Title: Loss of visceral fat is associated with a reduction in inflammatory status in patients with metabolic syndrome.

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Abbreviatures:

DXA: Dual-energy X-ray absorptiometry

VAT: Visceral adipose tissue

SAT: Subcutaneous adipose tissue

MD: Mediterranean diet

MetS; Metabolic syndrome

CVD: Cardiovascular diseases

BMI: Body mass index

PA: Physical activity

FFQ: Food frequency questionnaire

EVOO: Extra virgin olive oil

TNF-α: Tumor necrosis factor alpha

IL: Interleukin

GLP-1: Glucagon-like peptide 1

MCP-1: Monocyte chemoattractant protein-1

PAI-1: Plasminogen activator inhibitor-1

SFA: Saturated fatty acids

MUFA: Monounsaturated fatty acids

PUFA: Polyunsaturated fatty acids

RCT: Randomized clinical trial

Abstract (180 words)

Excessive visceral adipose tissue (VAT) is associated with higher secretion of pro-inflammatory molecules, contributing to systemic inflammation and obesity-related metabolic disturbances. This prospective analysis includes 117 overweight/obese adults (55-75 years) from the PREDIMED-Plus study. Fourteen inflammatory markers and adipokines were measured using a Bio-Plex assay with multiplex technology: insulin, glucagon, IL-6, visfatin, ghrelin, GLP-1, TNF-α, MCP-1, PAI-1, resistin, C-peptide, leptin, adipsin and adiponectin. Participants were categorized into tertiles according to changes in VAT after 1-year of follow-up, determined by dual-energy X-Ray absorptiometry. Participants allocated in tertile 3, which represent an increase of VAT content after 1-year of follow-up compared to tertile 1, showed significant differences in insulin (T3 vs. T1, fully adjusted model: $p = 0.037$, p for trend 0.042), PAI-1 (fully adjusted model: $p = 0.05$, p for trend 0.06), c-peptide (fully adjusted model: $p = 0.037$, p for trend 0.042), and TNF-α (fully adjusted model $p = 0.037$, p for trend 0.042). Our results evidenced that a reduction in VAT was associated with clinical improvements in several inflammatory and adiposity markers, mainly in insulin, c-peptide, and PAI-1 levels.

1. Introduction

The worldwide overweight and obesity incidence has increased at an alarming rate. In 2016, nearly 40% of adults were overweight and 13% were obese[1]. Overweight and obesity, especially excessive visceral adipose tissue (VAT), is closely related to the inflammatory response due to the imbalance in the secretion of adipokines and cytokines with anti- and/or pro-inflammatory properties[2]. Moreover, strong evidence highlights that body mass index (BMI) does not differentiate physiological and pathological states, especially in senior populations[3,4]. In this sense, VAT content demonstrates a more specific capacity to identify individuals with altered glucose

metabolism and lipid profile, mainly regarding modified triglyceride (TG) and high-density lipoprotein cholesterol levels[3,5-7].

Adipose tissue is an endocrine and paracrine organ with the capacity to produce cytokines, such as interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and adipokines or adipocytokines (e.g., leptin, adiponectin, resistin, and visfatin, among others)[8,9]. These molecules influence body weight homeostasis and are linked to inflammation, coagulation, and fibrinolysis. Even though the inflammatory response observed in obesity is not yet fully understood, the macrophages infiltration into adipose tissue is increased in obese individuals, increasing the number and recruitment of M1 macrophages and, consequently, increasing M1/M2 ratio. Both absolute number of M1 and M2 macrophages and the M1/M2 ratio may contribute to the persistence of the chronic inflammation and insulin resistance observed in obesity[10]. Under normal physiological conditions, adipocytes mainly produce anti-inflammatory adipokines, such as adiponectin. However, excess VAT is associated with higher secretion of pro-inflammatory adipocytokines and cytokines, leading to systemic inflammation and obesity-related metabolic disturbances, such as insulin resistance, dyslipidemia, hypertension, oxidative stress, and atherosclerosis[11,12]. This excess adipose tissue and inflammatory response can be reversed by lifestyle modification, including physical activity promotion and following a healthy dietary pattern, such as the Mediterranean Diet (MD)[11,13,14]. MD is rich in polyphenols, plant-derived bioactive compounds characterized by the presence of aromatic ring and attached hydroxyl groups, which are present in the main key-foods of this dietary pattern (extra virgin olive oil (EVOO), nuts, vegetables, wine, vegetables, fruits and whole-grain cereals). Its intake has been associated to anti-inflammatory and anti-oxidative properties, which may also improve obesity-related inflammatory response[15]. The MetS prevalence in Spanish population is around 22%, and there has been a relative increase of attributable death and disease burden due to metabolic risks (fasting glycaemia, total cholesterol, systolic blood pressure) by more than 15% from 2005 to 2015 globally[16,17]. Abdominal obesity and high blood pressure were the 2 most frequent components of MetS.

Our objective was to evaluate whether changes in VAT after a 12-month of follow-up were associated with greater improvements in obesity-related inflammatory and adipokine marker concentrations in older overweight or obese individuals with MetS.

2. Experimental section

2.1. Study design

The present study was a longitudinal analysis of data collected during the first year of the Prevención con Dieta Mediterránea (PREDIMED) Plus study, an ongoing large-scale, multicenter, parallel-group, intervention randomized controlled trial conducted in 23 centers of the National Spanish Health System designed to assess the effect of an energy-restricted MD and physical activity promotion on cardiovascular morbidity and mortality in individuals with MetS[18].

Volunteers were men (aged 55–75) and women (60–75) free of documented history of cardiovascular disease (CVD) with overweight or obesity (BMI ≥27 or <40 kg/m^2) who met at least three or more criteria of MetS: abdominal obesity (waist circumference >102 cm for men or ≥88 cm for women), hypertension, hypertriglyceridemia, low HDL cholesterol levels and hyperglycemia, or diagnosis of type 2 diabetes mellitus[19]. The recruitment period lasted from September 5, 2013, to October 31, 2016. The study protocol and the eligibility and exclusion criteria can be found at http://predimedplus.com. After randomization, the participants were assigned to an intervention or control group. The intervention arm promoted an energy-restricted MD and physical activity (PA) and provided behavioral support, whereas the control group received usual health care for CVD prevention and advice on following a traditional MD without energy restriction or PA promotion. The trial protocol was approved by the local institutional ethics committees and was registered under International Standard Randomized Controlled Trial number 89898870 (ISRCT: http://www.isrctn.com/ISRCTN89898870). All participants provided written informed consent before joining the study.

