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Original article

# Adherence to the Mediterranean diet is inversely associated with visceral abdominal tissue in Caucasian subjects



CLINICAL NUTRITION

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### A R T I C L E I N F O

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## SUMMARY

*Background:* & aim: Adherence to the Mediterranean dietary pattern (MDP) is inversely related with abdominal adiposity as detected by waist circumference but the specific association to subcutaneous and visceral abdominal tissue has not been investigated. To this purpose we evaluated the association between MDP, visceral (VAT) and subcutaneous (SAT) abdominal tissue in a large sample of Italian adults. *Methods:* A cross-sectional study was carried out on 4388 consecutive adults (73.2% women) followed as outpatients at Nutritional Research Centre in Milan, ICANS. VAT and SAT were measured by ultrasonography. MDP was evaluated using a Mediterranean dietary score (MEDscore) obtained from a validated 14-item questionnaire.

*Results:* At multiple linear regression adjusted for sex, age, smoking and physical activity, a 1-unit increase in MEDscore was associated with a  $-0.118 \text{ kg/m}^2$  decrease in BMI (p < 0.01), a -0.292 cm decrease in waist circumference (p < 0.01), a -0.002 cm:cm decrease in waist to height ratio (p < 0.001), a -1.125 mm decrease in the sum of 4 skinfolds (p < 0.001), and with a -0.045 cm decrease in VAT (p < 0.05). MEDscore was, however, not associated with SAT. Finally, the adherence to the MDP was a protective factor for obesity (OR = 0.717, 95%CI: 0.555-0.922) and VAT excess (OR = 0.717, 95%CI: 0.530 -0.971).

*Conclusion:* Our study confirms the inverse association between MDP, BMI and waist circumference and adds that the association with abdominal obesity as detected by waist circumference is due to an association with VAT and not with SAT.

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## 1. Introduction

Abdominal obesity is associated with metabolic syndrome and cardiometabolic diseases [1]. However, abdominal fat is composed of visceral abdominal fat tissue (VAT) and subcutaneous abdominal fat tissue (SAT) [2]. VAT plays a central role in the pathogenesis of metabolic syndrome [1,3] and is an independent predictor of cardiometabolic diseases [4]. Whether and to what degree subcutaneous abdominal fat tissue (SAT) contributes to cardiometabolic diseases is however the matter of substantial debate [3]. Computed tomography (CT) and magnetic resonance imaging (MRI) are the reference methods for the assessment of VAT and SAT. However, because of their high costs and exposure to ionizing radiation, they

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Abbreviation: CT, Computed tomography; MDP, Mediterranean dietary pattern; MRI, Magnetic resonance imaging; SAT, Subcutaneous abdominal tissue; SF4, Sum of four skinfolds; VAT, Visceral abdominal tissue; WC, Waist circumference; WHtR, Waist-to-height ratio.

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cannot be used in routine clinical practice and epidemiological research. Ultrasonography offers a validated, cheap and non-invasive alternative to reference methods [5–7] and it has already been used in previous epidemiological researches [8,9].

Diet and lifestyle play a major role in the development of general and abdominal obesity [10]. In particular, the Mediterranean dietary pattern (MDP), moderate alcohol consumption, daily physical activity and nonsmoking, are associated with lower prevalence of general and abdominal obesity [10]. MDP, characterized by high consumption of olive oil, fruits and vegetables, nuts, fish and legumes, low consumption of saturated fats and sugars, and moderate consumption of wine, has been reported to be inversely associated with BMI, waist circumference (WC) and waist-to-height ratio (WHtR) in many cross-sectional studies and some cohort studies [10–18]. These results suggest that MDP may be related to abdominal fat distribution. However, such studies employed WC as surrogate measure of abdominal fat but WC does not allow to separate VAT from SAT [19] so that the association between MDP, VAT and SAT is presently unknown.

The aim of this study was to evaluate the association between MDP and VAT and SAT measured by ultrasonography in a large sample of adults.

## 2. Subjects and methods

#### 2.1. Study design

4388 men and women were consecutively enrolled into the study at ICANS between June 2009 and September 2013. All came to ICANS to obtain a thorough nutritional assessment and/or to enter a structured weight loss program. Subjects aged  $\geq$ 18 years and  $\leq$ 80 years were studied. Exclusion criteria were: abdominal scars from previous surgery in the area of the ultrasound measurements, neurological, gastrointestinal, cardiac, renal and pulmonary failure, cancer, acute illness and use of medications known to cause lipodystrophy including steroids and antiretroviral agents. A very high activity level (participation in sports or training for greater than 12 h/week) was reason of exclusion as well. All measurements were performed on fasting subjects during the morning. A physician collected a clinical history and performed ultrasonographic assessment of VAT and SAT. A dietician performed anthropometric measurements and collected a structured interview on dietary and lifestyle habits (physical activity level and smoke attitude) [20]. Lastly, the patients were administered a validated questionnaire to assess MDP [21,22]. The study was carried out according to the

Table 1					
Measurements	of	the	study	subj	jects.

