Anthropometric variables as mediators of the association of changes in diet and physical activity with inflammatory profile

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Abbreviations list

- PA= physical activity
- MedDiet= Mediterranean Diet
- BMI= body mass index
- WC= waist circumference
- hs-CRP= high-sensitivity C-reactive protein
- IL-6= interleukin 6
- IL-8= interleukin 8
- IL-18= interleukin 18
- MCP-1= monocyte chemo-attractant protein-1
- RANTES= regulated on activation, normal T-cell expressed and secreted chemokine
- MVPA= moderate-to-vigorous physical activity
- CVD= cardiovascular disease
- SD= standard deviations
- MET= Metabolic Equivalent Task
- MET-min/d= metabolic equivalent task-minutes per day
- LDV= lowest detectable value

Abstract

BACKGROUND Mechanisms underlying the associations of high levels of physical activity (PA) and adherence to the Mediterranean Diet (MedDiet) with a better inflammatory profile remain unclear. Our objective was to assess the mediating role of changes in body mass index (BMI) and waist circumference (WC), as markers of body fat in the association of changes in PA and adherence to the MedDiet, with changes in the inflammatory profile.

METHODS This study included 489 adults, aged 55 to 75 years, from the PREDIMED-Plus multi-centre lifestyle intervention trial. An inflammatory score was calculated, based on 8 blood biomarkers: high-sensitivity C-reactive protein, interleukin 6, interleukin 8, interleukin 18, monocyte chemo-attractant protein-1, C-peptide, leptin, and regulated on activation, normal T-cell expressed and secreted chemokine (RANTES). Biomarkers, levels of PA, score of MedDiet adherence, BMI and WC were measured at baseline and at one-year follow-up. Linear regression models were fitted according to the Baron and Kenny framework for mediation analysis.

RESULTS Changes in BMI and WC mediated the association of both changes in PA and changes in the MedDiet adherence with the inflammatory score. BMI mediated 26% of the association of changes in total PA with the inflammatory profile, and 27% of the association of changes in the MedDiet, while WC mediated 13% and 12% of these associations, respectively.

CONCLUSION In older adults at high cardiovascular risk, increasing PA levels and adherence to a MedDiet during 1 year were associated with a lower inflammatory score, which was partly mediated by a reduction in body fat.

International Standard Randomized Controlled Trial registry (ISRCTN89898870; registration date 24/07/2014). Retrospectively registered.

Keywords

Body mass index, waist circumference, Mediterranean Diet, inflammation, mediation analysis.

Introduction

Chronic low-grade inflammation has been extensively associated with the pathogenesis of chronic diseases (1,2) and is a hallmark of aging. Therefore, it is imperative to identify the determinants and underlying mechanisms involved in low-grade inflammation. This is particularly relevant in older adults with increased body fat, because aging and obesity are independent risk factors for low-grade inflammation (3,4).

Lifestyle factors have the potential to act on systemic inflammation levels. Evidence indicates that physical activity (PA) interventions are effective at decreasing chronic inflammation in the general population (5,6). In older adults, prospective studies have shown inconsistent results (7–9) and the few available intervention trials have included a relatively small sample size (10–12). The Mediterranean diet (MedDiet) has been associated with lower levels of inflammation (13–18) and lower rates of inflammation-related diseases (19–21) in both midlife and older adults.

Although higher PA levels and MedDiet adherence are associated with a better inflammatory profile, the mechanisms underlying these associations remain unclear. Evidence indicates that these two lifestyle factors are strongly associated with a healthy body mass index (BMI) and waist circumference (WC) (22–24). On the other hand, anthropometric variables reflecting body fat have also shown to be directly associated with low-grade inflammation (25–29). It is therefore plausible to hypothesize that changes in BMI and WC could mediate the association between changes in PA/MedDiet adherence and changes in low-grade inflammation. However, to the best of our knowledge, only Park and colleagues have assessed this hypothesis with a cross-sectional study in the general population (30).

The present study aimed to assess, in a 12-month period, the extent to which changes in BMI and in WC mediate the association of changes in PA levels and MedDiet adherence with changes in an array of inflammatory plasma biomarkers in subjects aged 55 to 75 years. This is particularly relevant in older adults with increased body fat, because aging and obesity are independent risk factors for low-grade inflammation.