For the present analysis, out of the total sample of 6,874 randomized participants, only 1,569 participants from 7 recruiting center underwent total body dual X-ray absorptiometry (DXA) scans at baseline. Thirty-seven participants were excluded because they reported energy intake values outside the predefined limits measured by food frequency questionnaire (FFQ) (<3,347 kJ [800 kcal]/day or >17,573 kJ [4,000 kcal]/day for men; <2,510 kJ [500 kcal]/day or >14,644 kJ [3,500 kcal]/day for women) at baseline and during follow-up[20]. Participants were also excluded if they had missing data on dietary information (n = 159) or lacked DXA data (n = 178) at baseline and after 1 year of follow-up. Ultimately, 1,195 participants had available data on body composition parameters measured by DXA. Of these, 1,157 participants had available data on VAT at baseline. For the present analysis, a random subsample of 117 participants with VAT measurements at baseline and after 1 year of follow-up was selected for biomarkers analysis.

2.2. *Inflammatory and adipokine biomarkers*

Blood samples were collected after overnight fasting at baseline and the 1-year follow-up visit. These were centrifuged and stored at −80 ºC until analysis. The following biomarkers were analyzed: insulin, glucagon, IL-6, visfatin, ghrelin, glucagon-like peptide 1 (GLP-1), TNF-α, monocyte chemoattractant protein 1 (MCP-1), plasminogen activator inhibitor 1 (PAI-1), resistin, C-peptide, leptin, adipsin, and adiponectin. These biomarkers were measured using a Bio-Plex assay (Bio-Rad Laboratories Inc., Hercules, CA, USA) based on multiplex technology. First, samples were incubated, and beads were suspended and covered with antibodies specific to the mentioned molecules. Second, samples were washed, and third, biotinylated detection antibodies were applied to the samples. Fourth, the samples were incubated with streptavidin–phycoerythrin. Finally, Bio-Plex 200 was used to read the fluorescent sign.

2.3. *Body composition parameters*

Direct measures of body composition were performed with a DXA scanner (GE Healthcare/DXA Lunar Prodigy Primo and Lunar iDXA; Madison, WI, USA) connected to enCore^{TM} software. For VAT measurement, scans were reanalyzed using the validated CoreScan software application[21]. As described elsewhere[22], VAT (g) was subtracted from android fat mass. Body composition measures were preferably performed within two months from baseline and follow-up visit in. DXA scans were performed and calibrated daily by trained operators according to the standard protocols provided by the manufacturer. Participants were scanned wearing examination gown or light clothes.

2.4. *Covariable assessment*

Self-reported questionnaires were collected at baseline by trained staff and provided data on sex, age, smoking status, educational level, history of a medical condition, lifestyle habits, and medication use. Dietary information was assessed at baseline and after 1-year of follow-up by a validated 143-item semi-quantitative food-frequency questionnaire (FFQ)[23]. A 17-point score was used to assess MD adherence[24] at baseline and one-year follow-up visits. As described elsewhere[25], dietary polyphenol intake was estimated by multiplying polyphenol content in food (mg/100g of food) by the daily consumption of each food (g/day). Total polyphenol intake and polyphenol subclasses were calculated as the sum of all individual polyphenol intakes from the food sources reported from the FFQ. The validated Registre Gironí del Cor (REGICOR) short self-reported physical activity questionnaire was used to assess total leisure-time PA (MET min/week)[26] and the validated Spanish version of the Nurses' Health Study

questionnaire to assess sedentary behaviors[27] at baseline and after 1-year of follow-up. Sociodemographic and lifestyle variables were categorized as follows: educational level (three categories: primary, secondary, or high school), physical activity level (three categories: low, moderate, or high), BMI (three categories: 27.0–29.9 kg/m^2 or overweight, 30.0–34.9 kg/m^2 or obesity class I, and ≥35 or obesity class II kg/m^2), and smoking status (three categories: never, former, or current smoker).

2.5. Statistical analysis

Based on previous studies with similar a study population[28] the sample was determined with the Sample Size and Power Calculator design by the Program of Research in Inflammatory and Cardiovascular Disorders from the Institut Municipal d'Investigació Mèdica (IMIM), Barcelona, Spain. Accepting an alpha risk of 0.05 and a beta risk of 0.1 in a two-sided test, 68 subjects are necessary to recognize as statistically significant a difference greater than or equal to 0.05 units in IL-6 plasma levels, with a standard deviation of 1.2 assuming a maximum loss of 10% of participants. IL-6 was considered the primary outcome and was used to determine the sample size. Nonetheless, changes in all the parameters were of equal interest in this study.

The baseline characteristics of study participants are expressed as means and standard deviation (SD) for continuous variables and counts and percentages for categorical variables. Variables with a skewed distribution (as assessed with the Kolmogorov test) were transformed to their logarithm for analysis. Differences in baseline characteristics by tertiles of VAT changes at 1 year versus baseline were analyzed using one-way ANOVA. VAT changes were allocated into tertiles of change after 1 year, with tertile 1 (reduction in VAT) as the reference category.

The differences in inflammatory and adipokines parameters at baseline and after 1 year of follow-up between tertiles of VAT changes were assessed by multivariate linear regression models. The minimally adjusted model included for age, sex, intervention group, recruitment center, smoking status (three categories: never smoker, smoker, and former smoker), type 2 diabetes diagnose (yes/no), changes in BMI (1-year versus baseline, except for waist circumference) and baseline levels of each parameter (pg/mL). The fully adjusted model was further adjusted for educational level (three categories: primary, secondary, or high school), physical activity level (three categories: low, moderate and active), non-steroidal anti-inflammatory drug (NSAIDs) and cholesterol-lowering treatment (yes/no), energy intake (kcal/day), saturated fatty acid intake (g/day), trans fat intake (g/day), and fiber intake (g/day).

The differences in energy intake and nutrient density at baseline and after 1-year of follow-up according to tertiles of VAT changes were assessed by multivariate linear regression. The full-adjusted model included age, sex, intervention group, recruitment center, smoking status (three categories: never smoker, smoker, and former smoker), type 2 diabetes diagnose (yes/no), educational level (three categories: primary, secondary, or high school), physical activity level (three categories: low, moderate, and active), NSAIDs and cholesterol-lowering treatment (yes/no), and baseline levels of each nutritional parameter (g/day). To assess the linear trend (*p* for trend) across tertiles of VAT changes, the mean value was assigned to each tertile.