Declaration of Helsinki and all subjects gave written informed consent. The institutional review board approved the study procedures.

### 2.2. Anthropometry

Anthropometric measurements were performed following international guidelines [23]. Body weight was measured to the nearest 100 g using a Seca 700 scale and height was measured to the nearest 0.1 cm using a Seca 217 vertical stadiometer (Seca Corporation, Hanover, MD, USA). BMI was calculated as weight (kg)/height (m<sup>2</sup>) and classified according to the World Health Organization. Waist circumference was measured midway between the lower rib margin and the superior anterior iliac spine. WHtR was calculated as WC (cm) divided by height (cm). Skinfolds (triceps, biceps, subscapular and suprailiac) were measured using a Tanner-Whitehouse calliper (Holtain Ltd, Crymych, UK). The skinfolds were then summed to obtain the sum of four skinfolds (SF4). In our Centre, the intra-observer coefficient of variation for repeated measurements of these skinfolds is < 2.9%.

## 2.3. Abdominal ultrasonography

Abdominal US was performed on fasting patients by the same operator using a Logiq 3 Pro equipped with a 3.5 MHz convex-array probe and with a 7.5 MHz linear probe (GE Healthcare, Milwaukee, WI, USA). VAT and SAT were measured 1 cm above the umbilicus. The examination was performed at end-expiration and same pressure of the ultrasonographic probe was applied for all participants. SAT was measured with the 7.5 MHz linear probe as the distance between the epidermis and the external face of the *rectus abdominis* muscle, VAT was measured with the 3.5 MHz convexarray probe as the distance between the anterior wall of the aorta and the posterior surface of the *rectus abdominis* muscle [5]. Each measurement was performed three times and a mean was calculated. The within-dayintra-operator coefficient of variation for repeated measures of VAT and SAT in our laboratory is 0.8%.

## 2.4. Mediterranean dietary pattern

MDP was evaluated using a validated 14-item questionnaire [21,22], which is the extension of an original 9-item questionnaire [24]. A Mediterranean score (MEDscore) was obtained from this questionnaire following Estruch et al. [21]. Briefly, one point was attributed for each of the following: 1) olive oil as main cooking fat,

····· · · · · · · · · · · · · · · · ·			
	Women ( <i>n</i> = 3214)	Men ( <i>n</i> = 1174)	Total ( <i>n</i> = 4388)
Age (years)	45 (37–55)	47 (39–57)	46 (37–56)
Weight (kg)	70.8 (63.5-79.4)	89.6 (81.4-98.1)	75.2 (66.0-86.7)
Height (m)	1.62 (1.57-1.66)	1.75 (1.70-1.79)	1.64 (1.59-1.71)
BMI $(kg/m^2)$	27.2 (24.4-30.4)	29.3 (27.0-32.0)	27.9 (25.0-31.0)
BSF (mm)	15 (11-20)	10 (7-13)	13 (9–18)
TSF (mm)	28 (23-32)	17 (13–22)	25 (19-31)
SSF (mm)	28 (20-36)	30 (24–36)	29 (21-36)
SISF (mm)	37 (31–42)	40 (34-44)	38 (32-42)
SF4 (mm)	109 (88-128)	98 (81–113)	105 (86-124)
WC (cm)	89.6 (81.7-97.4)	103.5 (96.0-111.0)	93.2 (84.2-102.5)
WHtR (cm/cm)	0.55 (0.50-0.61)	0.59 (0.55-0.64)	0.57 (0.52-0.62)
VAT (mm)	3.97 (2.92-5.50)	6.87 (5.13-8.77)	4.61 (3.20-6.54)
SAT (mm)	2.69 (1.97-3.48)	2.48 (1.75-3.31)	2.63 (1.90-3.44)
MEDscore (units)	7.0 (5.0-8.0)	7.0 (6.0-8.0)	7.0 (5.0-8.0)

Values are reported as median and interquartile range (between parentheses).