Methods

Study design

This was a prospective study nested in the on-going PREDIMED-Plus clinical trial. A detailed description of the study protocol has been published elsewhere (31,32) and further information can be found at http://predimedplus.com/. The effect of the interventions on inflammation in the PREDIMED-Plus pilot study has also been described elsewhere (33). In short, the PREDIMED-Plus is a 6-year, multi-centre, randomized controlled trial conducted in Spain assessing the effect of a lifestyle intervention on the primary prevention of cardiovascular diseases (CVD). Participants were randomly allocated to one of two groups: an intensive weight-loss intervention program, composed of an energy-restricted MedDiet, PA promotion, and behavioural support, or the control group, receiving traditional health care and a MedDiet recommendation without energy restriction. This clinical trial was registered at the International Standard Randomized Controlled Trial registry (ISRCTN89898870; registration date 24/07/2014).

Study participants

From October 2013 to December 2016, 6874 participants were recruited from 23 health centers in Spain. Men aged 55 to 75 years and women aged 60 to 75 years at high risk

of CVD were included if they had overweight or obesity (BMI ≥27 and <40 kg/m^2) and met at least 3 components of Metabolic Syndrome diagnostic criteria, defined according to the International Diabetes Federation and the American Heart Association and National Heart, Lung and Blood Institute (34).

Of the first 1013 participants assessed for eligibility, 143 declined to participate, 36 met an exclusion criterion, and 136 did not meet inclusion or randomization criteria. Of the remaining 698 participants, 70 were excluded due to a protocol change in the pre-randomization requirements and 2 were excluded due to a cancer diagnosis. Finally, 134 participants with missing data in the variables included in the present study (at baseline and/or one-year follow-up) were excluded. Three participants reporting extreme changes in PA (>3 standard deviations from the mean) were excluded from the final sample. The final sample included 489 participants, with a mean age of 65.5±4.8 years **(Figure 1 in the Supplement).**

The Research Ethics Committees of all participating centres approved the study protocol, which was conducted following the standards of the Declaration of Helsinki. All participants provided written informed consent.

Assessment of the independent variables

The Registre Gironí del Cor (REGICOR) short-PA questionnaire, validated in Spain (35), was used to measure PA levels. This questionnaire assesses four dimensions of PA: frequency (days per conventional month), duration (minutes per day), intensity (MET [Metabolic Equivalent Task] assigned to each activity), and type (walking at a slow pace [4 METs], gardening [5 METs], brisk walking [5 METs], walking in the countryside [6 METs], climbing stairs [7 METs], and playing sports [11 METs]) (36). Total energy expenditure was quantified as MET minutes per day (MET-min/d),

calculated as the sum of the intensity, duration, and frequency assigned to each activity, divided by 30. The PA was later classified as light (<4 METs), moderate (4 to 5.5 METs), vigorous (≥6 METs), and moderate-to-vigorous physical activity (MVPA, >4 METs). Adherence to an energy-restricted MedDiet was assessed using the 17-item energy-restricted Mediterranean Diet Adherence screener (er-MEDAS), as a modified version of the validated 14-item Mediterranean Diet Adherence Screener (MEDAS) (37) which is used to assess adherence to a non-restricted MedDiet. The modified 17-item screener displays more restrictive cut-offs for caloric-dense items and additional items has been added focusing on reducing caloric intake (32).The list of items and its scoring criteria are presented in **Supplemental Table 5.** The total score ranges from 0 to 17, with higher scores indicating a higher adherence. Both exposure variables, PA and MedDiet, were collected by interview by trained dieticians at baseline and at one-year follow-up.

Assessment of the outcome variable

At baseline and at one-year follow-up, trained nurses collected 12-hour overnight fasting blood samples from participants. Samples were kept at -80 ºC until they were analyzed in a central laboratory. Interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 18 (IL-18), monocyte chemo-attractant protein-1 (MCP-1), C-peptide, leptin, and regulated on activation, normal T-cell expressed and secreted chemokine (RANTES) were simultaneously measured in serum with bead-based multiplexing technology using an XMAG-Luminex assay (Biorad, Hercules, California, USA) and serum levels of high-sensitivity C-reactive protein (hs-CRP) were measured using a wide-range latex-enhanced immunoturbidimetric assay on an ADVIA 2400 analyzer (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). The lowest detectable values (LDVs) for IL-6,

IL-8, IL-18, MCP-1, C-peptide, hs-CRP, leptin, and RANTES were 0.34 pg/ml, 0.36 pg/ml, 0.29 pg/ml, 0.4 pg/ml, 0.09 ng/ml, 0.4 mg/L, 0.88 ng/ml, and 0.19 ng/ml, respectively. Values below this LDV were imputed as LDV/2. IL-6, C-peptide, leptin, and hs-CRP had 101, 1, 1, and 22 values imputed at baseline and 114, 1, 1, and 28 values imputed at one-year follow-up, respectively.

