, −4.9)***	<0.001	−2.8 (−4.0, −1.5)***

Abbreviations: p, points; Ref., reference; GDF-15, Growth Differentiation Factor 15; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
[a] Model 1: Adjusted for sex (male, female), age, educational level (primary, secondary, university).
[b] Model 2: As Model 1 + for tobacco status (current, former, never), body mass index (kg/m^2), energy intake (kcal/day), type 2 diabetes mellitus, cardiovascular disease [myocardial infarction, stroke, or heart failure].
[c] Model 3: As Model 2 + for systolic blood pressure (mmHg), blood glucose (mg/dL), and blood LDL-cholesterol (mg/dL).

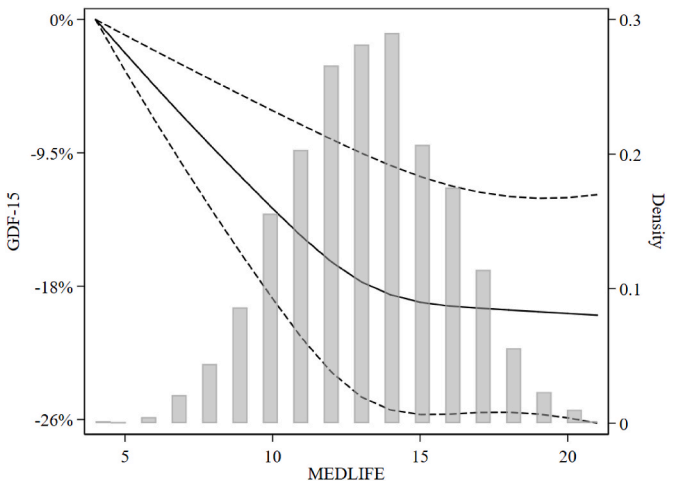

Fig. 1. Association of MEDLIFE index with GDF-15 (pg/mL).
Plotted values are mean percentage differences (95% CI) of GDF-15 obtained from restricted cubic spline linear regression models, adjusted as in Model 3 in Table 2. Histogram of frequencies of MEDLIFE (points) in the background.

percentage difference per each 2-point increase was −2.8% (−4.0, −1.5). In restricted cubic spline regression analyses, there was an inverse dose-response association until a plateau seemed to appear at highest MEDLIFE adherence (Fig. 1).

The results were robust in sensitivity analysis (using quintiles of the MEDLIFE Supplementary Table S3) and did not vary by sex, educational level, smoking status, BMI, CVD or diabetes status; although the associations appeared to be stronger in men, never smokers, and participants with lower education, normal weight or CVD; nevertheless, an interaction with age was found ($p = 0.016$), the association being stronger in older individuals (Supplementary Table S4). When we replicated analyses excluding those with CVD and type 2 diabetes, BMI≥30, or all three conditions, the results were in the same direction. (Supplementary Table S5).

3.2. *MEDLIFE blocks and items and GDF-15*

Block 3 ("Physical activity, rest, social habits and conviviality") was independently associated with GDF-15 concentrations; compared with participants in the lowest quartile of MEDLIFE index, mean percentages differences of GDF-15 (95% CI) were −6.4% (−10.6, −2.0) for the second quartile, −9.3% (−13.6, −4.9) for the third quartile and −10.3% (−14.4, −5.9) for the fourth quartile (p-trend<0.001) (Table 3). We did not find significant associations for the first and second MEDLIFE blocks; however, for most of the individual items GDF-15 levels showed a tendency in the expected direction, except for legumes and low-fat milk (Fig. 2). After removing those two items from Block 1, the association became stronger (4th vs 1st quartile: −3.9% [−8.1, 0.6]); per 2-point increase: −2.1% [−4.1, −0.0]) (Supplementary Table S6). White meat, doing sufficient physical activity, adequate sleep duration, and participating in collective sports were independently associated with lower levels of GDF-15 (Fig. 2).

4. Discussion

In this cohort of Spanish older adults, adherence to a Mediterranean lifestyle was associated with reduced concentrations of GDF-15. This association remained after excluding individuals with CVD, type 2 diabetes, and obesity, suggesting that even rather healthy individuals could also benefit from a Mediterranean lifestyle.

