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Prospective association of physical activity and inflammatory biomarkers in older adults from the PREDIMED-Plus Study with overweight or obesity and metabolic syndrome

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Short running head

Physical activity and inflammatory biomarkers

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- Number of figures: 0 (there are 2 supplemental figures)

IMPACT statement

1. We certify that this work is confirmatory of recent novel clinical research.
2. List of references to the relevant research that the work confirms.

a. Parsons TJ, Sartini C, Welsh P, et al. Physical Activity, Sedentary Behavior, and Inflammatory and Hemostatic Markers in Men. *Med Sci Sports Exerc*. 2017;49(3):459-465. doi:10.1249/MSS.0000000000001113.

b. LEE I-M, SESSO HD, RIDKER PM, MOUTON CP, STEFANICK ML, Manson JE. Physical Activity and Inflammation in a Multiethnic Cohort of Women. *Med Sci Sport*. 2012;44(6):1088-1096. doi:10.1249/MSS.0b013e318242b11a.

c. Wannamethee SG, Lowe GDO, Whincup PH, Rumley A, Walker M, Lennon L. Physical Activity and Hemostatic and Inflamatory Variables in Elderly Men. *Circulation*. 2002;105(15):1785-1790. doi:10.1161/01.CIR.0000016346.14762.71.

3. Statement about what this research specifically adds to the literature: We found an inverse association between changes in physical activity and changes in the inflammatory profile (a composite score of 8 inflammatory biomarkers) in older individuals at high cardiovascular risk. Our finding that moderate physical activity, independently of light and vigorous PA, was inversely associated with the inflammatory profile is important because moderate PA is a feasible option for many older adults, compared to a recommendation of vigorous activity.

Disclaimers, if any, and no conflict of interest

- The authors declare that they have no competing interests.

Abbreviations list

- 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
- MET-min/d= metabolic equivalent task-minutes per day
- MVPA= moderate-to-vigorous physical activity
- PA= physical activity
- PREDIMED-Plus= Prevención con dieta mediterránea plus
- RANTES= regulated on activation, normal T-cell expressed and secreted chemokine
- SD= standard deviations

Clinical Trial Registry

Clinical trial identifier: International Standard Randomized Controlled Trial 89898870.

Abstract

BACKGROUND There is limited prospective evidence on the association between physical activity (PA) and inflammation in older adults. Our aim was to assess the associations between changes in PA and changes in the inflammatory profile in older individuals who are overweight or obese.

METHODS This prospective study included 489 men and women, aged 55 to 75 years, from the PREDIMED-Plus trial. Levels of interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 18 (IL-18), monocyte chemo-attractant protein-1 (MCP-1), C-peptide, high-sensitivity C-reactive protein (hs-CRP), leptin, and regulated on activation, normal T-cell expressed and secreted chemokine (RANTES) were obtained from fasting blood samples and a composite inflammatory score based on these biomarkers was calculated. Physical activity was measured by a validated questionnaire. All measures were taken at baseline and one-year follow-up.

RESULTS Multiple linear regression models showed an association between an increase in total PA and a decrease in the inflammatory score (p=0.012), which was particularly driven by a decrease in C-peptide (p=0.037). Similarly, the inflammatory score decreased with increasing moderate PA (p=0.001), and moderate-to-vigorous PA (p=0.006).

CONCLUSIONS Increases in total PA, moderate and moderate-to-vigorous PA were associated with a decrease in the inflammatory profile of obese or overweight older

individuals. This finding is relevant for PA recommendations and public health strategies.

Keywords

Prospective study, light physical activity, moderate-to-vigorous physical activity, inflammation, and inflammatory score.

Introduction

Aging is associated with increased and persistent low-level inflammation (1) and obesity is associated with an increased pro-inflammatory state (2). Furthermore, strong evidence links low-grade inflammation with the pathogenesis of numerous chronic diseases, including dementia, cancer, and coronary heart disease (3–5). Therefore, it is paramount to identify the most appropriate strategies to improve the inflammatory profile.

Increased physical activity (PA) is associated with a decrease in the incidence of several chronic diseases in the general population (6) and in older adults (7,8). It has been suggested that these protective associations are partially due to the anti-inflammatory effect of PA (9).