Assessment of potential mediators

Anthropometric variables were measured by trained nurses following the established PREDIMED-Plus protocols. The participants wore light clothing and no shoes. A wall-mounted stadiometer and an electronic scale were used to measured height and weight, respectively. Body mass index was calculated by dividing the weight (kg) by the height squared (m^2). Waist circumference was measured midway between the lowest rib and the iliac crest after a normal exhalation, using an anthropometric non-elastic tape.

Assessment of confounders

Socio-demographic information (age, sex, and educational level) and smoking status were collected at baseline using a general questionnaire. Education was dichotomized as more or less than primary school completion. Smoking was dichotomized as smoker or non-smoker. Smokers included current smokers or individuals who had stopped smoking less than a year prior to the study baseline.

Statistical analysis

Change in the inflammatory score was obtained as follows: i) quintiles of each biomarker were obtained at baseline and after one-year follow-up, using the same cut-points (**Table 1 in the Supplement**), ii) the sum of the quintiles of the eight biomarkers

was calculated at baseline and at one-year follow-up, obtaining a baseline and a follow-up inflammatory score, and iii) the difference between the baseline and the follow-up inflammatory score was finally calculated. The score ranged from 8 (being in the first quintile for all biomarkers) to 40 (being in the fifth quintile for all biomarkers). Through the inspection of histograms and Q-Q plots, a normal distribution was observed for the baseline inflammatory score, follow-up inflammatory score, and the changes in the inflammatory score.

Linear regression models were fitted to analyse the associations of changes in PA (total, light and moderate to vigorous) and in MedDiet adherence with changes in the inflammatory score. Models included sex, age, intervention group, smoking status, educational level, trial centre, changes in the other lifestyle factor (models using changes in MedDiet score as the independent variable were adjusted for changes in total PA and vice versa), baseline levels of the corresponding independent and outcome variables, and, when appropriate, by changes in the intensities of PA. Including interaction terms in the linear regression models allowed the assessment of the influence of sex, age (≤65 and >65 years old), educational level (more or less than primary school completion), and study group (weight loss intervention or control) in the association between changes in the two lifestyle factors analyzed and changes in the inflammatory score. To detect if BMI and WC were potential mediators, we further adjusted the models for either BMI or WC. We primarily presented unstandardized coefficients, but also presented standardized coefficients as Supplemental material to compare the magnitude of the associations of PA and MedDiet. For this purpose, we standardized the continuous variables (changes in total PA, MVPA, light PA, and adherence to the MedDiet) and ran the models using the resulting z-scores.

Mediation analysis was performed to study the extent to which anthropometric variables (BMI and WC) were responsible for the association between changes in lifestyle factors (PA and MedDiet) and changes in the inflammatory profile. This analysis was based on the standardized steps proposed by Baron and Kenny (38) **(Figure 1 and Figure 2).** The following models were fitted: i) a linear regression assessing the association between changes in PA and in the inflammatory score, excluding the mediators (changes in BMI or in WC) from the model to check that there is an effect that may be mediated (path c); ii) a linear regression assessing the association between changes in PA and in the anthropometric markers (BMI or WC), excluding the inflammatory score from the model (path a); and iii) a linear regression assessing the association between change in the anthropometric markers (BMI or WC) and in the inflammatory score, with additional adjustment for changes in PA (path b). The same procedure was repeated using changes in light PA, in MVPA, and in MedDiet adherence as the independent variables. To assess the presence of collinearity between the independent variables included in the models, the variance inflation factor was calculated.

The existence of mediation was determined by analysing the direct effect, which represents the association between the independent and dependent variable while the mediator is held constant (path c'), and the indirect effect (path a * path b) which represents the amount of mediation exerted by the mediator variable (BMI or WC) in the association between the independent and dependent variable.

We further estimated the proportion mediated by BMI and WC, dividing the indirect effect by the sum of the direct and indirect effect. To calculate the significance of these estimations, confidence intervals (CIs) were obtained from bootstrapping analysis (1000 replications). Since the aim of this study was not to analyze the role of the intervention in the studied mediation, specific methods to address potential bias related to mediation

analysis in randomized clinical trials (39,40) were not required. Nevertheless, we tested the potential influence of the intervention arm by including an interaction term in one set of models, and by performing a sensitivity analysis removing the intervention variable from the models. Additionally, we performed sensitive analysis to test for bias due to missing data.

Associations were considered significant if $p<0.05$. Mediation was assessed by the R package "mediation" version 4.4.6. All the statistical analysis was performed with R, version 3.0.2.