To account for multiple comparisons, we applied the Simes method to interpret the results. Statistical analyses were performed using Stata v16.0 (StataCorp LLC, Texas, USA), and statistical significance was set at $p < 0.05$. The PREDIMED-Plus longitudinal database generated on June 26, 2020 (202006290731_PREDIMEDplus) was used.

3. Results

Baseline participant characteristics

Of the 117 participants included, 59 were randomly allocated to the energy-restricted MD intervention, and 51.3% were women. **Table 1** shows the baseline characteristics of study participants according to each tertile of VAT changes. Tertiles were well-balanced regarding BMI, smoking status, age, energy intake, physical activity levels, and MD adherence. Medication use and educational level were also similar among the three tertiles. Significant differences were observed in sex, for which tertile 3 consisted of significantly more women compared with tertile 1 ($p<0.001$), and waist circumference ($p = 0.036$).

Inflammatory and adipokine parameters

The baseline and 1-year mean changes for inflammatory and adipokines parameters are shown in **Table 2**. Significant differences between tertiles of VAT changes (Tertile 3 (T3) vs. Tertile 1 (T1)) after 1-year of follow-up were observed in waist circumference (T3 vs. T1, fully adjusted model: β= 4.62 [95% CI: 2.15 to 7.08], $p < 0.001$, p for trend <0.001), insulin (T3 vs. T1, fully adjusted model: β= 65.1 [95% CI: 17.5 to 112.8], $p = 0.037$, p for trend 0.042), c-peptide (T3 vs. T1, fully adjusted model: β= 1.16 [95% CI: 1.05 to 1.30], $p = 0.037$, p for trend 0.042), TNF-α (T3 vs. T1, fully adjusted model: β= 1.18 [95% CI: 1.05 to 1.35], $p = 0.037$, p for trend 0.042), and PAI-1 (T3 vs. T1, fully adjusted model: β= 366.5 [95% CI: 73.4 to 659.6], $p = 0.052$, p for trend 0.066). Tertile

3 (VAT increase after 1 year of follow-up) showed significant mean increases in ghrelin (15.5 pg/mL [95% CI: 0.5 to 30.4]), leptin levels (4399 pg/mL [95% CI: 702.4 to 8095]), PAI-1 (mean difference 215.9 pg/mL [95% CI: 8.72 to 423.1]), and resistin (369.4 pg/mL [95% CI: 152.8 to 585.9]). Moreover, significant reductions in glucagon (-24.2 pg/mL [95% CI: -41.0 to -7.3]), and insulin levels (-29.1 pg/mL [95% CI:-57.1 to -1.1]), were observed in Tertile 1.

Supplementary Table 1 shows the associations between inflammatory and adipokine parameters according to tertiles of changes in total fat mass (kg) after 1 year of follow-up. No significant differences were observed between tertiles of total fat mass changes after 1 year of follow-up. Tertile 3 (total fat mass increase after 1 year of follow-up) showed significant mean increases in c-peptide (mean difference 15.2 pg/mL [95% CI: 4.1 to 26.3]), and leptin (4044 pg/mL [95% CI: 593.2 to 7495]). Moreover, significant reductions in glucagon (-15.0 pg/mL [95% CI: -26.3 to -3.7]), were observed in Tertile 1.

Changes in dietary and polyphenol intake after 1 year of follow-up

The baseline and 1-year mean changes for dietary and main polyphenol family intake are shown in **Table 3**. Significant differences among tertiles of VAT changes after 1-year of follow-up were observed in energy intake (T3 vs. T1, fully adjusted model: β=292.2 [95% CI: 103.8 to 480.5], p = 0.003, p for trend = 0.002), *trans*-fat (T3 vs. T1, fully adjusted model: β= 0.06 [95% CI: 0.01 to 0.11], p = 0.024, p for trend = 0.020), fiber (T3 vs. T1, fully adjusted model: β= -1.45 [95% CI: -2.86 to -0.05], p = 0.043, p for trend = 0.028), and lignans intake (T3 vs. T1, fully adjusted model: β= -0.10 [95% CI: -0.19 to -0.02], p = 0.020, p for trend = 0.015). Tertile 1 showed a significant mean decrease in energy (mean difference -224.3 kcal/day [95% CI: -409.0 to -79.7]), carbohydrate (-8.4 g day/1000 kcal [95% CI: -13.3 to -3.5]), saturated fatty acids (SFA) (-1.1 g day/1000 kcal [95% CI: -1.8 to -0.5]), and trans-fat (-0.1 g day/1000 kcal [95% CI: -0.1 to -0.0]). Moreover, a significant increase was observed in protein (2.8 g day/1000 kcal [95% CI: 0.4 to 5.2]), total fat (2.2 g day/1000 kcal [95% CI: 0.1 to 4.3]), monounsaturated fatty acids (MUFA) (3.4 g day/1000 kcal [95% CI: 1.8 to 5.0]), polyunsaturated fatty acids (PUFA) (0.9 g day/1000 kcal [95% CI: 0.3 to 1.6]) and fiber (2.3 g day/1000 kcal [95% CI: 1.2 to 3.4]). Regarding dietary polyphenol intake, a significantly difference between tertiles was observed for lignans (T3 vs T1 p = 0.018, p for trend= 0.013).

The baseline and 1-year mean changes for MetS components are shown in **Supplementary Table 2**. No significant differences were observed among tertiles of changes in VAT after 1-year of follow-up, except for triglycerides (T3 vs T1 full-adjusted p-value 0.057, p for trend 0.037). However, significant differences were observed in participants who decreased VAT (T1) after 1-year of follow-up for HDL-cholesterol (1.9 mg/dL [95% CI 0.1 to 3.7]) and systolic blood pressure levels (-5.2 mmHg [95% CI -10.3 to -0.1]). Moreover, a tendency was observed in fasting glucose levels in participants who decreased VAT after 1-year of follow-up (-6.2 mg/dL [95% CI-13.3 to 0.8]).

4. Discussion

The present study analyzed data from 117 participants recruited into the PREDIMED-Plus study after 1 year of follow-up; and this study is the continuation of the work previously performed in the same study population[3,29,30]. we found significant associations between changes in VAT and circulating levels of insulin, c-peptide, and PAI-1 levels.