GDF-15 has been previously associated with CVD, non-CVD and all-cause mortality [29], and a meta-analysis of 31 prospective studies found that per each log-unit increment in GDF-15, CVD and all-cause mortality was 2.11 and 2.70 times higher, respectively [30]. This is important because even modest decreases in GDF-15, through small improvements in a healthy lifestyle, may have public health relevance.

Our findings were consistent across participant's groups (sex, age, educational level, smoking status, BMI categories). The interaction found with age was not surprising since GDF-15 steeply increases with age, and it is associated to age-related disorders, such as mitochondrial dysfunction [4], cognitive impairment [31], CVD [3,13], other processes related to longevity, inflammaging, and mortality [32]. Nonetheless, the protective association between MEDLIFE and GDF-15 was significant for both, those <75 and those ≥75 years, although it was stronger in older age.

To our knowledge, this is the first study evaluating the joint association of multiple lifestyle behaviors with serum GDF-15 concentrations in older adults. However, previous research has found associations between separate behaviors and GDF-15. In this same cohort, a higher adherence to healthy dietary patterns was associated with lower concentrations of GDF-15 [16], possibly due to the anti-inflammatory effects of several food components of diet. Although in this analysis we did not find a significant association for Block 1 ("Mediterranean food consumption"), when we excluded the two items (legumes and low-fat dairy) that showed unexpected associations, a 2-point increment in

Table 3
Mean (95% CI) percentage differences for the association of MEDLIFE blocks quartiles and GDF-15 ($N = 2504$).

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-trend	Per 2-point increase
Block 1: *Mediterranean food consumption*						
Model 1[a]	Ref.	0.2 (−5.4, 6.2)	−2.9 (−7.6, 2.0)	−3.9 (−9.3, 1.8)	0.495	−1.2 (−3.2, 0.9)
Model 2[b]	Ref.	−0.5 (−5.5, 4.9)	−0.7 (−5.1, 3.9)	−0.8 (−6.0, 4.6)	0.743	−0.9 (−2.8, 1.0)
Model 3[c]	Ref.	−1.3 (−6.3, 3.9)	−1.0 (−5.3, 3.5)	−1.0 (−6.1, 4.4)	0.731	−0.9 (−2.8, 1.0)
Block 2: *Mediterranean dietary habits*						
Model 1[a]	Ref.	−0.8 (−4.7, 3.4)	−2.4 (−7.9, 3.4)	−14.8 (−28.8, 1.9)	0.195	−2.5 (−6.9, 2.1)
Model 2[b]	Ref.	−1.4 (−5.0, 2.4)	−1.8 (−6.8, 3.5)	−14.1 (−27.1, 1.2)	0.197	−2.1 (−6.1, 2.2)
Model 3[c]	Ref.	−2.4 (−6.0, 1.2)	−3.0 (−7.9, 2.2)	−14.4 (−27.2, 0.6)	0.070	−3.2 (−7.2, 1.0)
Block 3: *Physical activity, rest, social habits and conviviality*						
Model 1[a]	Ref.	−8.6 (−13.1, −3.8)**	−12.9 (−17.4, −8.2)***	−15.7 (−20.0, −11.2)***	<0.001	−8.8 (−11.3, −6.3)***
Model 2[b]	Ref.	−6.3 (−10.6, −1.9)**	−9.6 (−13.9, −5.1)***	−10.6 (−14.7, −6.2)***	<0.001	−5.9 (−8.2, −3.5)***
Model 3[c]	Ref.	−6.4 (−10.6, −2.0)**	−9.3 (−13.6, −4.9)***	−10.3 (−14.4, −5.9)***	<0.001	−5.6 (−7.9, −3.2)***

Abbreviations: p, points; Ref., reference; GDF-15, Growth Differentiation Factor 15; ** p < 0.01, *** p < 0.001.
Each block has been adjusted for the remaining blocks.
[a] Model 1: Adjusted for sex (male, female), age, educational level (primary, secondary, university).
[b] Model 2: Model 1 + tobacco status (current, former, never), BMI (kg/m^2), energy intake (kcal/day), type 2 diabetes mellitus, cardiovascular disease [myocardial infarction, stroke, or heart failure].
[c] Model 3: Model 2 + systolic blood pressure (mmHg), blood glucose (mg/dL), and blood LDL-cholesterol (mg/dL).