Results

After one year of follow-up, participants reported a mean (standard deviation (SD)) change in total PA of +67 (372) MET-min/d (+87 (427) MET-min/d in men and +49 (314) MET-min/d in women). The mean change in light PA and MVPA was +6 (159) MET-min/d and +61 (362) MET-min/d, respectively, while the mean change in MedDiet adherence was 2.5 (3.1) points. Baseline characteristics of the participants are outlined in **Table 1**. The median (inter-quartile ranges) of the baseline levels of total, light, and moderate to vigorous PA were 316 (130 to 539), 64 (0 to 160), and 195 (40 to 412) METs-min/d, while the mean (SD) for the baseline levels of MedDiet score was 8.9 (2.5). At follow-up, those values were 375 (212 to 627), 80 (0 to 200), 247(44 to 503) for total, light, and moderate to vigorous PA, and 11.5 (2.8) for the MedDiet.

Changes in total PA were inversely associated with changes in the inflammatory profile, adjusted for sex, age, intervention group, smoking status, educational level, study centre, changes in MedDiet adherence, baseline PA levels and baseline inflammatory score (path c) **(Table 2)**. When additionally adjusting by BMI or WC, the magnitude of

the association decreased, but remained significant. Similar findings were observed when changes in total PA were replaced by changes in MVPA and in MedDiet adherence. To analyse the interaction between the exposure and sex, an interaction term was added in each of the 3 models presented in **Table 2**. Therefore, a total of 6 models was fitted: 3 models with changes in total PA and 3 models with changes in MedDiet as exposure variable. The same procedure was followed for the test of interaction with age, education, and intervention group. This analysis showed that the association between changes in the studied lifestyle factors and in the inflammatory score was not influenced by sex, age, education, or intervention group (p value of all interaction terms >0.05, data not shown). The standardized coefficients showed that changes in total, MVPA and in MedDiet adherence were associated with changes in the inflammatory profile to a similar extent **(Table 2 in the Supplement)**.

A summary of the mediation analysis is depicted in **Figure 1.** There was a significant association between changes in total PA and changes in BMI and WC (β coefficient -0.08, and -0.26 respectively, path a) and between changes in these anthropometric markers and in the inflammatory score (β coefficient 0.63 and 0.10, respectively, path b)**.** Regarding PA intensity, changes in MVPA were inversely associated with changes in BMI and in WC, whereas changes in light PA were only associated with changes in WC **(Table 3 in the Supplement)**. Similar significant associations were found when changes in MedDiet adherence were used as the independent variable. The VIF of each of the variables included in models a, b, and c was below 2 (data not shown).

After observing the statistically significant associations in paths a, b and c - with the exception of the non-significant association between changes in light PA and changes in BMI- we proceeded to fit the mediation model. Changes in BMI significantly mediated the association between changes in total PA and those in the inflammatory score,

explaining 26% (CI: 5 to 85 %) of the overall association **(Table 3)**. Additionally, a direct effect was observed between changes in total PA and those in the inflammatory score [β coefficient (95% CI) = -0.13 (-0.25 to -0.01)]. Changes in BMI mediated the association of changes in MVPA and in MedDiet adherence with changes in the inflammatory score [24% (CI: 4 to 94 %) and 27% (CI: 13 to 100%), respectively]. Changes in WC also mediated the association of changes in total PA, MVPA, and MedDiet adherence with changes in the inflammatory profile, accounting for 13% (CI: 4 to 44 %), 11% (CI: 3 to 43 %), and 12% (CI: 3 to 36 %) of the association, respectively. When removing the intervention arm from the mediation model, we observed a moderated increase in the proportion mediated by BMI and a moderate decrease in those mediated by WC. For example, in the association between changes total PA and changes in the inflammatory score the proportion mediated by changes in BMI increased from 26 to 32% and in the association between changes in the MedDiet and in the inflammatory score the proportion mediated by changes in BMI decreased from 27 to 12%.

(Table 4 in the Supplement).

Discussion

In this prospective study, we assessed the role of markers of global and abdominal adiposity as potential mediators of the association between changes in two lifestyle factors and in the inflammatory profile in older adults with metabolic syndrome. Body mass index acted as a partial mediator of the association of changes in total PA and in MedDiet adherence with changes in the inflammatory profile (the proportion mediated

was 26% and 27%, respectively). To a lesser extent, waist circumference also acted as a partial mediator of these associations (13% and 12%, respectively).

Despite the known associations of diet and physical activity with inflammation, and of adiposity with both lifestyle and inflammation, the mediating role of anthropometric variables in the association between lifestyle and inflammation has been little studied. Most studies have reported associations of either PA or dietary intake with inflammatory markers independently of anthropometric factors but did not evaluate mediation per se (5,27,41).