In the general adult population, randomized clinical trials provide convincing evidence of the anti-inflammatory effect of PA in normal weight and overweight/obese individuals (10–13). However, the majority of trials have been short-term and show inconsistent results (14–18). Moreover, the interventions have mainly been composed of structured sessions of aerobic, resistance, and strength exercises that are difficult to carry out in everyday life, especially for older individuals who are overweight or obese (19).

There is limited and inconsistent prospective evidence on the association between PA and inflammatory markers in older adults. Two studies have shown no significant association between these two variables (20,21); however, an earlier study found an inverse association between changes in PA and levels of inflammatory markers (22). We therefore analysed the association of one-year changes in total, light, moderate, vigorous, and moderate-to-vigorous PA (MVPA) with concurrent changes in the inflammatory profile, measured by a composite inflammatory score, in older individuals

who are overweight or obese and have metabolic syndrome. Furthermore, we analysed the association of changes in PA with changes in the levels of IL-6, IL-8, IL-18, MCP-1, C-peptide, hs-CRP, leptin, and RANTES.

Methods

Study Subjects

Participants were men (aged 55-75 years) and women (aged 60-75 years) at high risk of cardiovascular disease. Inclusion criteria included being overweight or obese (body mass index [BMI] ≥ 27 and < 40 kg/m^2) and meeting at least three of the Metabolic Syndrome criteria: abdominal obesity (waist circumference > 102 cm in men or ≥ 88 cm in women), hypertriglyceridemia (triglycerides ≥ 1.7 mmol/L or taking triglycerides-lowering medication), low HDL-cholesterol (< 1.0 mmol/L in men or < 1.3 mmol/L in women or taking medication to raise HDL-cholesterol), hypertension (systolic blood pressure ≥ 130 and /or diastolic blood pressure ≥ 85 mmHg or using antihypertensive medication), and hyperglycaemia (glucose ≥ 5.5 mmol/L or taking glycaemia-lowering medication) (23).

Study Design

The present study was a prospective cohort analysis in a subpopulation (the first 1013 recruited candidates) of the PREDIMED-Plus (PREvención con DIeta MEDiterránea Plus) survey. A detailed description of the trial can be found at http://predimedplus.com (24). Briefly, PREDIMED-Plus is a parallel, multicentre, randomized controlled trial designed to analyse the effect of an intensive lifestyle intervention on cardiovascular morbidity and mortality.

PREDIMED-Plus recruited 6874 participants from 23 centres of the National Spanish Health System, who were randomized into an intervention or a control group. The intervention consisted of an energy-restricted Mediterranean diet, PA promotion, and behavioural support. The control group received usual health care for cardiovascular prevention and advice on following a Mediterranean diet but with no energy restrictions or PA recommendations.

The PA intervention (25) was delivered by dieticians who received additional training on PA recommendations. During the first year of the trial, the intervention group received PA education during 12 face-to-face individual sessions, 3 group sessions related to PA (out of 12 sessions), and 12 telephone calls. In the individual sessions, they received personalized recommendations to increase PA levels. In the first 6 months, the dieticians recommended a progressive increase in PA level to achieve at least 150 minutes per week of MVPA. The goal was to walk at least 45 minutes per day, 6 days per week. The recommendations also included strength, flexibility, and balance exercises. A pedometer was given to all participants as a motivational and self-monitoring tool to encourage them to take 10,000 steps per day.

Ethics Committees of all participating centres approved the study protocol, which was based on the standards of the declaration of Helsinki. This study is registered at the International Standard Randomized Controlled Trial with number 89898870.

The recruitment occurred between September 2013 and November 2016; the PREDIMED-Plus trial is expected to last for six years. The present study includes data from baseline and one-year follow-up. All participants provided written informed consent.

Outcome Assessment

At baseline and one-year follow-up, blood samples were collected after the participants had fasted 12 hours overnight. Certified nurses collected the samples according to usual protocol in primary care centres. Aliquots were stored in freezers at -80ºC until analysis. Routine biochemical analysis was done in laboratories using standard methods. The following biomarkers of inflammation were analysed in a central laboratory: IL-6, IL-8, IL-18, MCP-1, C-peptide, hs-CRP, leptin, and RANTES. With the exception of hs-CRP, these biomarkers were simultaneously measured using a XMAG-Luminex assay (Biorad, Hercules, California, USA) with multiplex technology. First, all samples were incubated and beads were suspended and covered with antibodies specific to the molecules. Samples were washed, a cocktail of biotinylated detection antibodies was applied, and the samples were then incubated with streptavidin-phycoerythrin. We used a BioPlex 200 (Biorad) to read the fluorescent sign. Serum level of hs-CRP was measured by a wide-range latex-enhanced immunoturbidimetric assay on an ADVIA 2400 analyser (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA).