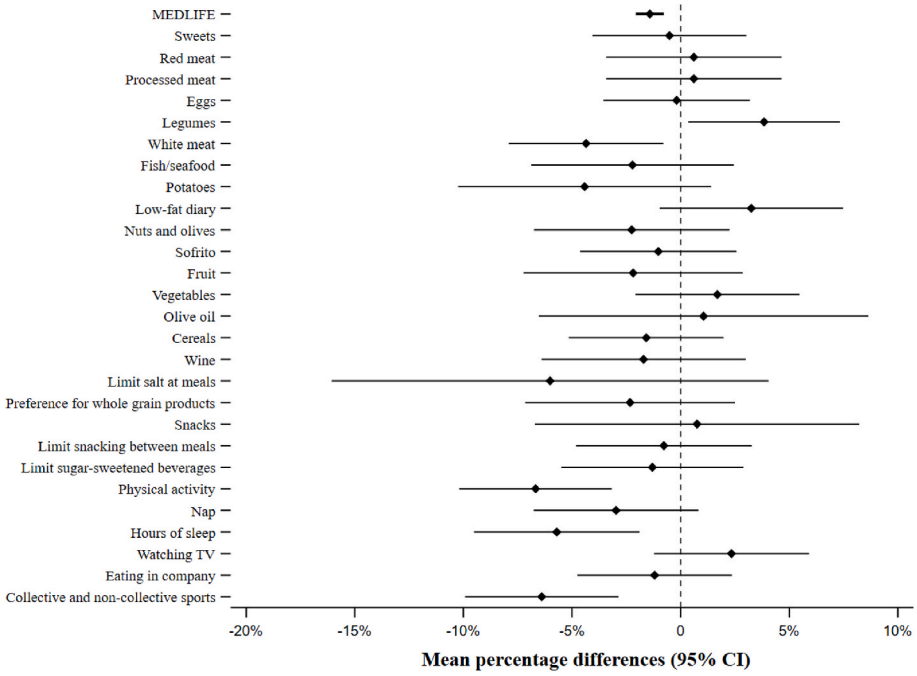

Fig. 2. Mean (95% CI) percentage differences for the association of each MEDLIFE item and GDF-15 (N = 2504).
Adjusted for sex (men, women), age, educational level (primary, secondary, university), tobacco status (current, former, never), body mass index, energy intake, type 2 diabetes mellitus, cardiovascular disease [myocardial infarction, stroke, or heart failure], systolic blood pressure, blood glucose, blood LDL-cholesterol and the MEDLIFE index excluding each item in each case.

Block 1 showed similar mean percentage difference in GDF-15 concentrations as the previously reported [16]. Interestingly, legumes are a polyphenol-rich food group with potential anti-inflammatory and antioxidants effects [33,34] but, the fact that in Spain legumes are often consumed as part of stews ("fabada", "cocido") rich in processed red meat (sausages, bacon) and salt [35,36], may have contributed to an association with a higher GDF-15 level. Some reverse causation may also explain these results: legumes can cause gastrointestinal discomfort due to flatulence, so individuals with chronic bowel diseases or disorders like diabetes whose treatment (e.g., metformin) produce flatulence may reduce or avoid its consumption. Regarding dairy, there is still not enough evidence to suggest a pro- or anti-inflammatory effect. While some recent systematic reviews found no effect of milk and dairy consumption on chronic systemic inflammation [37], others suggest neutral to potential anti-inflammatory effects [38–40]. Similarly, as in a previous study focusing on frailty [21], we did not find independent associations concerning Block 2 of MEDLIFE ("Mediterranean dietary habits") and GDF-15, probably because older adults are a subpopulation with high adherence to Block 2 components: 97% limited salt intake at meals, 94% limited snacking and 80% limited sugar-sweetened beverages (Supplementary Table S1).