One study by Fedewa and colleagues (5) analysed anthropometric markers as moderators of the causal effect between PA interventions and CRP levels in a meta-analysis of 83 intervention trials. They found that when PA interventions yielded a decrease in BMI, the magnitude of CRP reduction was greater than when PA interventions did not result in decreased BMI; nonetheless, the CRP decrease was significant in both BMI groups. Similarly, Richard and colleagues (18) reported that a high MedDiet adherence effectively reduced inflammation, and this effect was amplified when WC decreased. These findings could indicate that changes in BMI and in WC explain partly the association between PA/MedDiet adherence and inflammation. In the same line, the present study showed that the association between changes in PA/MedDiet adherence and in inflammation was reduced after adjusting for changes in BMI or in WC, supporting the hypothesis that changes in BMI and/or WC could be a mechanism by which PA/MedDiet adherence is related to inflammation.

To the best of our knowledge, only Park and colleagues (30) have performed a mediation analysis, although they used an observational cross-sectional design. In 4700 individuals aged 20 to 90 years, they assessed BMI and WC mediation of a cross-sectional association between MedDiet adherence and inflammatory markers. They

found that WC mediated the association of MedDiet adherence with white blood cell count and fibrinogen (16.9% and 9.6%, respectively), while BMI mediated a nonsignificant proportion of these associations (13.1% and 7.6%, respectively). On the contrary, in the present study we found a greater mediation effect of BMI than WC. This finding was not expected because the accumulation of visceral fat has shown a greater correlation with inflammation, compared to the accumulation of general body fat (42). One possible explanation is the greater inaccuracy in WC measurements in comparison with height and weight alone, particularly in subjects with abdominal obesity (43). Moreover, despite commonly thought to be a good marker of visceral fat, WC is also a marker of abdominal subcutaneous fat (44). This is supported by Mayr and colleagues (22), who found that a MedDiet intervention was associated with a decrease in WC but not in visceral fat. This is important because the changes in WC observed in our study were not necessarily related to visceral adipose tissue, which is more closely associated with inflammation than is general adipose tissue.

The non-significant association between changes in light PA and changes in the inflammatory score could be explained by the minor changes reported in light PA during the study period (mean change +6 (159) MET-min/d). On the other hand, previous studies (45,46) have also shown that light PA is not consistently associated with inflammation; therefore, the recommendation of moderate and vigorous PA when aiming to reduce the levels of inflammation seems more appropriate.

Adipose tissue has endocrine functions and is an active secretor of pro-inflammatory and chemotactic compounds (47). Our findings add further evidence to the notion that changes in surrogate markers of body fat play a crucial role in the mechanism involved in the association between changes in lifestyle factors and the inflammatory profile.

Nevertheless, it should be noted that BMI and WC mediated less than 30% of the associations studied. Several mechanisms other than changes in anthropometric variables could explain the association of changes in PA and MedDiet adherence with concurrent changes in the inflammatory profile. According to Bailey and Holscher (48), changes in the gastrointestinal microbiota and a decrease in the circulating levels of bacterial endotoxins could be two additional mechanisms explaining the association between a high adherence to the MedDiet and inflammation. On the other hand, Casas and colleagues (16,17) suggested that the synergistic effect of key foods in the MedDiet, including nuts and extra virgin olive oil, could explain this anti-inflammatory effect. Mechanisms that could explain the association between PA and inflammation include a PA-related antioxidant and antiatherogenic effect (49), an improved endothelial function, and enhanced insulin sensitivity (50). Finally, it has been suggested that high levels of PA and MedDiet adherence are associated with a reduction in the expression of pro-inflammatory and pro-atherogenic genes, including epigenetic changes that are among the mechanisms responsible for the decrease in inflammation (51,52).

The use of eight biomarkers and the prospective nature of the analysis are strengths of the study. The main limitations in this study were i) the potential multiplicity derived by the simultaneous testing of multiple mediation hypothesis, ii) the potential bias induced by the indirect nature of the measurements of abdominal obesity, and iii) the alternative analysis, other than mediation, that could address the complex association between lifestyle behaviours, anthropometric markers, and inflammation that were not tested in this study. Finally, although using self-reported questionnaires to measure PA and adherence to the MedDiet can lead to errors and misclassification, the errors were most likely random, which would underestimate our results.

In conclusion, this study showed that changes in PA and in MedDiet adherence, associated with changes in the inflammatory score, were partly mediated by changes in anthropometric measures. The monitoring of surrogate markers of body fat, in addition to the promotion of PA and MedDiet adherence, could be an effective strategy to control the inflammatory profile.