The lowest detectable values 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 under these limits at baseline and at year one were imputed to be half of the lowest detected value (26). Therefore, 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.

Exposure variable

The validated REGICOR short PA (sPA) questionnaire (27) was administered to participants by trained stuff at baseline and at one-year follow-up. Participants indicated the frequency (number of days per month) and duration (minutes per day) of performing

six types of activities during a usual month: walking at a slow or normal pace, walking at a fast pace (brisk walking), walking in the countryside or in the mountains, climbing stairs, gardening, and exercising or playing sports. Each of these activities had a specific intensity, expressed in metabolic equivalent task (MET) units. Energy expenditure in total PA was measured in MET-minutes per day (MET-min/d), calculated as the sum of the frequency, duration, and intensity of each activity, divided by 30 days per month. We estimated PA according to total, light (≤4 METs), moderate (4–5.5 METs), vigorous (≥6 METs), and MVPA (>4 METs) intensity, at baseline and one-year follow-up. The REGICOR sPA questionnaire was created and validated in the framework of a large-scale Spanish study (27), which found high reliability and reasonable validity (Intraclass correlation coefficient for total PA = 0.82 and Spearman correlation coefficient for total PA= 0.39, respectively), and sensitivity in detecting changes from baseline to the last visit of their 27-week study in both moderate and vigorous PA.

Covariables

At baseline, a general questionnaire collected information on sex, age, smoking status, education level, history of medical conditions, and medication used. Education level was dichotomized as having an education level higher or lower primary school. Smoking status was dichotomized as smoker or non-smoker. Smokers were defined as current smokers and those who had stopped smoking less than a year before the baseline visit. A 17-item questionnaire, which was a modified version of a previously validated 14-item questionnaire (28), was used to assess the adherence to an energy-restricted Mediterranean diet (29). Weight and height were measured annually by trained stuff using a calibrated beam scale and a wall-mounted stadiometer, respectively. The BMI

was calculated by dividing the weight (kg) by the height squared (m^2). All these covariables were measured at baseline and follow-up visits.

Statistical analysis

Participants' baseline characteristics are shown in Table 1. To estimate the overall inflammatory profile, a composite inflammatory score of eight inflammatory markers (IL-6, IL-8, IL-18, MCP-1, C-peptide, hs-CRP, leptin, and RANTES) was created. For this purpose, we created quintiles of each biomarker at baseline and at follow-up. For each biomarker, the same cut points were used at both time points. By summing these quintiles, we obtained a score at baseline and a score at follow-up. The change in the inflammatory score was the difference between the baseline score and a year after score. The score ranged from 8 (all biomarkers in the first quintile) to 40 (all biomarkers in the fifth quintile); a higher score indicated a higher inflammatory profile. Multiple linear regression models were fitted to estimate the associations between changes in PA and changes in the inflammatory score, IL-6, IL-8, IL-18, MCP-1, C-peptide, hs-CRP, leptin and RANTES. Final models were adjusted by sex, age, intervention group, change in BMI, smoking status, education level, adherence to an energy-restricted Mediterranean diet, baseline PA, and the corresponding inflammatory marker or inflammation score at baseline. To analyse the independent association between changes in each PA intensity level, inflammation markers, and the inflammatory score, total PA was replaced by light, moderate, vigorous, and MVPA in mutually adjusted models. In addition, general linear models were used to analyse the associations between changes in the inflammatory score and quintiles of the changes in total PA.. Linear trend was measured by polynomial contrast and a post hoc Bonferroni correction was performed for multiple comparisons.

Interaction analyses were performed for sex, age, intervention group, smoking, and BMI. To assess the number of biomarkers that explained the associations between the inflammatory score and changes in PA, we created a new score, excluding the biomarkers that had a significant association with changes in PA, and repeated the analysis. Finally, we used multiple linear regression analysis with cubic spline models to assess the dose–response association of the changes in total, light and moderate-to-vigorous levels of PA with changes in the inflammatory score. For this purpose, we set the reference cut-point at 0 MET-min/d, corresponding to no change in PA. For this analysis we used the 'gam' package in R, version 3.0.2. All final models were adjusted by sex, age, intervention group, change in BMI, smoking status, education level, adherence to an energy-restricted Mediterranean diet, baseline inflammation score, and baseline total, light PA or MVPA as appropriate; all different intensities of PA were mutually adjusted.