We found that the Block 3 ("Physical activity, rest, social habits and conviviality") was independently associated with lower concentrations of GDF-15 and was the main contributor to the overall MEDLIFE association with GDF-15. Of note is that, in the SUN cohort, Block 3 also showed the strongest association among MEDLIFE blocks with other outcomes, such as mortality [41]. Within this block, the items physical activity, hours of sleep and collective and non-collective sports had the strongest association in the current analysis. There is robust evidence of the health benefits of even small amounts of physical activity on many chronic diseases [42]. Our findings are in line with a previous report in this cohort where accelerometer-measured moderate-to-vigorous physical activity was associated with lower GDF-15; interestingly, an strong association was also observed for light-intensity physical activity, which was linked to lower GDF-15 in the less active individuals [43]. This is important because older adults reduce their physical activity resistance and intensity as they age, but light and small amounts of activity may still be beneficial for their health. Another previous study also found an inverse association between physical activity and GDF-15 at any age (18–83 years, in 228 participants) [14].

With regard to sleep, we found a significant decrease in GDF-15 levels associated with sleeping 6–8 h a day; however, Ortolá et al. [43] did not find such association. Previous metanalyses showed a J-shaped association of short (<6 h) and long (>8 h) sleep duration with mortality [44,45], which may be mediated by inflammatory biomarkers and lifestyle factors [46]. Discrepancies between studies might be due to the way sleep has been analysed: using a single healthy category of sleep (6–8 h/day) in our study versus a continuous variable in others. Nonetheless, the components of the Mediterranean lifestyle most probably have a synergistic effect, because despite we found significant associations for a few MEDLIFE items, none of them considered separately was sufficient to explain the overall beneficial association between MEDLIFE and GDF-15. In fact, the inverse association between the overall MEDLIFE index and GDF-15 doubles that previously found when only diet was evaluated; this highlights the importance of an integral lifestyle approach to reduce GDF-15 and its associated disease burden.

4.1. *Underlying mechanisms*

The plausible mechanisms of the association MEDLIFE to reduce circulating levels of GDF-15 may result from the synergy of its multiple components. First, the Mediterranean diet has recognized anti-inflammatory and antioxidant properties, mostly from consumption of fruits and vegetables [33], virgin olive oil and nuts [47] and fish, which are rich in polyphenols (e.g., quercetin, oleuropein, hydroxityrosol, resveratrol, etc.), mono and polyunsaturated fatty acids, and vitamins that play a pivotal role in the inflammation process through various mechanisms (including changes in the microbiota) [48]. Second, although during acute exercise many cytokines are secreted by several metabolically-active tissues such as the muscle during contraction, chronic exercise has been associated with lower levels of systemic inflammatory markers [49,50]. In addition, social support-social integration are significantly related to lower levels of inflammation, while the opposite may occur for loneliness and social isolation [51,52]. Similarly, short and long sleep duration may be associated with inflammatory burden [53].

4.2. Strengths and limitations

There are some limitations to our study. First, the cross-sectional design does not allow drawing causal inferences. Second, diet, physical activity, and several chronic diseases were self-reported, which could lead to measurement error and underestimate the actual association between MEDLIFE and GDF-15. However, self-reported data on these traits have been widely used in epidemiological studies, showing good reliability and validity, and predicting many outcomes. Specifically, we used a diet history comprising a variety of foods and cooking methods with good correlation with seven 24-h recalls over 1 year [23]; and a validated physical activity questionnaire, and a validated MEDLIFE index that showed good validity and reproducibility [54]. Third, there may be some measurement error in GDF-15; nonetheless, measurement error bias tends to be non-differential and drives the association toward the null value. Fourth, although we adjusted for many potential confounders, residual confounding cannot be completely ruled out. Five, due to the discrete nature of the MEDLIFE index, categorization is sometimes difficult, and the distribution of the sample may be irregular, but results were robust in different sensitivity analyses and previous work have found strong associations with other health outcomes. Finally, these results cannot be generalized to other populations because our study was conducted in older adults of a Mediterranean country.