Abbreviations: BMI = body mass index; BSF = biceps skinfold; TSF = triceps skinfold; SSF = subscapular skinfold; SISF = suprailiac skinfold; SF4 = sum of four skinfolds; WC = waist circumference; WHtR = waist to height ratio; VAT = visceral adipose tissue; SAT = subcutaneous adipose tissue.

Table 2					
Nutritional status	lifestyle and	dietarv	characteristics	of recruited	sample.

	Womer	1	Men		Total	
	N	%	N	%	N	%
BMI classes						
Normal	967	30.1	120	10.2	1087	24.8
Overweight	1347	41.9	546	46.5	1893	43.1
Obesity class 1	684	21.3	397	33.8	1081	24.6
Obesity class 2	183	5.7	99	8.4	282	6.4
Total	33	100.0	1174	100.0	45 4388	100.0
Smoke	5214	100.0	11/4	100.0	4300	100.0
No	2490	77.5	788	67.1	3278	74.7
Yes	724	22.5	386	32.9	1110	25.3
Total	3214	100.0	1174	100.0	4388	100.0
Physical activity	1020	60.2	622	52.0	2571	50.0
INU Ves	1939	00.3 30.7	632 542	23.8 46.2	2371 1817	28.0 /1/
Total	3214	100.0	1174	100.0	4388	100.0
Olive oil as main co	oulinary fo	it .				
No	84	2.6	40	3.4	124	2.8
Yes	3130	97.4	1134	96.6	4264	97.2
Total	3214	100.0	1174	100.0	4388	100.0
Onve on $\geq 4$ tablesp	200ns/aay	62.9	917	60.6	2000	65 /
Yes	1163	36.2	357	30.4	1520	34.6
Total	3214	100.0	1174	100.0	4388	100.0
Vegetables $\geq 2$ serv	ings/day					
No	1433	44.6	605	51.5	2038	46.4
Yes	1781	55.4	569	48.5	2350	53.6
Total	3214	100.0	1174	100.0	4388	100.0
Fruits ≥3 servirigs/0	2760	86.2	1008	85.0	3777	86.1
Yes	445	13.8	166	14.1	611	13.9
Total	3214	100.0	1174	100.0	4388	100.0
Red or processed m	neat <1 se	rving/day				
No	998	31.1	417	35.5	1415	32.2
Yes	2216	68.9	757	64.5	2973	67.8
I OTAI Butter or cream or	3214 margarin	100.0 e <1/day	11/4	100.0	4388	100.0
No	11101 gui 111 82	2.6	27	23	109	2.5
Yes	3132	97.4	1147	97.7	4279	97.5
Total	3214	100.0	1174	100.0	4388	100.0
Soda drinks <1/day	,					
No	503	15.7	231	19.7	734	16.7
Yes	2/11	84.3	943 1174	80.3	3054 1200	83.3
Wine $>3$ glasses/w	eek	100.0	11/4	100.0	4300	100.0
No	2436	75.8	606	51.6	3042	69.3
Yes	778	24.2	568	48.4	1346	30.7
Total	3214	100.0	1174	100.0	4388	100.0
Legumes $\geq$ 3 serving	gs/week	000	1000	00.5	44	05.0
N0 Voc	3091	96.2	1086	92.5	41//	95.2
Total	3214	100.0	1174	100.0	4388	100.0
Fish/seafood $\geq 3$ set	vings/wee	ek		10010	1500	10010
No	2912	90.6	1080	92.0	3992	91.0
Yes	302	9.4	94	8.0	396	9.0
Total	. 3214	100.0	1174	100.0	4388	100.0
Sweets and confect	1402	/week	504	42.0	1007	1E E
Ves	1495	40.5 53.5	504 670	42.9 57.1	2391	45.5 54.5
Total	3214	100.0	1174	100.0	4388	100.0
Nuts $\geq 1$ /week						
No	2812	87.5	1009	85.9	3821	87.1
Yes	402	12.5	165	14.1	567	12.9
Total White more than r	3214	100.0	1174	100.0	4388	100.0
No	24 meat () 1233	38.4	587	50.0	1820	41 5
Yes	1233	61.6	587	50.0	2568	58.5
Total	3214	100.0	1174	100.0	4388	100.0
Sofrito sauce $\geq 2/w$	veek					
No	1784	55.5	574	48.9	2358	53.7
Yes Total	1430	44.5	600 1174	51.1 100.0	2030 1300	46.3
MEDscore	5214	100.0	11/4	100.0	4000	100.0
1	2	0.1	1	0.1	3	0.1

Table 2 (continued)