It is well-established that VAT is associated with CVD and metabolic disturbances, including non-alcoholic fatty liver disease, but the mechanisms underlying these effects are still unclear[14]. This excess VAT may induce chronic low-grade systemic inflammation mediated by macrophage infiltration and the secretion of several proinflammatory cytokines.[8,9] Clinical studies with insulin-resistant obese participants described adipose tissue macrophage infiltration, initiating the recruitment of other immune cells to promote the secretion of several cytokines to regulate the inflammatory response. In addition, macrophage infiltration has been postulated as a potential mechanism underlying the insulin resistance, pro-inflammatory response, and metabolic dysfunction observed in obese patients[31,32]. Moreover, the activation of these macrophages induces the secretion of several proinflammatory mediators, such as MCP-1, IL-6, and TNF-a[31,33]. Besides metabolic disturbances, an excess VAT accumulation may indicate a dysfunctional adipose tissue without the capacity to effectively store the excessive energy intake [34]. Moreover, this efficient fat storage is translated to accumulate the excess of energy in subcutaneous adipose tissue, which is more insulin-sensitive, and it protects from metabolic disturbances associated to obesity, which can also explain why we found significant differences in VAT changes instead of total fat mass. In this sense, our findings are aligned with improvements in MetS components, mainly triglycerides, HDL-cholesterol, and systolic blood pressure.

The beneficial effects observed in individuals with higher VAT reduction on some circulating proinflammatory parameters related to obesity, such as leptin and MCP-1, were also reported by Salas-Salvadó *et al.* in the same study population[29]. Human studies conducted in the context of bariatric surgery- or lifestyle interventions focus on weight loss suggest that although adipose tissue inflammation is not sufficient to induce insulin resistance on its own, it is a major contributor to systemic insulin resistance, and substantial improvements in insulin sensitivity are associated with a reduction in VAT [35,36]. Weight loss achievements through dietary intervention alone or energy-restricted diet and physical activity promotion resulted in decreased circulating IL-6, CRP, PAI-1, TNF-α, soluble TNF receptor, P-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and IL-18 in men and women of various age groups and BMIs [37]. In our study, we are comparing MetS participants with overweight or obesity, while most of the finding were observed comparing obese versus normal weight or healthy individuals.

Despite its physiological functions, leptin is considered a pro-inflammatory cytokine. Its secretion is proportional to fat depots, and its inflammatory response is mediated by the activation of monocytes, leukocytes, and macrophages to secrete IL-6, TNF-α, along with increases in reactive oxygen species (ROS) [38,39]. No differences in changes from baseline for inflammatory adipocytokines related to M1 macrophages were observed. Similar results were reported by Paquette *et al.* in a randomized controlled trial with non-diabetic overweight and obese adults after dietary intervention with strawberry and cranberry polyphenols [40]. Similar to leptin, resistin may play a pro-inflammatory role, inducing the expression of pro-inflammatory cytokines by nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signaling and inflammation markers, such as MCP-1.

High serum adiponectin levels are negatively associated with pro-inflammatory markers, such as IL-6 or TNF-α, as well as insulin resistance [41]. Its anti-inflammatory properties are mainly mediated by the inhibition of NF-kB [42,43]. In this sense, TNF-α is overexpressed in the individuals with overweight, while IL-6 is linked to the obese state. Even though a reduction of VAT is observed, their BMI slightly changed, which may explain the null findings in IL-6. It should be noted that the secretion of adiponectin is suppressed in chronic obesity [44], and this might explain the non-significant changes we observed in adiponectin levels.

Expression of C-peptide has been directly linked with insulin resistance and CVD risk, and significant inverse associations were observed between C-peptide levels and

physical activity in the same study population [30]. Interestingly, the association between physical activity levels and C-peptide are independent of body composition parameters [45,46], whereas our results suggest a potential association between VAT changes and C-peptide. We found a significant increase in participants who increased VAT after one year of follow up in ghrelin levels, when this hormone secretion is decreased in obese individuals. Ghrelin is a gut hormone that modulates the activation of adipose tissue macrophages and might be involved in the pathogenesis of obesity-related inflammation[10]. No significant associations were observed for the rest of hormones related to food intake. For GLP-1, in a study with 40 patients with morbid obesity and type 2 diabetes undergoing bariatric surgery, no significant associations were found between GLP-1 receptors in adipose tissue and weight loss after surgery [47]. Thus, GLP-1 secretion is dependent on nutrient intake and its use has been postulated as a potential treatment for obesity or type 2 diabetes.

Regarding dietary factors, our results showed a significant reduction in energy, total fat, SFA, and trans-fat intake in participants who decrease their VAT deposits. These nutrient intake changes can be explained by the reduction in the consumption of refined cereals, red meat, pastries, cakes, and sweets[29]. Dietary polyphenol intake has been associated with several CVD benefits and mediates inflammatory processes and ROS production [48,49]. In the case of inflammation, several meta-analyses evaluated the effects on inflammatory mediators after resveratrol supplementation, showing non-significant effects on TNF-α and IL-6 but significant reductions in high-sensitivity C-reactive protein [50,51]. In line with these findings, another meta-analysis assessing the effects of vegetable and fruit intake on inflammatory biomarkers observed similar effects on C-reactive protein and TNF-α [52]. In the case of protein, the type of protein (plant-based protein versus animal protein) may influence inflammatory status more than total protein intake [53]. Because several dietary components, such as β-carotene, lycopene, dietary fiber, and polyphenols, have beneficial effects, their combination, which is naturally found in fruits and vegetables, can enhance their anti-inflammatory properties [54].

The strengths of this study are that DXA has been considered the gold standard method for body composition measurement; thus, VAT was objectively measured with a validated imaging technique [55], and the assessment of fourteen biomarkers at baseline and after 1 year of follow-up. The main limitation of the present study is the prospective design, which does not allow attributing the conclusions to plausible causes. Moreover, the sample size is limited. Other limitations include potential

residual confounding, reverse causation bias, and the lack of generalizability of the results to other populations.

In conclusion, a reduction in VAT was associated with improvements in several inflammatory and adipokines levels, mainly in insulin, C-peptide, and PAI-1 levels. These improvements may contribute to a reduction in cardiometabolic disturbances observed in obesity.

Reporting dose & administration details: This information does not apply for the present study.

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Data sharing: There are restrictions on the availability of data for the PREDIMED-Plus trial, due to the signed consent agreements around data sharing, which only allow access to external researchers for studies following the project purposes. Requestors wishing to access the PREDIMED-Plus trial data used in this study can make a request to the PREDIMED-Plus trial Steering Committee predimed_plus_scommittee@googlegroups.com.