5. Conclusions

Higher adherence to the Mediterranean lifestyle was associated with lower circulating levels of GDF-15 in older adults, suggesting that a combination of multiple lifestyles may be an integral approach to reduce systemic inflammation and age-associated disease burden. Future prospective studies should examine and confirm the effects of an overall lifestyle on the circulating GDF-15 concentrations.

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Declaration of competing interest

Authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.freeradbiomed.2022.12.090.

References

[1] World Health Organization, Decade of healthy ageing: plan of action [Internet]. [cited 2021 Aug 19]. Available from: https://www.who.int/publications/m/item/decade-of-healthy-ageing-plan-of-action, 2020.
[2] I. Martínez de Toda, N. Ceprián, E. Díaz-Del Cerro, M. De la Fuente, The role of immune cells in oxi-inflamm-aging, Cells 10 (11) (2021) 2974.
[3] B. Srour, R. Kaaks, T. Johnson, L.C. Hynes, T. Kühn, V.A. Katzke, Ageing-related markers and risks of cancer and cardiovascular disease: a prospective study in the EPIC-Heidelberg cohort, Eur. J. Epidemiol. 37 (1) (2022) 49–65.
[4] Y. Fujita, Y. Taniguchi, S. Shinkai, M. Tanaka, M. Ito, Secreted growth differentiation factor 15 as a potential biomarker for mitochondrial dysfunctions in aging and age-related disorders: GDF15 in aging and age-related disorders, Geriatr. Gerontol. Int. 16 (2016) 17–29.
[5] V. Xanthakis, D.M. Enserro, J.M. Murabito, J.F. Polak, K.C. Wollert, J.L. Januzzi, et al., Ideal cardiovascular health: associations with biomarkers and subclinical disease and impact on incidence of cardiovascular disease in the framingham offspring study, Circulation 130 (19) (2014) 1676–1683.
[6] R. Adela, S.K. Banerjee, GDF-15 as a target and biomarker for diabetes and cardiovascular diseases: a translational prospective, J. Diabetes Res. 2015 (2015) 1–14.
[7] A. Carballo-Casla, E. García-Esquinas, A. Buño-Soto, E.A. Struijk, E. López-García, F. Rodríguez-Artalejo, et al., Metabolic syndrome and Growth Differentiation Factor 15 in older adults. GeroScience(2022) 44 (2):867-880.
[8] S. Desmedt, V. Desmedt, L. De Vos, J.R. Delanghe, R. Speeckaert, M.M. Speeckaert, Growth differentiation factor 15: a novel biomarker with high clinical potential, Crit. Rev. Clin. Lab Sci. 56 (5) (2019) 333–350.
[9] X. Bao, Y. Borné, B. Xu, M. Orho-Melander, J. Nilsson, O. Melander, et al., Growth differentiation factor-15 is a biomarker for all-cause mortality but less evident for cardiovascular outcomes: a prospective study, Am. Heart J. 234 (2021) 81–89.
[10] B.M. May, M. Pimentel, L.I. Zimerman, L.E. Rohde, GDF-15 as a biomarker in cardiovascular disease [Internet] Arq. Bras. Cardiol. (2021) 116 (3): 494-500 [cited 2021 Nov 23]; Available from: http://abccardiol.org/article/gdf-15-como-biomarcador-em-doencas-cardiovasculares/.
[11] K.C. Wollert, T. Kempf, L. Wallentin, Growth differentiation factor 15 as a biomarker in cardiovascular disease, Clin. Chem. 63 (1) (2017) 140–151.
[12] S. Ehlers, S.H.E. Kaufmann, Infection, inflammation, and chronic diseases: consequences of a modern lifestyle, Trends Immunol. 31 (5) (2010) 184–190.
[13] Y. Kim, S. Lu, J.E. Ho, S. Hwang, C. Yao, T. Huan, et al., Proteins as mediators of the association between diet quality and incident cardiovascular disease and all-cause mortality: the framingham heart study, J. Am. Heart Assoc. 10 (18).