	Womer	ı	Men		Total	
	N	%	N	%	N	%
2	13	0.4	2	0.2	15	0.3
3	64	2.0	26	2.2	90	2.1
4	229	7.1	92	7.8	321	7.3
5	506	15.7	169	14.4	675	15.4
6	696	21.7	265	22.6	961	21.9
7	757	23.6	252	21.5	1009	23.0
8	524	16.3	195	16.6	719	16.4
9	286	8.9	106	9.0	392	8.9
10	106	3.3	50	4.3	156	3.6
11	24	0.7	11	0.9	35	0.8
12	7	0.2	5	0.4	12	0.3
Total	3214	100.0	1174	100.0	4388	100.0

2) olive oil  $\geq$ 4 tablespoons/day, 3) vegetables  $\geq$ 2 servings/day ( $\geq$ 1 portion raw or on salad), 4) fruits  $\geq$ 3 servings/day, 5) red or processed meat <1 serving/day, 6) butter or cream or margarine <1/ day, 7) soda drinks <1/day, 8) wine  $\geq$ 3 glasses/week, 9) legumes  $\geq$ 3 servings/week, 10) fish/seafood  $\geq$ 3 servings/week, 11) commercial sweets and confectionery <3/week, 12) nuts  $\geq$ 1/week, 13) white more than red meats (yes) and, 14) use of sofrito sauce  $\geq$  2/week. Subjects with a MEDscore  $\geq$ 9 points have been considered to have a high adherence to the MDP [25,26].

#### 2.5. Statistical analysis

Continuous variables are reported as 25th, 50th and 75th percentiles because of non-Gaussian distributions. Discrete variables are reported as counts and percentages. The associations between the continuous outcomes of interest (BMI, WC, WHtR, SF4, VAT and SAT) and the continuous predictor (MEDscore) was evaluated using multiple linear regression with correction for age (continuous, years), sex (discrete, 0 = female; 1 = male), physical activity (discrete, 0 = no structured physical activity; 1 = structured physical activity) and smoke attitude (discrete, 0 = not currently smoking; 1 = currently smoking). Multivariable regression splines were used to model non-linear relationships. Because there was evidence of violation of the homoscedasticity assumption for some regression models, we calculated robust confidence intervals for all models. We also used multinomial logistic regression adjusted for sex, age, smoking and physical activity, to evaluate the association between adherence to the MDP and to each of 14 food items and the risk for obesity and VAT (dependent variable, quartiles:  $\leq$  3.2, 3.3-4.6, 4.7-6.5, >6.5 cm) and SAT (dependent variable, guartiles: <1.9, 2.0–2.6, 2.7–3.4, >3.4 cm) excess. Statistical analysis was performed using STATA 13.0 and SPSS version 22.

# 3. Results

The continuous measurements of the 4388 study subjects are given in Table 1.

Women made up 73.2% of the study population. 43.1% of the subjects were overweight and 32.1% were obese. The distribution of the BMI classes, lifestyle descriptors, MEDscore and its components are reported in Table 2.

The relationships between the outcomes of interest (BMI, WC, WHtR, SF4, VAT and SAT) and MEDscore are reported in Table 3.

At multiple linear regression adjusting for sex, age, smoking and physical activity, a 1-unit increase in MEDscore was associated with a  $-0.118 \text{ kg/m}^2$  decrease in BMI (p < 0.01), a -0.292 cm decrease in waist circumference (p < 0.01), a -0.002 cm:cm decrease in waist to height ratio (p < 0.001), a -1.125 mm decrease in the sum of 4