Table 1: Baseline characteristics according to tertiles of change in VAT (g) at 1-year in a sub-sample.

1 year – baseline VAT (g), median (min to max)	**Total**	**T1 -507g (-3114 to -283)**	**T2 -150g (-283 to 32.1)**	**T3 240g (32.1 to 1643)**	***P*-value**
N	117	39	39	39	
Waist circumference, *cm*	107.2±8.6	109.9±8.0	105.0±9.8	106.8±7.4	0.036
Age, years	65.2±4.6	63.8±4.9	65.7±3.9	66.2±4.7	0.049
Intervention arm, *n (%)*	59 (50.4)	22 (56.4)	14 (35.9)	23 (59.0)	0.083
Women, *n (%)*	60 (51.3)	10 (25.6)	22 (56.4)	28 (71.8)	<0.001
BMI, kg/m^2	32.8±3.1	32.8±3.2	32.3±2.7	33.2±3.3	0.376
Fasting glucose levels, *mg/dL*	115.7±30.1	117.4±30.3	114.8±32.3	114.9±28.4	0.910
HDL-c, *mg/dL*	45.9±9.59	43.6±10.3	47.4±8.06	46.7±10.0	0.169
Triglycerides, *mg/dL*	145.7±57.7	147.2±62.4	137.4±51.6	152.3±58.9	0.515
Systolic blood pressure, *mmHg*	140.0±16.1	141.6±15.4	137.2±16.1	141.1±16.7	0.434
Diastolic blood pressure, *mmHg*	79.2±10.5	79.6±10.3	78.3±10.7	79.6±10.6	0.812
Adherence to ER-MedDiet, 17p score	8.6±2.5	8.1±2.8	9.0±2.3	8.6±2.3	0.264
Total energy intake, *kcal/day*	2391±504	2454±549	2309±427	2409±530	0.436
Type 2 diabetes prevalence, n (%)	40 (34.2)	16 (41.0)	10 (25.6)	14 (35.9)	0.351
Current smokers, *n (%)*	21 (17.9)	9 (23.1)	7 (17.9)	5 (12.8)	0.084
Physical activity,(METS.min/week)	2753±2301	2342±2177	3183±2763	2735±1849	0.274
Leptin, *pg/dL*	17891±12114	16766±11445	18077±9702	18860±14878	0.750
Medication, *n (%)*					
Antihypertensive agents	98 (83.8)	34 (87.2)	32 (82.0)	32 (82.0)	0.782
Cholesterol-lowering agents	63 (53.8)	20 (51.3)	23 (59.0)	20 (51.3)	0.739
Insulin	3 (2.6)	2 (5.1)	1 (2.6)	0 (0.0)	0.365
Metformin	32 (27.3)	13 (33.3)	9 (23.1)	10 (25.6)	0.578
Other hypoglycemic agents	32 (27.3)	14 (35.9)	8 (20.5)	10 (25.6)	0.305
Aspirin or antiplatelet agents	17 (14.5)	8 (20.5)	6 (15.4)	3 (7.7)	0.276
NSAIDS	25 (21.4)	6 (15.4)	5 (12.8)	14 (35.9)	0.024
Vitamin and minerals	8 (6.8)	3 (7.7)	2 (5.1)	3 (7.7)	0.877
Sedative or tranquilizer agents	29 (24.8)	8 (20.5)	10 (25.6)	11 (28.2)	0.731
Hormonal treatment (only women)	4 (3.4)	1 (2.6)	0 (0.00)	3 (7.7)	0.166
Educational level, *n (%)*					0.866

Primary school	62 (53.0)	17 (43.6)	23 (59.0)	22 (56.4)
Secondary school	26 (22.2)	14 (35.9)	4 (10.3)	8 (20.5)
University and other studies	29 (24.7)	8 (20.5)	12 (30.8)	9 (23.1)

Continue variables are expressed as mean (±SD). Categorical variables are expressed as number (n) and percentage (%). Analysis of variance–one factor was used for continuous variables. ER: Energy-restricted; MET: Metabolic Equivalent of Task; NSAIDs: Non-steroidal anti-inflammatory drugs.

Table 2: Changes in inflammatory parameters according to tertiles of changes in visceral adipose tissue after 1-year of follow-up.

		T1	T2	T3	Minimally adjusted *P* value	Full-adjusted *P*-value	*P* for trend
		-507g (-3114 to -283)	-150g (-283 to 32.1)	240g (32.1 to 1643)	(T3 vs T1)	(T3 vs T1)	
Waist circumference, cm	Baseline	109.9±8.0	105.0±9.8	106.8±7.4	0.224	0.663	0.868
	1-y Mean changes	-4.8 (-6.7; -2.9)	-2.1 (-3.5; -0.6)	-0.8 (-2.5; 0.8)	0.007	<0.001	<0.001
Insulin, *pg/mL*	Baseline	163.4±82.7	116.2±60.6	146.9±88.7	0.890	0.608	0.680
	1-y Mean changes	-29.1 (-57.1; -1.1)	7.8 (-13.8; 29.4)	31.9 (-2.7; 66.4)	0.014	0.037	0.042
C-peptide, *pg/mL*	Baseline	132.6±56.8	116.6±46.8	124.5±58.7	0.323	0.348	0.319
	1-y Mean changes	-17.2 (-33.2; -1.20)	9.6 (-2.9; 22.0)	16.1 (-0.9; 33.1)	0.019	0.037	0.042
Glucagon, *pg/mL*	Baseline	186.7±56.9	181.2±46.1	168.7±67.2	0.220	0.464	0.436
	1-y Mean changes	-24.2 (-41.0; -7.3)	2.8 (-11.3; 16.8)	10.4 (-5.3; 26.1)	0.122	0.389	0.476
GLP-1, *pg/mL*	Baseline	11.9±8.9	8.4±5.0	9.4±8.3	0.657	0.538	0.553
	1-y Mean changes	0.6 (-3.6; 5.0)	1.4 (-1.8; 4.5)	-1.9 (-6.1; 2.3)	0.434	0.996	0.988
Visfatin, *pg/mL*	Baseline	2886±2517	3341±2194	3592±2397	0.822	0.988	0.986
	1-y Mean changes	87.8 (-2046; 2221)	-52.6 (-693.2; 588.0)	72.6 (-318.3; 463.5)	0.673	0.855	0.846
Ghrelin, *pg/mL*	Baseline	111.4±79.4	105.5±55.4	86.9±51.0	0.140	0.085	0.074
	1-y Mean changes	6.8 (-5.8; 19.5)	16.2 (-1.2; 33.5)	15.5 (0.5; 30.4)	0.131	0.146	0.137
TNF-α, *pg/mL*	Baseline	114.9±142.4	170.1±160.6	125.1±157.6	0.592	0.628	0.579
	1-y Mean changes	3.21 (-32.3; 38.7)	33.2 (2.3; 64.1)	12.2 (-16.4; 40.8)	0.028	0.037	0.042
IL-6, *pg/mL*	Baseline	12.3±18.1	14.5±14.7	14.4±21.1	0.981	0.743	0.852
	1-y Mean changes	5.6 (-0.4; 11.6)	3.7 (-4.6; 12.0)	-0.0 (-7.6; 7.6)	0.595	0.903	0.988
MCP-1, *pg/mL*	Baseline	160.9±176.6	224.3±202.2	195.8±213.9	0.706	0.598	0.591
	1-y Mean changes	-12.0 (-43.9; 19.7)	17.3 (-12.4; 47.0)	-3.71 (-28.3; 20.9)	0.059	0.214	0.252
Leptin, *pg/mL*	Baseline	16766±11445	18077±9702	18860±14878	0.466	0.376	0.330
	1-y Mean changes	-1769 (-4317; 779.1)	1528 (-1012; 4067)	4399 (702.4; 8095)	0.182	0.311	0.322
Adipsin, *pg/mL*	Baseline	3168±1290	3180±2054	2906±1276	0.745	0.682	0.658
	1-y Mean changes	-145.4 (-604.0; 313.0)	-189.1 (-690.1; 311.9)	-32.5 (-401.4; 335.4)	0.703	0.855	0.846