[14] M. Conte, M. Martucci, G. Mosconi, A. Chiariello, M. Cappuccilli, V. Totti, et al., GDF15 plasma level is inversely associated with level of physical activity and correlates with markers of inflammation and muscle weakness, Front. Immunol. 11 (2020) 915.
[15] R. Ortolá, E. García-Esquinas, A. Buño-Soto, A. Carballo-Casla, M. Sotos-Prieto, J. R. Banegas, et al., Alcohol consumption patterns and growth differentiation factor 15 among lifetime drinkers aged 65+ years in Spain: a cross-sectional study, Addiction (2022), 15809.
[16] R. Ortolá, E. García-Esquinas, A. Buño-Soto, M. Sotos-Prieto, E.A. Struijk, F. F. Caballero, et al., Healthy dietary patterns are associated with lower concentrations of growth differentiation factor 15 in older adults, Am. J. Clin. Nutr. 113 (6) (2021) 1619–1626.
[17] M. Krintus, F. Braga, M. Kozinski, S. Borille, J. Kubica, G. Sypniewska, et al., A study of biological and lifestyle factors, including within-subject variation, affecting concentrations of growth differentiation factor 15 in serum, Clin. Chem. Lab. Med. 57 (7) (2019) 1035–1043.
[18] A. Mata-Fernández, M.S. Hershey, J.C. Pastrana-Delgado, M. Sotos-Prieto, M. Ruiz-Canela, S.N. Kales, et al., A Mediterranean lifestyle reduces the risk of cardiovascular disease in the "Seguimiento Universidad de Navarra" (SUN) cohort, Nutr. Metabol. Cardiovasc. Dis. 31 (6) (2021) 1728–1737.
[19] M. Delgado-Velandia, V. Gonzalez-Marrachelli, A. Domingo-Relloso, M. Galvez-Fernandez, M. Grau-Perez, P. Olmedo, et al., Healthy lifestyle, metabolomics and incident type 2 diabetes in a population-based cohort from Spain, Int. J. Behav. Nutr. Phys. Activ. 19 (1) (2022) 8.
[20] M. Sotos-Prieto, R. Ortolá, M. Ruiz-Canela, E. Garcia-Esquinas, D. Martínez-Gómez, E. Lopez-Garcia, et al., Association between the Mediterranean lifestyle, metabolic syndrome and mortality: a whole-country cohort in Spain, Cardiovasc. Diabetol. 20 (5) (2021) 12.
[21] J. Maroto-Rodriguez, M. Delgado-Velandia, R. Ortolá, E. García-Esquinas, D. Martinez-Gomez, E.A. Struijk, et al., A Mediterranean Lifestyle and Frailty Incidence in Older Adults: the Seniors-ENRICA-1 Cohort, The Journals of Gerontology: Series A, 2021, p. glab292.
[22] F. Rodríguez-Artalejo, A. Graciani, P. Guallar-Castillón, L.M. León-Muñoz, M. C. Zuluaga, E. López-García, et al., Rationale and methods of the study on nutrition and cardiovascular risk in Spain (ENRICA), Rev Esp Cardiolog English ed 64 (10) (2011) 876–882.
[23] P. Guallar-Castillón, J. Sagardui-Villamor, T. Balboa-Castillo, A. Sala-Vila, M. J. Ariza Astolfi, M.D. Sarrión Pelous, et al., Validity and reproducibility of a Spanish dietary history. Tomé D, editor, PLoS One 9 (1) (2014), e86074.
[24] N.J. Wareham, R.W. Jakes, K.L. Rennie, J. Schuit, J. Mitchell, S. Hennings, et al., Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study, Publ. Health Nutr. 6 (4) (2003) 407–413.
[25] M.A. Martínez-González, C. López-Fontana, J.J. Varo, A. Sánchez-Villegas, J. A. Martinez, Validation of the Spanish version of the physical activity questionnaire used in the Nurses' health study and the health professionals' follow-up study, Publ. Health Nutr. 8 (7) (2005) 920–927.
[26] M. Sotos-Prieto, G. Santos-Beneit, P. Bodega, S. Pocock, J. Mattei, J.L. Peñalvo, Validation of a questionnaire to measure overall Mediterranean lifestyle habits for research application: the MEDiterranean LIFEstyle index (MEDLIFE), Nutr. Hosp. (3) (2015) 1153–1163.