	DMI (120/202)	14/U (cm)	(united (cm)	() PES	WAT (cm.)	CAT (cm)
	DIVIL (Kg/III )			SF4 (IIIIII)		
MEDscore (1-unit increase)	$-0.12^{**}$ $[-0.20, -0.04]$	$-0.29^{**} \left[-0.48, -0.10 ight]$	$-0.00^{***}$ [ $-0.00, -0.00$ ]	$-1.13^{***}$ $[-1.61, -0.64]$	$-0.05^{*}$ [ $-0.08, -0.01$ ]	-0.01 [ $-0.04,0.01$ ]
Age (1-year increase)	0.08**** [0.07,0.09]	0.35*** [0.32,0.37]	0.00**** [0.00,0.00]			
Male Gender (Yes)	$1.82^{***}$ $[1.54, 2.09]$	$12.94^{***}$ [12.25,13.63]	0.03*** [0.03,0.04]	$-10.69^{***}$ $[-12.35, -9.02]$	2.51*** [2.36,2.65]	$-0.12^{**}$ $[-0.19, -0.04]$
Physical activity (Yes)	$-1.55^{***}$ $[-1.80, -1.29]$	$-4.25^{***}$ [-4.87,-3.62]	$-0.03^{***}$ [ $-0.03, -0.02$ ]	$-10.97^{***}$ $[-12.59, -9.35]$	$-0.77^{***}$ [ $-0.88, -0.65$ ]	$-0.27^{***}$ [ $-0.34, -0.21$ ]
Smoke (Yes)	0.12 [-0.18, 0.42]	$0.73^{*}$ [0.00,1.46]	0.00 [-0.00,0.01]	-0.53 $[-2.34, 1.27]$	0.21** [0.07,0.35]	-0.05[-0.13,0.02]
Age (years) SP1	, I	1	1	3.46*** [2.59,4.34]	$0.92^{***}$ [0.86,0.98]	$-0.07^{***}$ [-0.10,-0.03]
Age (years) SP2	1	1	1	1.30** [0.48,2.11]	$-0.07^{*}$ [ $-0.12, -0.01$ ]	0.10*** [0.07,0.13]
Constant	$25.24^{***}$ [24.60,25.88]	77.65*** [76.13,79.17]	0.47*** [0.46,0.47]	$119.08^{***}$ [115.72,122.44]	$5.00^{***}$ [4.76,5.25]	2.98*** [2.83,3.12]
Observations	4388	4388	4388	4388	4388	4388
p < 0.05, ** p < 0.01, ** p < 0.00						

Table 3

Abbreviations: SP1 = spline knot 1; SP2 = spline knot 2.

Values are regression coefficients and robust 95% confidence intervals obtained from multiple linear regression adjusted for age, gender, physical activity and smoking status (in brackets). When the association of age with the e is to be interpreted as the effect of the increase of 1 year of age. This = -0.05\*MEDscore + 0.92\*Age\_SP1 - 0.07\*Age\_SP2 + 2.51\*Male outcome is linear, e.g. for the BMI model (BMI =  $-0.12^{-}$ )MEDscore + 0.08\*Age + 1.82\*Adale - 1.55\*Activity + 0.12\*Smoking + 25.24), the effect of age is to intermetation does not hold if the association of age with the outcome is non linear and splines are used, e.g. for the VAT model (VAT = -0. 0.77\*Activity + 0.21\*Smoke + 5.00). skinfolds (p < 0.001), and with a -0.045 cm decrease in VAT (p < 0.05). MEDscore was however not associated with SAT.

Finally, we studied the associations between adherence to the MDP and to each of 14 food items and BMI classes and guartiles of VAT and SAT using a multinomial logistic regression adjusted for sex, age, smoking and physical activity (Table 4). First, the high adherence to the MDP was inversely associated to the risk of developing obesity (OR = 0.717, 95%CI: 0.555–0.922). Moreover, a high consumption of red wine, fish and nuts and a low consumption of red meat and soda drinks were protective factors for obesity. A higher olive oil, sofrito and white meat consumption increased the risk of obesity. In addition, the high adherence to the MDP was a protective factor for VAT (OR = 0.717, 95%CI: 0.530-0.971), but not SAT (OR = 0.907, 95%CI: 0.701-1.172) excess. When we analysed all 14 food items separately, we found that a low consumption of red meat, butter, carbonated beverages and sweets, and a high consumption of vegetables, shellfish and nuts were protective factors for VAT excess. Contrarily, a high consumption of olive oil and sofrito were risk factors for higher VAT. However, the prevalent use of olive oil as culinary fat was protective from VAT excess. Only red wine and fish consumption were protective factors for SAT excess whereas olive oil and white meat increased the risk.

## 4. Discussion

In the present cross-sectional study, performed in a large sample of Italian adults, we found that MDP was inversely associated with VAT and not associated with SAT. This is the first time that these associations are explored and our results expand the available knowledge on the association between MDP and abdominal obesity.