Declarations

a) Funding

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b) Conflicts of interest/Competing interests

The authors declare that they have no competing interests.

c) Ethics approval (include appropriate approvals or waivers)

The Research Ethics Committees of all participating centres approved the study protocol, which was conducted following the standards of the Declaration of Helsinki.

d) Consent to participate (include appropriate statements)

All participants provided written informed consent.

e) Consent for publication (include appropriate statements)

Not aplicable

f) Availability of data and material (data transparency)

The data that support the findings of this study are available from the corresponding author, HS and MF, upon reasonable request.

g) Code availability (software application or custom code)

Not aplicable

h) Authors' contributions

HS, MF and GC designed research; CL, JW, MG, JS-S, MAMG, DC, LSM, and JK conducted research; RE, JAM, XP, CV, JV, JAT, ADL, and HL provided essential reagents; HS and GC analysed data; HS and GC wrote the paper; HS, MF and GC had primary responsibility for final content. All authors read and approved the final manuscript.

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Table 1 Baseline characteristics of the study participants (n=489) [a]

Characteristic	
Women, n	259 (53.0%)
Age, years	65.5 (65.0 to 65.9)
Study group, n	
-Intervention	256 (52.4%)
-Control	233 (47.6%)
More than primary education, n	237 (48.5%)
BMI, kg/m^2 [b]	32.4 (32.1 to 32.7)
WC, cm	106.8 (106.0 to 107.7)
Smokers, n [c]	68 (13.9%)
MedDiet score [d]	8.9 (8.7 to 9.1)
PA, MET-min/d	
-Total	316 (130 to 539)
-Light	64 (0 to 160)
-Moderate to vigorous	195 (40 to 412)
Markers of inflammation	
IL-6 pg/ml	1.33 (0.55 to 2.15)
IL-8 pg/ml	8.07 (5.91 to 10.63)
IL-18 pg/ml	80.26 (58.10 to 107.49)
MCP-1 pg/ml	67.18 (47.44 to 88.37)
C- peptide ng/ml	1.55 (1.50 to 1.60)
Hs-CRP mg/L	2.37 (1.28 to 4.95)
Leptin ng/ml	14.80 (8.21 to 26.34)
RANTES ng/ml	10.13 (9.99 to 10.28)

Note. BMI = Body mass index, hs-CRP = High-sensitivity C-reactive protein, IL-6 = Interleukin 6, IL-8 = Interleukin 8, IL-18 = Interleukin 18, MCP-1= Monocyte chemo-attractant protein-1, MedDiet score = Adherence to an energy-restricted Mediterranean diet, MET-min/d= Metabolic equivalent of task minutes per day, mg/L = Milligram per liter, ng/ml = Nanogram/ millilitre, PA= Physical activity, pg/ml = Picogram/ millilitre, RANTES = Regulated on activation, normal T-cell expressed and secreted chemokine, WC= Waist circumference.

[a] Categorical, continuous normal, and continuous non-normal distributed variables are expressed as number (proportion), mean (confidence interval), and median (interquartile range), respectively.

[b] BMI was calculated by dividing the weight (kilograms) by the square of the height (meters).

[c] Smokers included current smokers and ex-smokers who stopped smoking less than a year before baseline.

[d] MedDiet score ranges from 0 (minimum adherence) to 17 (maximum adherence).

*$p<0.05$ in bold typeface.

Table 2 Association between changes in lifestyle factors (adherence to the MedDiet and total, light, and MVPA) and changes in the inflammatory score [a]

	Δ Inflammatory score [b]		
	Model 1	Model 2	Model 3
	β coefficient (95% CI)	β coefficient (95% CI)	β coefficient (95% CI)
Δ PA (100 MET-min/d)			
-Total	**-0.19 (-0.30 to -0.07)**	**-0.14 (-0.26 to -0.03)**	**-0.17 (-0.29 to -0.06)**
-Light	0.005 (-0.29 to 0.30)	0.06 (-0.23 to 0.35)	0.04 (-0.25 to 0.33)
-Moderate to vigorous	**-0.19 (-0.31 to -0.08)**	**-0.15 (-0.27 to -0.04)**	**-0.18 (-0.29 to -0.06)**
Δ MedDiet score [c]	**-0.25 (-0.41 to -0.10)**	**-0.18 (-0.34 to -0.04)**	**-0.23 (-0.39 to -0.08)**

Note. MedDiet = Mediterranean diet; PA= Physical activity; MET-min/d= Metabolic equivalent of task minutes per day; β coefficient (95% CI) = β coefficients (95% confidence interval); BMI = Body mass index.