PAI-1, *pg/mL*	Baseline	1541±559.9	1452±623.5	1358±674.0	0.092	0.034	0.035
	1-y Mean changes	-220.5 (-444.6; 3.50)	-48.1 (-197.1; 100.9)	215.9 (8.72; 423.1)	0.019	0.052	0.066
Adiponectin, *pg/mL*	Baseline	127332±66797	133653±87595	138053±77241	0.871	0.603	0.636
	1-y Mean changes	-1240 (-20767; 18288)	3750 (-20907; 28406)	-2076 (-22381; 18229)	0.367	0.410	0.414
Resistin, *pg/mL*	Baseline	1885±1200	1568±1069	1316±683.4	0.028	0.023	0.021
	1-y Mean changes	-68.7 (-420.4; 283.0)	77.0 (-134.7; 288.7)	369.4 (152.8; 585.9)	0.122	0.196	0.198

N=117 participants (N=39 each tertile). Values are means±SD and mean changes are expressed as mean (95% IC). P-values and P for trend were respectively calculated by multivariate lineal regression models. Minimally adjusted model included age, sex, intervention group, recruitment center, smoking status (three categories: never smoker, smoker, and former smoker), type 2 diabetes diagnose (yes/no), changes in BMI (1-year versus baseline, except for waist circumference) and baseline levels of each parameter (pg/mL). The fully adjusted model was further adjusted for educational level (three categories: primary, secondary, or high school), physical activity level (three categories: low, moderate, and active), NSAIDs and cholesterol-lowering treatment (yes/no), energy intake (kcal/day), saturated fatty acid intake (g/day), trans fat intake (g/day), and fiber intake (g/day). Linear trend (p for trend) was assessed across tertiles of VAT, and the mean value was assigned to each tertile. IL: Interleukin; MCP-1: Monocyte Chemoattractant Protein 1; GLP-1: Glucagon-Like Peptide-1; TNF-α: Tumor Necrosis Factor alpha; PAI-1: Plasminogen Activator Inhibitor-1.

Table 3: Changes in total energy intake and nutrient density according to tertiles of change in visceral adipose tissue after 1-year of follow-up

		T1	T2	T3	Unadjusted *P*-value	Full-adjusted *P*-value	*P* for trend
		-509g (-3114 to -283)	-159g (-281 to 32.1)	242g (59.4 to 1643)	(T3 vs T1)	(T3 vs T1)	
Energy intake, *kcal/day*	Baseline	2454±549.5	2309±427.1	2409±529.9	0.699	0.148	0.138
	1-y Mean changes	-224.3 (-409.0; -79.7)	-81.6 (-204.9; 41.6)	-35.2 (-29.1; 188.7)	0.091	0.003	0.002
Carbohydrates, *g day/1000 kcal*	Baseline	95.6±14.6	98.1±13.8	100.2±17.0	0.182	0.343	0.344
	1-y Mean changes	-8.4 (-13.3; -3.5)	-9.5 (-14.2; -4.9)	-8.5 (-14.8; -2.2)	0.976	0.281	0.243
Protein, *g day/1000 kcal*	Baseline	40.7±6.9	42.2±7.0	39.8±8.3	0.593	0.123	0.107
	1-y Mean changes	2.8 (0.4; 5.2)	1.3 (-1.0; 3.6)	2.5 (-0.2; 5.2)	0.877	0.207	0.194
Total fat*, g day/1000 kcal*	Baseline	46.4±5.3	44.7±6.2	46.4±7.5	0.994	0.883	0.958
	1-y Mean changes	2.2 (0.1; 4.3)	3.4 (1.3; 5.5)	2.7 (0.1; 5.3)	0.785	0.240	0.224