In agreement with previous studies [25,26], only 13.6% of our subjects had a MEDscore indicative of high adherence to the MDP. This differs from other clinical settings and this discrepancy is explained in part by the younger age of our subjects, because MDP is known to increase with increasing age [27]. Also in agreement with previous studies [10,13–18], we found an inverse association between MEDscore and BMI, WC, WHtR and SF4. Although most studies reported an inverse association between MEDscore and BMI, no association was found in a large study of 497308 subjects recruited from 10 different European countries [14]. However, the same study found an inverse association between MDP and WC and the authors speculate that systematic differences between Northern and Southern European populations may be responsible for the lack of association between MDP and BMI. We too were not able to detect any BMI-MEDscore association in a previous study [25]. This may partly be explained by the analytical technique employed as in that study we categorized BMI as quintiles while we evaluated as continuous in the present study. However, and more importantly, in that study we did not separate VAT from SAT as we did here [25]. An inverse association has been recently reported between MDP (measured by a different questionnaire than that employed here) and percent body fat measured by dual-energy X-ray absorptiometry [18]. The same study reported that a 1-unit increase of MDP was associated with a -2.6 mm decrease of the sum of skinfolds. Even if we used a different instrument to measure MDP, our findings are similar as we found that a 1-unit increase of MDP was associated with a -1.125 mm decrease of the same of four skinfolds. Our findings also confirm the existence of an inverse association between MDP and WHtR as reported by Martinez-Gonzalez et al. in the PREDIMED study [13]. Interestingly, we found an inverse association between MEDscore and VAT but no association between MEDscore and SAT. In detail, a 1-unit increase of MDP was associated with a -0.045 cm decrease of VAT (p < 0.05). Although the decrease of VAT seems relatively small, a high adherence to the

#### Table 4

Association between adherence to the MDP and to each of 14 food items and nutritional status and abdominal adiposity.

