Appendix 1.

AlkaMeSy Study

The AlkaMeSy (Alkam Metabolic Syndrome) Study is a retrospective, observational study performed in collaboration with 10 Primary Care Physicians (PCPs) of the National Health Service (SIMG: Società Italiana di Medicina Generale) on 13,195 individuals from the city of Alcamo, Western Sicily. The aims of AlkaMeSy Study were 1) to investigate the prevalence of Metabolic Syndrome (MetS) evaluated according to ATP III criteria and related cardiovascular events; 2) to quantify the dispersion of MetS parameters in PCP clinical practice, using a computed system. WC was measured at the midpoint between the west rib and the iliac crest. Blood chemistry analyses were performed in accredited laboratories of the National Health Service in Alcamo.

Appendix 2.

Calculation of Model of Adipose Distribution (MOAD) and Visceral Adiposity Index (VAI)

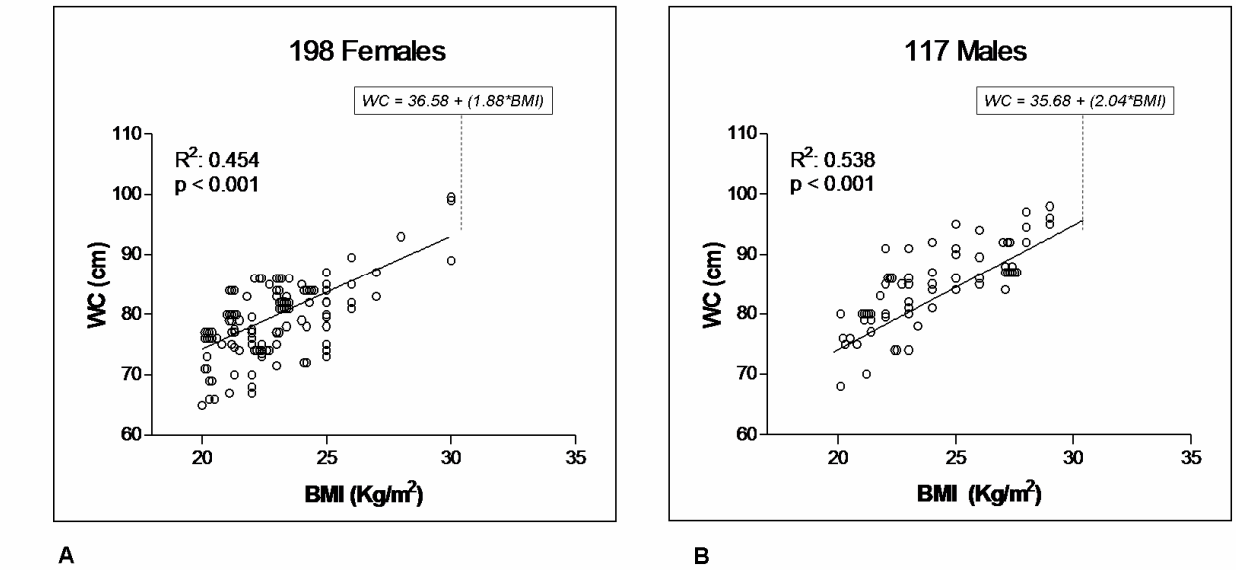
315 subjects (117 males and 198 females), with BMI between 20 and 30 Kg/m2 and aged 43.46 ± 14.30 years (range 19-83) were selected from the 1,498 PC patients for the absence of: diabetes mellitus or FPG > 5.6 mmol/l, high blood pressure, dyslipidemia, MetS and cardiovascular disease (CVD).

In this selected group of healthy subjects, a highly significant positive linear correlation between WC and BMI was observed both in males (coefficient of determination R2: 0.538; F-ratio: 133.81; $p < 0.001$) and females (coefficient of determination R2: 0.453; F-ratio: 162.66; $p < 0.001$), as described in the following linear equations:

$$Males:\ WC = 39.68 + (1.88 \times BMI)$$

$$Females:\ WC = 36.58 + (1.89 \times BMI)$$

where the constants 39.68 and 36.58 correspond to the respective intercepts and the constants 1.88 and 1.89 correspond to the respective regression line slopes (Fig. 1A; Fig. 1B).