MUFA, *g day/1000 kcal*	Baseline	24.6±4.3	23.5±4.7	24.3±4.7	0.809	0.381	0.416
	1-y Mean changes	3.4 (1.8; 5.0)	4.4 (2.6; 6.2)	3.5 (1.5; 5.5)	0.934	0.519	0.531
PUFA, *g day/1000 kcal*	Baseline	7.6±1.7	7.8±1.8	8.0±2.2	0.313	0.473	0.464
	1-y Mean changes	0.9 (0.3; 1.6)	0.8 (0.2; 1.5)	0.3 (-0.5; 1.1)	0.195	0.374	0.386
SFA, *g day/1000 kcal*	Baseline	11.8±2.1	10.9±1.8	11.5±2.2	0.566	0.736	0.868
	1-y Mean changes	-1.1 (-1.8; -0.5)	-0.5 (-1.1; 0.1)	-0.2 (-1.0; 0.5)	0.055	0.519	0.531
Trans fat, *g day/1000 kcal*	Baseline	0.3±0.2	0.2±0.1	0.3±0.1	0.768	0.957	0.971
	1-y Mean changes	-0.1 (-0.1; -0.0)	-0.1 (-0.1; -0.0)	-0.1 (-0.1; -0.0)	0.562	0.007	0.006
Fiber, *g day/1000 kcal*	Baseline	10.8±3.2	11.3±3.2	11.8±4.2	0.236	0.287	0.296
	1-y Mean changes	2.3 (1.2; 3.4)	1.9 (0.7; 3.1)	0.5 (-0.7; 1.8)	0.029	0.496	0.442
Alcohol, *g day/1000 kcal*	Baseline	5.3±5.9	5.1±6.3	3.2±5.2	0.104	0.913	0.851
	1-y Mean changes	0.3 (-1.2; 1.8)	0.3 (-0.8; 1.4)	-0.0 (-1.6; 1.6)	0.730	0.935	0.956
Total polyphenol, m*g day/1000 kcal*	Baseline	398.0±103.8	367.2±106.5	385.6±107.4	0.605	0.244	0.296
	1-y Mean changes	-12.2 (-40.0; 15.6)	-12.9 (-46.4; 20.6)	-42.3 (-80.3; -4.3)	0.199	0.201	0.222
Flavonoids, m*g day/1000 kcal*	Baseline	221.2±88.3	221.7±93.0	230.1±94.7	0.673	0.542	0.555
	1-y Mean changes	-9.2 (-43.0; 24.5)	-4.3 (-32.1; 23.6)	-27.6 (-59.2; 4.1)	0.403	0.083	0.078
Phenolic acids, m*g day/1000 kcal*	Baseline	136.0±61.1	113.6±50.2	118.5±53.4	0.165	0.273	0.348
	1-y Mean changes	-0.6 (-19.1; 17.8)	-13.6 (-29.7; 2.5)	-12.3 (-30.2; 5.5)	0.340	0.421	0.544
Lignans, m*g day/1000 kcal*	Baseline	0.7±0.3	0.7±0.2	0.6±0.2	0.664	0.146	0.138
	1-y Mean changes	0.0 (-0.0; 0.1)	0.0 (-0.0; 0.1)	-0.0 (-0,1; 0.0)	0.278	0.077	0.065
Stilbenes, m*g day/1000 kcal*	Baseline	1.1±1.3	1.0±1.7	0.6±1.3	0.146	0.759	0.724
	1-y Mean changes	0.2 (-0.3; 0.6)	0.5 (0.5; 1.0)	0.1 (-0.3; 0.4)	0.740	0.608	0.547

N=117 participants (N=39 each tertile). Values are means±SD and mean changes are expressed as mean (95% IC). P-values and P for trend were respectively calculated by multivariate lineal regression models. Full-adjusted model was adjusted for age, sex, intervention group, recruitment center, smoking status (three categories: never smoker, smoker, and former smoker), type 2 diabetes diagnose (yes/no), educational level (three categories: primary, secondary, or high school), physical activity level (three categories: low, moderate, and active), NSAIDs and cholesterol-lowering treatment (yes/no), and baseline levels of each nutritional parameter (g/day). Linear trend (p for trend) was assessed across tertiles of VAT, and the mean value was assigned to each tertile. MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; SFA: Saturated fatty acids.

Supplementary 1: Changes in inflammatory parameters according to tertiles of changes in total fat mass after 1-year of follow-up.

		T1	T2	T3	Minimally adjusted *P* value	Full-adjusted *P*-value	*P* for trend
		-4.5 kg (-15.6 to -3.1)	-1.5 kg (-3.0 to -0.3)	0.4 kg (-0.1 to 7.9)	(T3 vs T1)	(T3 vs T1)	
Waist circumference, cm	Baseline	108.3±7.8	108.0±8.6	105.3±9.3	0.732	0.844	0.729
	1-y Mean changes	-5.1 (-7.0; -3.1)	-3.3 (-4.7; -2.0)	0.7 (-0.6; 2.0)	<0.001	<0.001	<0.001
Insulin, *pg/mL*	Baseline	145.9±75.6	150.0±94.6	129.8±67.3	0.163	0.122	0.121
	1-y Mean changes	-6.0 (-32.0; 20.0)	4.6 (-31.0; 39.9)	11.1 (-14.2; 36.4)	0.875	0.757	0.761
C-peptide, *pg/mL*	Baseline	130.7±59.2	126.6±59.3	116.2±41.8	0.346	0.456	0.448
	1-y Mean changes	-8.9 (-21.7; 3.9)	3.4 (-17.4; 24.1)	15.2 (4.1; 26.3)	0.752	0.723	0.761
Glucagon, *pg/mL*	Baseline	197.8±61.6	174.6±47.3	164.4±57.6	0.774	0.731	0.727
	1-y Mean changes	-15.0 (-26.3; -3.7)	2.9 (-13.8; 19.8)	1.6 (-17.7; 20.9)	0.752	0.757	0.761
GLP-1, *pg/mL*	Baseline	9.6±7.6	10.3±7.0	9.8±8.5	0.731	0.435	0.451
	1-y Mean changes	-0.3 (-5.1; 4.5)	1.9 (-1.2; 5.0)	-1.2 (-3.4; 1.0)	0.865	0.757	0.761
Visfatin, *pg/mL*	Baseline	3865±2800	3189±2302	2659±1751	0.836	0.548	0.548
	1-y Mean changes	163.1 (-1826; 2152)	-351.8 (-985.5; 281.9)	301.8 (-258.3; 861.9)	0.875	0.978	0.981
Ghrelin, *pg/mL*	Baseline	112.4±61.3	99.0±75.4	92.3±52.2	0.729	0.719	0.717
	1-y Mean changes	11.0 (-2.6; 24.5)	17.3 (-1.3; 35.9)	10.1 (-2.3; 22.5)	0.752	0.757	0.761
TNF-α, *pg/mL*	Baseline	112.7±131.5	150.4±156.4	148.7±174.3	0.250	0.656	0.649
	1-y Mean changes	21.3 (-18.7; 61.3)	21.5 (-8.9; 51.9)	4.6 (-15.6; 24.7)	0.752	0.723	0.761
IL-6, *pg/mL*	Baseline	12.0±15.9	10.2±14.1	20.3±22.8	0.138	0.326	0.285
	1-y Mean changes	7.1 (0.5; 13.7)	7.0 (-0.3; 14.3)	-7.2 (-13.9; -0.5)	0.865	0.978	0.981
MCP-1, *pg/mL*	Baseline	172.6±195.1	184.5±170.7	225.2±226.5	0.477	0.881	0.876
	1-y Mean changes	-5.8 (-37.9; 26.2)	18.9 (-12.2; 50.0)	-11.7 (-33.0; 9.55)	0.875	0.978	0.981
Leptin, *pg/mL*	Baseline	21148±14526	17927±11428	14422±8884	0.563	0.883	0.885
	1-y Mean changes	-1626 (-4240; 987.9)	1789 (-924.0; 4501)	4044 (593.2; 7495)	0.836	0.865	0.871
Adipsin, *pg/mL*	Baseline	3365±1675	3074±1336	2815±1648	0.930	0.703	0.705
	1-y Mean changes	-445.8 (-916.3; 24.7)	18.5 (-485.6; 522.5)	72.5 (-261.3; 406.4)	0.752	0.723	0.761
PAI-1, *pg/mL*	Baseline	1550±668.2	1467±569.8	1335±613.8	0.536	0.506	0.505
	1-y Mean changes	-16.1 (-235.2; 203.0)	-70.9 (-283.6; 141.9)	39.9 (-143.3; 223.1)	0.752	0.757	0.761