	Normal weight	(Reference)	Overweight	Obesity
	riormai rreight	(increment)	OR (95% CI)	OR (95% CI)
Olive oil as main culinary fat (Ves)	1		1000(0633-1581)	0.951(0.570-1.586)
Olive oil $(>4 \text{ spoons/day})$	1		1.600(0.000 - 1.301)	$1.277 (1.068 - 1.527)^{**}$
Vegetables (>2 servings/day)	1		0.871(0.744 - 1.019)	0.900(0.758 - 1.069)
Fruit (>2 corvings/day)	1		0.071(0.744 - 1.015)	1142(0.901 - 1.465)
Pod most ( $<1$ coming/day)	1		0.555(0.755-1.164) 0.741(0.624, 0.870)**	1.142(0.851 - 1.403)
Red filed (<1 serving/day)	1		0.741(0.024-0.079)	1.011(0.501-0.726)
Butter (<1 serving/day)	1		1.061 (0.646-1.742)	1.011 (0.592–1.726)
Soda drink (<1 glass/day)	1		$0.787(0.634-0.977)^{\circ}$	0.717 (0.567–0.905)
Wine ( $\geq$ 3 glasses/day)	1		0.747 (0.625–0.894)**	0.528 (0.432–0.644)***
Legumes ( $\geq$ 3 servings/day)	1		0.969 (0.670-1.403)	0.824 (0.546-1.242)
Fish/Seafood ( $\geq$ 3 servings/day)	1		0.701 (0.543-0.905)**	0.583 (0.435-0.782)***
Sweets (<3 times/day)	1		0.950 (0.813–1.111)	0.980 (0.825–1.163)
Nuts ( $\geq 1$ servings/day)	1		0.736 (0.588-0.922)**	0.559 (0.433–0.721)***
White meat (Yes)	1		1.135 (0.970-1.329)	1.289 (1.084–1.533)**
Sofrito ( $\geq 2 \text{ times/day}$ )	1		1.270 (1.086-1.486)**	1.424 (1.200-1.690)***
Adherence to the MDP (MEDscore $\geq$ 9)	1		0.826 (0.656-1.041)	0.715 (0.555-0.922)*
	Ouartile 1 (VAT $< 2.2$ cm)	Quartile 2 (VAT 2.2, 4.6 cm)	$O_{\mu\nu}$ or $2(VAT 47, 65 \text{ cm})$	Ouartilo $A(VAT > 6.5 cm)$
	Qualtile I (VAI $\leq$ 5.2 CII)	Qualtile 2 (VAI $5.5-4.6$ CIII)	Qualitie 5 (VAI $4.7-0.5$ CIII)	Qualtile 4 (VAI $>0.5$ CIII)
	(Reference)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Olive oil as main culinary fat (Yes)	1	0.405 (0.238-0.687)**	0.529 (0.291-0.694)*	0.819 (0.666-1.008)
Olive oil (>4 spoons/day)	1	1.174 (0.980-1.406)	1.271 (1.051-1.537)*	1.357 (1.093-1.686)**
Vegetables (>2 servings/day)	1	0.864 (0.726-1.028)	0.692 (0.576-0.832)***	0.635 (0.515–0.783)***
Fruit (>3 servings/day)	1	0.809(0.624 - 1.051)	0.835(0.639 - 1.093)	0.833(0.619 - 1.120)
Red meat (<1 serving/day)	1	0.986(0.818 - 1.187)	0 726 (0 597–0 882)**	$0.589(0.471-0.736)^{***}$
Butter ( $<1$ serving/day)	1	1195(0651-2193)	0.728(0.414 - 1.315)	$0.503(0.171-0.938)^{*}$
Soda drinks ( $<1$ glass/day)	1	0.800(0.632 - 1.013)	0.751 (0.584-0.965)*	$0.563 (0.271 \ 0.345)^{***}$
Wine (>3 glasses/day)	1	11/3 (0.032 - 1.013)	0.825(0.665-1.025)	$0.903(0.423 \ 0.743)$
Logumos(>2 sorvings/day)	1	1506 (0.000 2.270)	0.810 (0.506 1.200)	0.327(0.733(1.172))
Eight $(\geq 3 \text{ set vings/day})$	1	1.500(0.555-2.270)	0.510(0.300-1.255)	0.737(0.442 - 1.227)
$r_{1}r_{2}r_{2}r_{3}r_{3}r_{3}r_{3}r_{3}r_{3}r_{3}r_{3$	1	1.010(0.024 - 1.103)	0.338(0.402-0.774)	0.737(0.518 - 1.050)
Sweets (<5 times/uay)	1	1.010 (0.851–1.199)	0.829 (0.691-0.994)	1.049 (0.851-1.294)
Nuts $(\geq 1 \text{ servings/day})$	1	0.886(0.685 - 1.146)	$0.759(0.579-0.995)^{\circ}$	$0.601(0.442 - 0.818)^{10}$
white meat (Yes)	1	0.949(0.797 - 1.130)	0.886(0.737 - 1.066)	0.986(0.797 - 1.218)
Sofrito ( $\geq 2$ times/day)	1	1.216 (1.022–1.447)*	1.706 (1.421–2.048)***	1.923 (1.561–2.368)***
Adherence to the MDP (MEDscore $\geq$ 9)	1	0.980 (0.755–1.272)	0.704 (0.532-0.931)*	0.717 (0.530–0.971)*
	Quartile 1 (SAT $\leq$ 1.9 cm)	Quartile 2 (SAT 2.0–2.6 cm)	Quartile 3 (SAT 2.7–3.4 cm)	Quartile 4 (SAT >3.4 cm)
	(Reference)	OR (95%CI)	OR (95%CI)	OR (95%CI)
		0.072 (0.520 1.420)		
Olive oil as main culinary fat (Yes)	1	0.872 (0.529–1.438)	0.907 (0.545–1.511)	1.137 (0.672–1.921)
Olive oil ( $\geq 4$ spoons/day)	1	1.195 (0.998–1.431)	1.355 (1.132–1.621)**	1.280 (1.069–1.534)**
Vegetables (≥2 servings/day)	1	0.920 (0.776–1.091)	0.899 (0.758–1.067)	0.989 (0.833–1.174)
Fruit (≥3 servings/day)	1	0.814 (0.635-1.042)	0.984 (0.773–1.253)	1.015 (0.793–1.299)
Red meat (<1 serving/day)	1	1.089 (0.906-1.310)	0.877 (0.731-1.051)	0.901 (0.752-1.080)
Butter (<1 serving/day)	1	0.845 (0.497-1.434)	1.038 (0.596-1.806)	1.062 (0.609-1.852)
Soda drinks (<1 glass/day)	1	1.182 (0.935-1.495)	0.897 (0.716-1.122)	1.027 (0.819-1.287)
Wine ( $\geq$ 3 glasses/day)	1	1.051 (0.872-1.267)	0.914 (0.756-1.107)	0.659 (0.540-0.805)***
Legumes ( $\geq$ 3 servings/day)	1	1.131 (0.783-1.634)	0.993 (0.675-1.461)	0.691 (0.450-1.062)
Fish/Seafood (>3 servings/day)	1	0.914 (0.694-1.204)	0.724 (0.540-0.971)*	0.712 (0.527-0.962)*
Sweets (<3 times/day)	1	1.021 (0.861-1.211)	0.964 (0.812-1.144)	0.977 (0.823-1.159)
Nuts (>1 servings/dav)	1	0.844 (0.661-1.076)	0.848 (0.663-1.085)	0.767 (0.593-0.993)*
White meat (Yes)	1	0.959(0.808 - 1.138)	1.097(0.923 - 1.304)	$1.205(1.013 - 1.434)^*$
Sofrito (>2 times/day)	1	1 091 (0 921–1 292)	1 172 (0 988-1 389)	1116(0941-1324)
Adherence to the MDP (MEDscore $> 0$ )	1	0.977 (0.767 - 1.244)	0.898(0.701 - 1.152)	0.907 (0.701 - 1.172)
$\frac{1}{2}$		0.577 (0.707 1.244)	0.000 (0.701 1.102)	0.007 (0.701 1.172)