A Model of Adipose Distribution (MOAD) was calculated according to these linear relationships between WC (expression of visceral fat) and BMI (expression of generalized fat):

$$Males: MOAD = WC/\left[39.68 + (1.88 \times BMI)\right];$$
$$Females: MOAD = WC/\left[36.58 + (1.89 \times BMI)\right];$$

assuming MOAD = 1 in subjects with normal ratio between subcutaneous and visceral adipose tissue.

This assumption offers ideal approximation when obtained by evaluation of the central tendency and dispersion of MOAD in the 315 healthy, non-obese subjects (mean 0.99; median 1.00; mode 0.97; range 0.86-1.12; standard deviation 0.050; standard error 0.002; variance 0.003).

To correct fat distribution (MOAD) for fat function, TG and HDL levels were introduced in the formula. This was defined as Visceral Adiposity Index or VAI. Median TG and HDL values of the 315 healthy subjects were used (Males: median TG = 1.03 mmol/l, median HDL = 1.31 mmol/l; Females: median TG = 0.81 mmol/l, median HDL = 1.52 mmol/l). Visceral adipose dysfunction was arbitrarily set for TG values higher than median values of healthy population and HDL values lower than median values of healthy population.

VAI is shown in the following equations:

$$Males: \mathbf{VAI} = \left(\frac{WC}{39.68 + (1.88 \times BMI)}\right) \times \left(\frac{TG}{1.03}\right) \times \left(\frac{1.31}{HDL}\right);$$
$$Females: \mathbf{VAI} = \left(\frac{WC}{36.58 + (1.89 \times BMI)}\right) \times \left(\frac{TG}{0.81}\right) \times \left(\frac{1.52}{HDL}\right);$$

Assuming VAI = 1 in healthy, non-obese subjects with normal ratio between subcutaneous and visceral adipose tissue and normal levels of TG and HDL.

Appendix 3.

MRI visceral and subcutaneous fat evaluation

Image acquisition was performed on a 1.5 T clinical MRI scanner GE Signa EXCITE® HD (Milwaukee, WI, USA), using body coil and a 2D dual-echo fast gradient after each RF excitation with water and fat signals in the in-phase (IP) and opposed-phase (OP), respectively. Slices were acquired cranio-caudally, including abdomen from the xyphoid to the pubis. The scan parameters used were: TR=150ms, TE1=2.0ms, TE2=5.3ms, flip angle 80°, matrix 320 x 192, FOV 430, slice thickness 4 mm. Scan time was 18 sec for each acquisition. A fully automated algorhythm for VAT and SAT sequence segmentation was developed based on a set of sequential processing steps applied to each image, without any image intensity correction.

A brief description of each processing step is reported below:

1) Input Image. Processed image is an MRI image of the acquired sequence;
2) Region of Interest (ROI) segmentation. A binary image is obtained splitting the tissue area from the background area. Image binarisation is obtained applying a dynamic threshold maximising the tissue area on the centre of each image;
3) SAT segmentation. A priori geometrical information for adipose tissue is exploited to locate and select a pixel seed to be used in an automatic region growing procedure. First, full image histogram is used for a course grain SAT segmentation. Sequentially, a seed pixel, located on ROI border mask, is selected among the previous segmented pixels. The seed has a significant value on the image histogram, and it is close to the highest bimodality histogram maximum value. The stop criteria are selected considering the distance between the bimodality histogram threshold value and the two bimodality histogram maximum values. As result of this fine grain segmentation procedure, the subcutaneous adipose tissue pixels are segmented.
4) VAT segmentation. The previous procedure is applied for the internal visceral adipose tissue (VAT). The segmented region is used with a NOT operator to split the VAT and the SAT pixels. After the course grain VAT segmentation, and the fine grain VAT segmentation (region growing procedure), the visceral adipose tissue pixels are segmented.

The DICOM image Pixel Spacing attribution is used to determine the SAT and VAT area (cm^2) in each slice. SAT and VAT area estimation and DICOM image Slice Thickness is attributed and then used to determine the SAT and VAT volume (cm^3) in the whole image sequence.