Associations were considered significant if $p<0.05$. With the exception of the spline analysis, all statistical analysis was done with SPSS for Windows version 22, using the complete PREDIMED-Plus database available as of March 27, 2018.

Results

The present study includes data from baseline and one-year follow-up. This study included the first 1013 candidates recruited to the PREDIMED-Plus study. Of these, 172 did not meet the inclusion or randomization criteria and 143 declined to participate. After allocation to one of the two groups, the first 70 randomized participants were excluded due to a protocol change in the pre-randomization requirement and 2 participants were excluded due to a cancer diagnosis. Of the 626 remaining participants, 134 did not have complete data of all inflammatory markers at baseline and follow-up.

Changes in PA generated few extreme values, but these were influential. We eliminated three extreme values that exceeded 3 standard deviations [SDs] from the mean changes in PA, obtaining a final sample of 489 participants. The final sample was 489 individuals (230 men and 259 women), with a mean age of 65.47±4.8 (**Supplemental Figure 1)**. After a year of follow-up, participants reported a mean ± 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. Characteristics of the participants at baseline and at one-year follow-up are outlined in **Table 1**.

At one-year follow-up, a significant linear association was observed between an increase in total PA and a decrease in c-peptide (p=0.001) and leptin (p=0.008) in multiple linear regression models adjusted by sex and age **(Table 2)**. The inverse association between changes in C-peptide and in total PA did not change meaningfully after additional adjustment by intervention group, change in BMI, smoking status, education level, adherence to an energy–restricted Mediterranean diet, and the baseline values for total PA and for the corresponding inflammatory marker.

In fully adjusted models, an inverse linear association was observed between changes in MVPA and changes in C- peptide, whereas changes in light PA were not significantly associated with changes in either biomarker **(Supplemental Table 1)**.

Table 3 shows the association between changes in total, light, moderate, vigorous, and MVPA and changes in the inflammatory score, assessed by multiple linear regression models. An increase in total PA and MVPA was inversely associated with changes in the inflammatory score (p linear trend <0.05 for both) after adjusting for sex, age, intervention group, change in BMI, smoking status, education level, adherence to the energy-restricted Mediterranean diet, baseline inflammatory score, and the

corresponding type of PA intensity at baseline. In the case of MVPA, further adjustment for light PA did not affect this association. In further analysis, changes in moderate PA showed an independent and inverse association with changes in the inflammatory score ($p=0.001$). The inclusion of changes in intakes of energy, vitamin C, vitamin E, omega-3 fatty acids, vegetables, and fruits in the regression models did not affect the direction and magnitude of the association of changes in the inflammatory score with changes in total PA and MVPA **(Supplemental Table 2)**

Interaction analysis found that sex, age, intervention group, smoking, and BMI did not significantly modulate the association between changes in PA and changes in the inflammatory score (all p-values of interaction terms >0.05, data not shown). To assess whether the association between changes in the inflammatory profile and changes in PA was determined only by those biomarkers that were significantly associated with changes in total PA, we created a new 7-variable score, excluding C-peptide from the analysis. Although the magnitudes of the associations were attenuated, they were similar in direction to those identified using the 8-variable score **(Supplemental Table 3).** Further analysis showed that changes in the inflammatory score were inversely associated with quintiles of the changes in total PA (p for linear trend <0.002) **(Supplemental Table 4).**

The dose-response curve of changes in PA (light and MVPA) and changes in the inflammatory score obtained by multiple linear regression analysis with cubic spline models is shown in **Supplemental Figure 2**. We set the reference point as no change in PA. The splines showed a linear association between increases in total PA and MVPA during the study period and decreases in the inflammatory score, whereas decreases in MVPA were linearly associated with increases in the inflammatory score. A

nonsignificant association between changes in light PA and in the inflammatory score was observed.

Discussion

In the present study, an increase in total PA was inversely associated with changes in the inflammatory score and in the levels of C-peptide. The inflammatory score decreased with increasing level of moderate PA and MVPA, but not light and vigorous PA, at one-year follow-up.