Values are odd-ratios (OR) and 95% confidence intervals (95% CI) obtained from multinomial logistic regression adjusted for age, sex, physical activity and smoking status. \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.01; \*\*\*p < 0.01.

MDP (MEDscore = 14) compared with a low adherence to the MDP (MEDscore = 0) would lead to a theoretical VAT decrease of 0.63 cm, corresponding to a reduction of 13.6% of median value found in our population. This result seems to be higher if compared with that one obtained for WC, for which a high adherence to the MDP would lead to a theoretical reduction of 4.4% of the median value. Moreover, we found that the high adherence to the MDP was a protective factor of VAT, but not SAT, excess. Therefore, although association does not imply causation, our findings are compatible with the hypothesis that MDP may be a protective factor for VAT accumulation. Future longitudinal studies should be focused on the effect of changes in VAT thickness on the more relevant metabolic syndrome components (e.g. triglycerides, HDL-cholesterol, glucose and blood pressure). Several studies have reported a decrease in obesity or greater weight loss with the adoption of a Mediterranean

diet [28,29] but other studies have reported no association [30,31]. Comparing these studies is difficult, however, because of the heterogeneity of study designs, recruitment methods and diagnostic assessments. A recent cohort study found the same incidence of abdominal obesity detected by WC across tertiles of MDP [11]. However, WC does not allow to separate VAT from SAT and thus the relative contribution of VAT and SAT to abdominal obesity remains unclear [19]. Intervention studies are needed to test the hypothesis that an increase in the mediterraneity of the diet can actually reduce VAT.

In addition to the observed inverse association between the high adherence to the MDP and visceral obesity, when we individually assessed each of the 14 items included in the score, many of them were associated with a lower risk of obesity and VAT excess and, in some case, also with SAT. It is not unexpected that at the highest levels of olive oil consumption the risk of excessive BMI, SAT, VAT was increased. Moreover, being olive oil one of the ingredients of sofrito, the positive association between sofrito and risk of obesity and VAT and SAT excess could depend on the excess of olive oil consumption. Interestingly, however, the prevalent use of olive oil as culinary fat was protective from VAT and not from SAT excess. These observational data in a large independent cohort, are consistent with the findings of Babio et al., who found that a controlled olive oil supplementation determined a significant decrement of central obesity [32].

Two points of strength of this study are the large sample size and the use of a validated MDP questionnaire [13,21,24-26]. However, our study has also some limitations. First, a crosssectional study alone cannot prove cause-effect relationships and cohort studies are needed to test whether MDP changes are associated with VAT changes. Second, we studied self-referred men and women coming to our Research Centre to obtain a thorough nutritional assessment and/or to enter a structured weight loss program. As such, our findings may not apply to the general population. Third, we measured VAT and SAT using ultrasonography, which is not the reference method for the evaluation of abdominal adipose tissue. However, many studies have shown a good correlation (0.7-0.81) between ultrasound thicknesses and areas of abdominal adipose tissue measured by CT and MRI [5–7], suggesting a good quality of ultrasound measurements of VAT and SAT [2]. Nevertheless, our results need to be confirmed by further studies using reference methods for the evaluation of abdominal fat. Finally, a further potential limitation is the estimation of the total body fat depot using four skinfolds excluding leg skinfolds. However, previous studies found comparable risk marker associations with the estimation of adiposity with these four skinfolds and with specific fat depots quantified by MRI [33,34].

In conclusion, our study confirms the inverse association between MDP, BMI and WC and adds that the association with abdominal obesity as detected by WC is due to an association with VAT and not with SAT.

## Authors' contributions

SB, AL and AB: designed the research; SB, AL, LV, AS, and AV: conducted the research; SB, AL and GB: performed statistical analysis; SB, AL and GB: wrote the manuscript; MAMG and MBR: gave their intellectual support, interpretation of the results and manuscript revision; SB: had primary responsibility for final content; and all authors: read and approved the final manuscript.

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## **Conflict of interest**

All authors have no conflicts of interest to declare.

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