[a] Linear regression models were designed as follows: Model 1 was adjusted by sex, age, intervention group, smoking status, educational level, trial centre, changes in the other lifestyle factor (MedDiet adherence adjusted by PA and vice versa), baseline levels of the corresponding independent variable (adherence to the MedDiet and total, light, and moderate to vigorous PA), baseline level of the inflammatory score, and,

when appropriate, by changes in the different intensities of PA. Model 2 was adjusted for all covariates in Model 1 and for changes in BMI. Model 3 was adjusted by all covariates in Model 1 and for changes in waist circumference. Values indicate the β coefficient (95% CI) of the change in the inflammatory score, occurring with each increase in 100 MET-min/d of PA or 1 point in the adherence to the MedDiet. After including interaction terms of sex, age, intervention group, and education with the independent variable (i.e. sex* changes in PA) separately, none significant interaction effect was found.

[b] The inflammatory score ranged from 8 (minimum inflammatory state) to 40 (maximum inflammatory state).

[c] MedDiet score ranged from 0 (minimum adherence) to 17 (maximum adherence).

*$p<0.05$ in bold typeface.

16 **Table 3** Mediation analysis for the association between changes in lifestyle factors (MedDiet adherence and total, light, and moderate-to-

17 vigorous PA) and changes in the inflammatory score, through anthropometric measures [a].

Independent variable	**Mediator**	**Outcome variable** [b]	**Indirect effect**	**Direct effect**	**Proportion mediated**
			β coefficient (95% CI)	β coefficient (95% CI)	%
Δ PA (100 MET-min/d)					
-Total	Δ BMI	Δ Inflammatory score	**-0.05 (-0.08 to -0.01)**	**-0.13 (-0.25 to -0.01)**	**25.8 % (5.4 to 85.4 %)**
-Light	Δ BMI	Δ Inflammatory score	-0.06 (-0.12 to 0.00)	0.07 (-0.20 to 0.34)	11.8 % (-100.0 to 100.0 %)
-Moderate to vigorous	Δ BMI	Δ Inflammatory score	**-0.05 (-0.09 to -0.01)**	-0.14 (-0.26 to 0.00)	**23.7 % (4.2 to 93.7 %)**
Δ MedDiet score[c]	Δ BMI	Δ Inflammatory score	**-0.07 (-0.12 to -0.03)**	-0.18 (-0.32 to 0.00)	**26.7 % (13.3 to 100.0%)**
Δ PA (100 MET-min/d)					
-Total	Δ WC	Δ Inflammatory score	**-0.03 (-0.05 to -0.01)**	**-0.16 (-0.27 to -0.05)**	**12.8 % (4.0 to 43.8 %)**
-Light	Δ WC	Δ Inflammatory score	**-0.05 (-0.10 to 0.00)**	0.05 (-0.22 to 0.30)	13.9 % (-100.0 to 10.00 %)
-Moderate to vigorous	Δ WC	Δ Inflammatory score	**-0.02 (-0.05 to -0.01)**	**-0.17 (-0.29 to -0.04)**	**11.1 % (2.8 to 42.6 %)**
Δ MedDiet score [c]	Δ WC	Δ Inflammatory score	**-0.03 (-0.06 to -0.01)**	**-0.22 (-0.36 to -0.04)**	**11.8 % (3.4 to 36.2 %)**

Note. MedDiet = Mediterranean diet; PA= Physical activity; MET-min/d= Metabolic equivalent of task minutes per day; BMI = Body mass index; WC= Waist circumference; β coefficient (95% CI) = β coefficients (95% confidence interval).

[a] The following models were fitted for mediation analysis: 1) a linear regression with changes in total physical activity as the independent variable and changes in the inflammatory score as the outcome variable, excluding the mediator from the model,(path c) 2) a linear regression with changes in total physical activity as the independent variable and changes in the anthropometric marker (BMI or WC) as outcome variable, excluding the inflammatory score from the model (path a), and 3) a linear regression with changes in the anthropometric marker (BMI or WC) as independent variable and changes in the inflammatory score as outcome variable, adjusted by changes in total PA (path b). The same procedure was repeated using light PA, MVPA, and adherence to a MedDiet as independent variables. All three models were further adjusted by sex, age, intervention group, smoking status, educational level, trial centre, changes in the other lifestyle factor when one of them was used as the independent variable (MedDiet adherence by PA and vice versa), baseline levels of the corresponding independent and outcome variables, and. when appropriate, by changes in the intensities of PA. The direct effect represents the association between the independent and dependent variable while the mediator is held constant (path c'), and the indirect effect is calculated as path a * path b). Values indicate the β coefficient (95% CI) of the change in the inflammatory score, occurring with each increase in 100 MET-min/d of PA or 1 point in the adherence to the MedDiet.