Only a few studies have assessed the prospective association between PA and inflammatory markers (20–22,30,31). Two of these studies were performed in the general adult population and used models that included changes in PA and not in the inflammatory biomarkers. A study with 1754 younger individuals (aged 36 to 64 years) analysed the association between changes in PA and in inflammatory biomarkers in the group aged 60-64 years. Compared to individuals who were inactive at all time-points, those who were active throughout the study had 15% lower levels of IL-6 and 18.3% lower levels of leptin (30). Similarly, Hamer and colleagues studied 4289 individuals from the Whitehall II study, aged 35 to 55 years old, and reported that those who increased their PA levels showed 2% lower levels of IL-6 and 5% lower levels of CRP, compared to individuals who maintained their PA levels throughout the 10 years of follow-up (31). This suggests increasing levels of PA as a possible predictor of lower levels of inflammatory markers in the adult population.

There is little prospective evidence of an association between PA and inflammation in older adults (20–22). Lee and colleagues assessed PA and six inflammatory biomarkers in 1355 women aged 50-79 years, at baseline and after three years. They found a cross-sectional and inverse association between PA and the inflammatory markers at both

time-points. As in our study, however, they did not find a significant association between changes in total PA and changes in CRP, IL-6, leptin, adiponectin, and resistin (21) . Parsons and colleagues studied the association between one-year change in accelerometer-determined PA and inflammatory markers (CRP and IL-6) in 490 older men. They found no significant relationship between IL-6 and CRP levels and changes in PA. The authors attributed these findings to the very small changes in PA during the study period (20). In a 20-year follow-up, however, Wannamethee and colleagues reported an inverse association between changes in PA and levels of CRP and white blood cells at year 20 (22). In our study, the finding that only changes in C-peptide were significantly associated with changes in PA also could be due to power limitations, explained in part by the low variability in the changes that occurred in each biomarker. It seems improbable that one inflammatory biomarker could represent the overall inflammatory state of an individual. Therefore, to better capture the overall individual inflammatory profile, we created an inflammatory score based on the sum of the changes in all eight biomarkers. To our knowledge, the association between PA and the overall inflammatory profile has not previously been assessed in a prospective study. A cross-sectional study in middle-aged individuals (40 to 69 years old) found an inverse association between accelerometer-assessed total PA and an overall inflammation index composed of the levels of five cytokines (32). In our study, the association between changes in PA and changes in a 7-biomarker score (excluding C-peptide), although attenuated, was similar in direction to those identified using the original 8-biomarker score. This finding confirmed that the association between these two variables was not driven by a single biomarker, the inclusion of eight biomarkers was pertinent, and this approach identified further additive effects.

Several studies have found an inverse association of light PA with all-cause mortality and the incidence of obesity (33,34). However, its association with low-level inflammation is not widely studied (35–37). Cross-sectional evidence in these three studies showed an inverse association between light PA and inflammatory markers, ranging from 16% to 66% of the biomarkers studied, in older individuals. In contrast, Nilsson and colleagues observed that replacing sedentary time with light PA was associated with lower fibrinogen levels, but not with lower CRP, in older women (38). If the goal is to control inflammatory parameters in older individuals, MVPA intensity PA seems to be most beneficial. However, in the present study, increments in moderate PA seemed to be enough to decrease the overall inflammatory profile in this population. In older adults, moderate PA could be a feasible and safe option to control low-level inflammation. Brisk walking (4.3 METs) or walking at a normal pace (5.3 METs) are accessible activities requiring no special equipment, and therefore offer a valid option for older individuals to include in their daily lives.

Changes in fat mass could be a mechanism explaining the association between changes in PA and changes in inflammation. However, our findings indicated that this association was independent of changes in BMI. Additionally, Vella and colleagues found a cross-sectional association between higher levels of PA and a decrease in pro-inflammatory cytokines, independently of objectively measured visceral fat (39). Along the same line, the inverse association found by Hamer and colleagues between 10-year changes in PA and inflammatory biomarkers was independent of changes in BMI (31). Additionally, healthy diet patterns such as the Mediterranean diet exhibit anti-inflammatory properties (40), which might attenuate the association between physical activity and inflammation. However, in the present study adjustment for changes in nutrients or foods with anti-inflammatory properties had no meaningful effect on the

association of the inflammatory score with total PA and MVPA. All these findings seem to indicate that other mechanisms could partially explain these associations.