[27] J.R. Banegas, A. Graciani, J.J. de la Cruz-Troca, L.M. León-Muñoz, P. Guallar-Castillón, A. Coca, et al., Achievement of cardiometabolic goals in aware hypertensive patients in Spain: a nationwide population-based study, Hypertension 60 (4) (2012) 898–905.
[28] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin. Chem. 18 (6) (1972) 499–502.
[29] L.B. Daniels, P. Clopton, G.A. Laughlin, A.S. Maisel, E. Barrett-Connor, Growth-Differentiation factor-15 is a robust, independent predictor of 11-year mortality risk in community-dwelling older adults: the rancho bernardo study, Circulation 123 (19) (2011) 2101–2110.
[30] S. Xie, L. Lu, L. Liu, Growth differentiation factor-15 and the risk of cardiovascular diseases and all-cause mortality: a meta-analysis of prospective studies, Clin. Cardiol. 42 (5) (2019) 513–523.
[31] T. Fuchs, J.N. Trollor, J. Crawford, D.A. Brown, B.T. Baune, K. Samaras, et al., Macrophage inhibitory cytokine-1 is associated with cognitive impairment and predicts cognitive decline - the Sydney Memory and Aging Study, Aging Cell 12 (5) (2013) 882–889.
[32] K.M. Eggers, T. Kempf, L. Wallentin, K.C. Wollert, L. Lind, Change in growth differentiation factor 15 concentrations over time independently predicts mortality in community-dwelling elderly individuals, Clin. Chem. 59 (7) (2013) 1091–1098.
[33] F. Zhu, B. Du, B. Xu, Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: a review, Crit. Rev. Food Sci. Nutr. 58 (8) (2018) 1260–1270.
[34] M.F. Juárez-Chairez, O.G. Meza-Márquez, Y.K. Márquez-Flores, C. Jiménez-Martínez, Potential anti-inflammatory effects of legumes - a review, Br. J. Nutr. (2022) 1–36.
[35] K. Esposito, C.M. Kastorini, D.B. Panagiotakos, D. Giugliano, Prevention of type 2 diabetes by dietary patterns: a systematic review of prospective studies and meta-analysis, Metab. Syndr. Relat. Disord. 8 (6) (2010) 471–476.
[36] R. Iqbal, M. Dehghan, A. Mente, S. Rangarajan, A. Wielgosz, A. Avezum, et al., Associations of unprocessed and processed meat intake with mortality and cardiovascular disease in 21 countries [Prospective Urban Rural Epidemiology (PURE) Study]: a prospective cohort study, Am. J. Clin. Nutr. 31 (2021) nqaa448.
[37] J.M. Hess, C.B. Stephensen, M. Kratz, B.W. Bolling, Exploring the links between diet and inflammation: dairy foods as case studies, Adv. Nutr. 12 (Supplement_1) (2021) 1S–13S.
[38] A. Bordoni, F. Danesi, D. Dardevet, D. Dupont, A.S. Fernandez, D. Gille, et al., Dairy products and inflammation: a review of the clinical evidence, Crit. Rev. Food Sci. Nutr. 57 (12) (2017) 2497–2525.
[39] K.M. Nieman, B.D. Anderson, C.J. Cifelli, The effects of dairy product and dairy protein intake on inflammation: a systematic review of the literature, J. Am. Coll. Nutr. 40 (6) (2021) 571–582.
[40] S.M. Ulven, K.B. Holven, A. Gil, O.D. Rangel-Huerta, Milk and dairy product consumption and inflammatory biomarkers: an updated systematic review of randomized clinical trials, Adv. Nutr. 10 (suppl_2) (2019) S239–S250.
[41] M.S. Hershey, A. Fernandez-Montero, M. Sotos-Prieto, S. Kales, A. Gea, L. Ruiz-Estigarribia, et al., The association between the mediterranean lifestyle index and all-cause mortality in the seguimiento Universidad de Navarra cohort, Am. J. Prev. Med. 59 (6) (2020) e239–e248.