Adiponectin, *pg/mL*	Baseline	134872±65979	131067±85069	133099±81013	0.545	0.188	0.189
	1-y Mean changes	-9839 (-31386; 11709)	4260 (-19586; 28106)	6012 (-12815; 24840)	0.752	0.757	0.761
Resistin, *pg/mL*	Baseline	1744±1095	1655±1114	1381±859.6	0.761	0.660	0.650
	1-y Mean changes	201.6 (-66.8; 470.0)	29.1 (-299.0; 357.3)	98.3 (-125.5; 322.0)	0.993	0.978	0.981

N=117 participants (N=39 each tertile). Values are means±SD and mean changes are expressed as mean (95% IC). P-values and P for trend were respectively calculated by multivariate lineal regression models. Minimally adjusted model included age, sex, intervention group, recruitment center, smoking status (three categories: never smoker, smoker, and former smoker), type 2 diabetes diagnose (yes/no), changes in BMI (1-year versus baseline, except for waist circumference) and baseline levels of each parameter (pg/mL). The fully adjusted model was further adjusted for educational level (three categories: primary, secondary, or high school), physical activity level (three categories: low, moderate, and active), NSAIDs and cholesterol-lowering treatment (yes/no), energy intake (kcal/day), saturated fatty acid intake (g/day), trans fat intake (g/day), and fiber intake (g/day). Linear trend (p for trend) was assessed across tertiles of VAT, and the mean value was assigned to each tertile. IL: Interleukin; MCP-1: Monocyte Chemoattractant Protein 1; GLP-1: Glucagon-Like Peptide-1; TNF-α: Tumor Necrosis Factor alpha; PAI-1: Plasminogen Activator Inhibitor-1.

Supplementary 2: Changes in MetS criteria according to tertiles of changes in visceral adipose tissue after 1-year of follow-up.

		T1 -509g (-3114 to -283)	T2 -159g (-281 to 32.1)	T3 242g (59.4 to 1643)	Minimally adjusted *P* value (T3 vs T1)	Full-adjusted *P*-value (T3 vs T1)	*P* for trend
Waist circumference, cm	Baseline	109.9±8.0	105.0±9.8	106.8±7.4	0.352	0.451	0.413
	1-y Mean changes	-4.8 (-6.7; -2.9)	-2.1 (-3.5; -0.6)	-0.8 (-2.5; 0.8)	0.007	0.554	0.518
Fasting plasma glucose, mg*/mL*	Baseline	117.4±30.3	114.8±32.3	114.9±28.4	0.944	0.788	0.847
	1-y Mean changes	-6.2 (-13.3: 0.8)	-3.1 (-10.6; 4.3)	2.8 (-3.6; 9.2)	0.519	0.320	0.300
Triglycerides, m*g/mL*	Baseline	147.2±62.4	137.4±51.6	152.3±58.9	0.279	0.870	0.822
	1-y Mean changes	-11.6 (-34.1; 11.0)	-14.0 (-26.8; -1.2)	8.2 (-20.6; 37.0)	0.094	0.057	0.037
HDL-cholesterol*, mg/dL*	Baseline	43.6±10.3	47.4±8.06	46.7±10.0	0.601	0.379	0.375
	1-y Mean changes	1.9 (0.1; 3.7)	3.4 (1.1; 5.7)	0.5 (-1.2; 2.2)	0.631	0.269	0.193
Systolic BP, mm*Hg*	Baseline	141.6±15.4	137.2±16.1	141.1±16.7	0.959	0.997	0.872

	1-y Mean changes	**-5.2 (-10.3; -0.1)**	-3.2 (-8.6; 2.1)	2.0 (-2.1; 6.1)	0.727	0.573	0.666
Diastolic BP, *mmHg*	Baseline	79.6±10.3	78.3±10.7	79.6±10.6	0.333	0.414	0.391
	1-y Mean changes	-3.0 (-6.2; 0.1)	-1.5 (-4.3; 1.3)	-2.0 (-4.0; 0.0)	0.218	0.183	0.171

N=117 participants (N=39 each tertile). Values are means±SD and mean changes are expressed as mean (95% IC). P-values and P for trend were respectively calculated by multivariate lineal regression models. Minimally adjusted model included age, sex, intervention group, recruitment center, smoking status (three categories: never smoker, smoker, and former smoker), type 2 diabetes diagnose (yes/no), changes in BMI (1-year versus baseline) and baseline levels of each parameter (pg/mL). The fully adjusted model was further adjusted for educational level (three categories: primary, secondary, or high school), physical activity level (three categories: low, moderate, and active), NSAIDs and cholesterol-lowering treatment (yes/no), energy intake (kcal/day), saturated fatty acid intake (g/day), trans fat intake (g/day), and fiber intake (g/day). We additionally adjusted for antidiabetic treatment when assessing glycemia, and antihypertensive treatment when assessing systolic and diastolic blood pressure. Linear trend (p for trend) was assessed across tertiles of VAT, and the mean value was assigned to each tertile. HDL: High density lipoprotein; BP: Blood pressure.