Increased BMI, unhealthy diet, smoking, and increasing age have been associated with a worse inflammatory profile. Our finding that improved levels of total PA and MVPA were associated with a better inflammatory profile, independently of all these covariables, is of importance. The independent and protective association of PA with inflammation has been linked, in part, to changes in the epigenetic profile: chronic moderate PA seems to be associated with the regulation of gene expression, promoting a decrease in pro-inflammatory markers, an increase in anti-inflammatory markers, and a decrease in the cell response to pro-inflammatory cytokines (41,42).

Our study observed a linear association between changes in PA and changes in C-peptide. This biomarker has been directly associated with insulin resistance and cardiovascular disease (43,44). Previous cross-sectional studies showing an association between PA and C-peptide have found inconsistent results (45–47). Alessa and colleagues reported that objectively measured total PA was inversely associated with C-peptide only when not adjusted by BMI (46), while others have shown this association to be independent of surrogate markers of body fat (45,47).

The strengths of this study are the prospective design and the assessment of eight biomarkers and PA at baseline and one-year follow-up. A study limitation is the measurement of PA and diet quality by self-reported questionnaires. Recall and reporting biases are inherent limitations of self-reported data and can lead to misclassification. The measurement error of these self-reported data is most likely to be random, which would attenuate the association of the exposure variables with the outcome.

In conclusion, an increase of total, moderate and moderate-to-vigorous levels of PA was associated with an improved inflammatory profile, measured by a composite score of eight inflammatory markers. These associations were independent of inflammation-related confounders.

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1) Conflict of Interest:

The authors declare that they have no competing interests.

2) Authors' contributions

HS, MF and GC designed research; OC, JW, PBC, JS-S, DC, LSM, and DR conducted research; RE, JAM, XP, CV, JV, JAT, FA, and MB provided essential reagents; HS, GC, IS analysed data; HS, MF 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 Characteristics of participants at baseline and at follow-up (n=489) [a]

Characteristic	Baseline	Follow-up
Men, n	230 (47.0%)	230 (47.0%)
Age, years	65.5±4.8	66.6 ±4.8
Study group, n		
Intervention group	256 (52.4%)	256 (52.4%)
Control group	233 (47.6%)	233 (47.6%)
More than primary education, n	237 (48.5%)	
BMI, kg/m^2 [b]	32.4 (32.1 to 32.7)	31.7 (31.3 to 32.0)
Smokers, n [c]	68 (13.9%)	55 (11.2%)
MedDiet score [d]	8.9 (8.7 to 9.1)	11.5 (11.2 to 11.7)
PA, MET-min/d		
Total	316 (130 to 539)	375 (212 to 627)
Light	64 (0 to 160)	80 (0 to 200)
Moderate	70 (0 to 271)	120 (0 to 300)
Vigorous	23 (0 to 168)	32 (1 to 205)
MVPA	195 (40 to 408)	247 (44 to 504)
Markers of inflammation		
IL-6 pg/ml	1.33 (0.55 to 2.15)	1.16 (0.39 to 2.27)
IL-8 pg/ml	8.07 (5.91 to 10.63)	7.95 (5.95 to 10.67)
IL-18 pg/ml	80.26 (58.10 to 107.49)	78.07 (59.21 to 102.51)
MCP-1 pg/ml	67.18 (47.44 to 88.37)	64.02 (43.88 to 87.92)
C- peptide ng/ml	1.55 (1.50 to 1.60)	1.49 (1.44 to 1.54)
Hs-CRP mg/L	2.37 (1.28 to 4.95)	2.30 (1.11 to 4.43)
Leptin ng/ml	14.80 (8.21 to 26.34)	13.71 (7.44 to 23.82)

Characteristic		
RANTES ng/ml	10.13 (9.99 to 10.28)	10.21 (10.05 to 10.37)

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/day= Metabolic equivalent of task minutes per day, mg/L = milligram per litre, ng/ml = nanogram/ millilitre, PA= Physical activity, pg/ml = picogram/ millilitre, RANTES = Regulated on activation, normal T-cell expressed and secreted chemokine.

[a] Categorical, continuous normal, and continuous non-normal distributed variables are expressed as n (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).