[42] D.E.R. Warburton, S.S.D. Bredin, Health benefits of physical activity: a systematic review of current systematic reviews, Curr. Opin. Cardiol. 32 (5) (2017) 541–556.
[43] R. Ortolá, E. García-Esquinas, A. Buño-Soto, V. Cabanas-Sánchez, D. Martínez-Gómez, M. Sotos-Prieto, et al., Associations of device-measured sleep, sedentariness and physical activity with growth differentiation factor 15 in older adults, J Cachexia Sarcopenia Muscle (2022) 113 (6): 1619-1626 jcsm.12924.
[44] C.S. Kwok, E. Kontopantelis, G. Kuligowski, M. Gray, A. Muhyaldeen, C.P. Gale, et al., Self-reported sleep duration and quality and cardiovascular disease and mortality: a dose-response meta-analysis [Internet] J. Am. Heart Assoc. (2018) 7 (15): e008552[cited 2022 Feb 28];7(15). Available from: https://www.ahajournals.org/doi/10.1161/JAHA.118.008552.
[45] C. Krittanawong, A. Tunhasiriwet, Z. Wang, H. Zhang, A.M. Farrell, S. Chirapongsathorn, et al., Association between short and long sleep durations and cardiovascular outcomes: a systematic review and meta-analysis, Eur Heart J Acute Cardiovasc Care 8 (8) (2019) 762–770.
[46] M.H. Hall, S.F. Smagula, R.M. Boudreau, H.N. Ayonayon, S.E. Goldman, T. B. Harris, et al., Association between sleep duration and mortality is mediated by markers of inflammation and health in older adults: the health, aging and body composition study, Sleep 38 (2) (2015) 189–195.
[47] M. Urpi-Sarda, R. Casas, G. Chiva-Blanch, E.S. Romero-Mamani, P. Valderas-Martínez, S. Arranz, et al., Virgin olive oil and nuts as key foods of the Mediterranean diet effects on inflammatory biomarkers related to atherosclerosis, Pharmacol. Res. 65 (6) (2012) 577–583.
[48] A. Nani, B. Murtaza, A. Sayed Khan, N.A. Khan, A. Hichami, Antioxidant and anti-inflammatory potential of polyphenols contained in mediterranean diet in obesity: molecular mechanisms, Molecules 26 (4) (2021) 985.
[49] J. Dupont, L. Antonio, L. Dedeyne, T.W. O'Neill, D. Vanderschueren, G. Rastrelli, et al., Inflammatory markers are associated with quality of life, physical activity, and gait speed but not sarcopenia in aged men (40–79 years), J Cachexia Sarcopenia Muscle (2021), 12 (6): 1818-1831.
[50] E. Wawrzyniak-Gramacka, N. Hertmanowska, A. Tylutka, B. Morawin, E. Wacka, M. Gutowicz, et al., The association of anti-inflammatory diet ingredients and lifestyle exercise with inflammaging, Nutrients 13 (11) (2021) 3696.
[51] B.N. Uchino, R. Trettevik, R.G. Kent de Grey, S. Cronan, J. Hogan, B.R.W. Baucom, Social support, social integration, and inflammatory cytokines: a meta-analysis, Health Psychol. 37 (5) (2018) 462–471.
[52] K.J. Smith, S. Gavey, N.E. Riddell, P. Kontari, C. Victor, The association between loneliness, social isolation and inflammation: a systematic review and meta-analysis, Neurosci. Biobehav. Rev. 112 (2020) 519–541.
[53] S.F. Smagula, K.L. Stone, S. Redline, S. Ancoli-Israel, E. Barrett-Connor, N.E. Lane, et al., Actigraphy- and polysomnography-measured sleep disturbances, inflammation, and mortality among older men, Psychosom. Med. 78 (6) (2016) 686–696.
[54] M. Sotos-Prieto, B. Moreno-Franco, J.M. Ordovás, M. León, J.A. Casasnovas, J. L. Peñalvo, Design and development of an instrument to measure overall lifestyle habits for epidemiological research: the Mediterranean Lifestyle (MEDLIFE) index, Publ. Health Nutr. 18 (6) (2014) 959–967.