[b] The inflammatory score ranged from 8 (minimum inflammatory state) to 40 (maximum inflammatory state).

[c] MedDiet score ranged from 0 (minimum adherence) to 17 (maximum adherence).

*p<0.05 in bold typeface.

Fig. 1 Schematic presentation of the mediation models. Waist circumference and body mass index as mediators of the association between changes in total physical activity and changes in the inflammatory score.

Adjusted by sex, age, intervention group, smoking status, educational level, trial centre, changes in the other lifestyle factor analysed (MedDiet adherence adjusted by PA and vice versa), and by baseline levels of the corresponding independent and outcome variable.

BMI = Body mass index; MedDiet = Mediterranean diet; PA= Physical activity; WC= Waist circumference; β = β coefficients; MET-min/d= Metabolic equivalent of task minutes per day

*= $p<0.05$; **= $p<0.001$; Italics= when waist circumference is used as mediator variable

Fig. 2 Schematic presentation of the mediation models. Waist circumference and body mass index as mediators of the association between changes in MedDiet adherence and changes in the inflammatory score.

Adjusted by sex, age, intervention group, smoking status, educational level, trial centre, changes in the other lifestyle factor analysed (MedDiet adherence adjusted by PA and vice versa), and by baseline levels of the corresponding independent and outcome variable.

61 BMI = Body mass index; MedDiet = Mediterranean diet; PA= Physical activity; WC= Waist circumference; β = β coefficients; MET-min/d=

62 Metabolic equivalent of task minutes per day

63 *= $p<0.05$; **= $p<0.001$; Italics= when waist circumference is used as mediator variable

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Figure 1

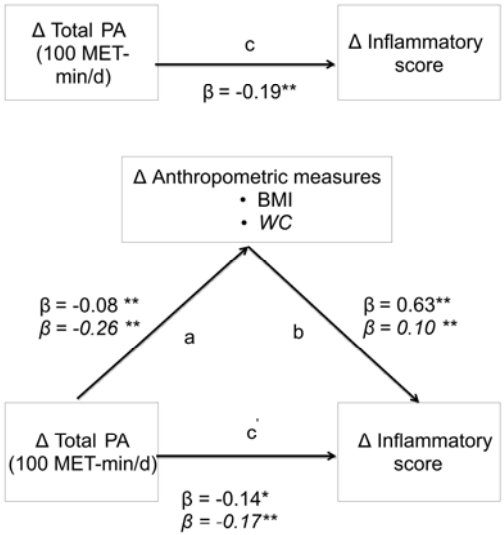
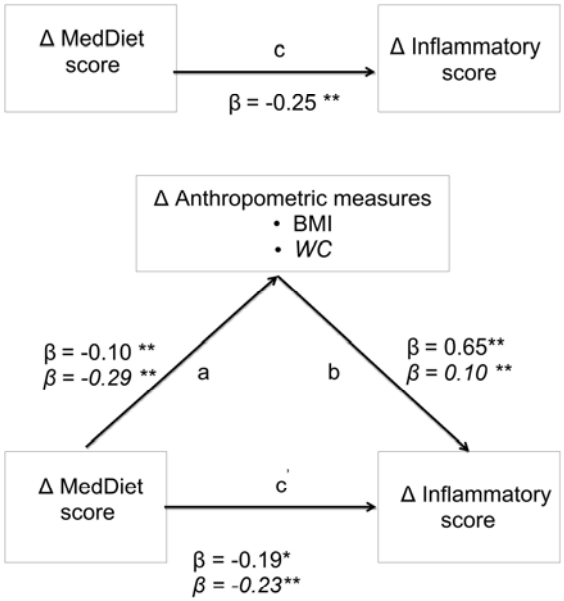

Δ Total PA (100 MET-min/d)
c
β = -0.19**
Δ Inflammatory score
Δ Anthropometric measures
• BMI
• WC
β = -0.08 **
β = -0.26 **
a
b
β = 0.63**
β = 0.10 **
Δ Total PA (100 MET-min/d)
c'
Δ Inflammatory score
β = -0.14*
β = -0.17**

Figure 2


Δ MedDiet score
c
β = -0.25 **
Δ Inflammatory score
Δ Anthropometric measures
• BMI
• WC
β = -0.10 **
β = -0.29 **
a
b
β = 0.65**
β = 0.10 **
Δ MedDiet score
c'
Δ Inflammatory score
β = -0.19*
β = -0.23**