Table 2 Association between one-year changes in total PA and in inflammatory markers [a] (n=489)

	Δ total PA (100 MET-min/d)	
Δ inflammatory markers	B coefficient (95% CI)	P for linear trend
IL-6 pg/ml		
Model 1	-0.02 (-0.15 to 0.11)	0.771
Model 2	-0.01 (-0.14 to 0.11)	0.843
IL-8 pg/ml		
Model 1	0.12 (-0.23 to 0.47)	0.503
Model 2	0.15 (-0.14 to 0.43)	0.311
IL-18 pg/ml		
Model 1	-0.56 (-1.20 to 0.08)	0.086
Model 2	-0.61 (-1.33 to 0.10)	0.094
MCP-1 pg/ml		
Model 1	-0.29 (-1.12 to 0.54)	0.491
Model 2	-0.83 (-1.78 to 0.11)	0.084
C- peptide ng/ml		
Model 1	-0.02 (-0.03 to -0.006)	**0.001**
Model 2	-0.01 (-0.02 to -0.0007)	**0.037**
Hs-CRP mg/L		
Model 1	-0.10 (-0.33 to 0.14)	0.413
Model 2	-0.06 (-0.27 to 0.15)	0.577
Leptin ng/ml		
Model 1	-0.29 (-0.51 to -0.08)	**0.008**
Model 2	-0.08 (-0.30 to 0.15)	0.501

	Δ total PA (100 MET-min/d)	
Δ inflammatory markers	B coefficient (95% CI)	P for linear trend
RANTES ng/ml		
Model 1	0.003 (-0.03 to 0.04)	0.865
Model 2	-0.03 (-0.07 to 0.01)	0.346

Note. CI= confidence interval, 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, MET-min/day= Metabolic equivalent of task minutes per day, mg/L = milligram per litre, ng/ml = nanogram/ millilitre, PA= Physical activity, pg/ml = picogram/ millilitre, RANTES = Regulated on activation, normal T-cell expressed and secreted chemokine.

[a] Linear regression models were used in this analysis. Model 1 was adjusted by sex and age. Model 2 was additionally adjusted by intervention group, changes in body mass index, smoking status, education level, adherence to an energy–restricted Mediterranean diet, by the level of the corresponding inflammatory marker at baseline, and by total PA at baseline.

Table 3 Association between one-year changes in the inflammatory score and in PA (total, light, moderate-to-vigorous, moderate, and vigorous) [a] (n=489)

Δ inflammatory score[b]	B coefficient (95% CI)	P for linear trend
	Δ total PA (100 MET-min/d)	
Model 1	-0.12 (-0.22 to -0.02)	**0.023**
Model 2	-0.15 (-0.26 to -0.03)	**0.012**
	Δ light PA (100 MET-min/d)	
Model 1	0.17 (-0.07 to 0.42)	0.162
Model 2	0.10 (-0.19 to 0.39)	0.495
	Δ MVPA (100 MET-min/d)	
Model 1	-0.16 (-0.27 to -0.05)	**0.003**
Model 2	-0.16 (-0.27 to -0.05)	**0.006**
	Δ moderate PA (100 MET-min/d)	
Model 1	-0.31 (-0.47 to -0.16)	**<0.001**
Model 2	-0.27 (-0.43 to 0.11)	**0.001**
	Δ vigorous PA (100 MET-min/d)	
Model 1	-0.03 (-0.19 to 0.12)	0.661
Model 2	-0.05 (-0.22 to 0.13)	0.604

Note. CI= confidence interval, MET-min/day= Metabolic equivalent of task minutes per day, MVPA = Moderate to vigorous physical activity, PA= Physical activity.

[a] Linear regression models were used in this analysis. Model 1 was adjusted by sex and age. Model 2 was additionally adjusted by intervention group, changes in body mass index, smoking status, education level, adherence to an energy–restricted Mediterranean diet, inflammation score at baseline, the corresponding type of PA at baseline, and finally all different intensities of PA were mutually adjusted.

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

Supplemental Figure 1: Flow Chart of the eligible participants.

MedDiet= Mediterranean diet, wk= week

Supplemental Figure 2: Dose-response association between changes in the inflammatory score and changes in A) total, B) moderate-to-vigorous intensity, and C) light PA.

Image obtained by multiple linear regression analysis with cubic spline models.

All models were adjusted by sex, age, intervention group, changes in body mass index, smoking status, education level, adherence to an energy–restricted Mediterranean diet, inflammation score at baseline, the corresponding type of PA at baseline, and finally all different intensities of PA were mutually adjusted.

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

Physical activity was measured in MET-min/day= Metabolic equivalent of task minutes per day (MET-mins/day).

MVPA= moderate-to-vigorous physical activity