Supplemental Table 1. Characteristics of 1,498 subjects grouped according to VAI Quintiles

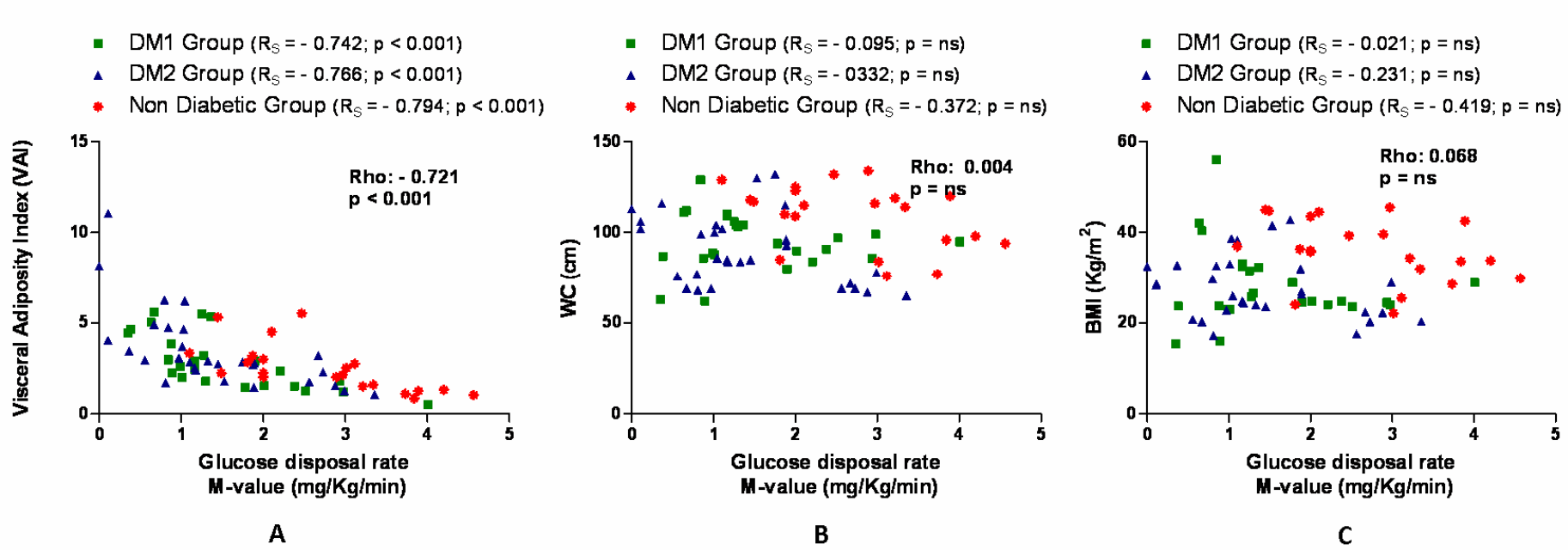
Characteristic	Quintile 1 (No. 299)	Quintile 2 (No. 300)	Quintile 3 (No. 300)	Quintile 4 (No. 300)	Quintile 5 (No. 299)	*P Value for trend*[†]
VAI						
Mean ± SD	0.71± 0.12	1.03 ± 0.08	1.31 ± 0.09	1.70 ± 0.15	3.11 ± 1.30	-
Median	0.73	1.04	1.31	1.66	2.67	-
Range	0.28 - 0.88	0.89 - 1.17	1.18 - 1.47	1.48 - 2.02	2.03 - 10.42	-
Age (years)						
Mean ± SD	36.94 ± 15.12	39.97 ± 16.51	43.63 ± 17.40	51.28 ± 16.57	57.59 ± 16.91	*< 0.001*[††]
Median	34	36.5	41	51.5	58	
Range	19-85	16-87	18-98	19-90	19-99	
	No. (%)	*No. (%)*	*No. (%)*	*No. (%)*	*No. (%)*	
Females	162 (54.2)	214 (71.3)	223 (74.3)	214 (71.3)	212 (70.9)	*< 0.001*
Males	137 (45.8)	86 (28.7)	77 (25.7)	86 (28.7)	87 (29.1)	
Metabolic Syndrome[§]	-	5 (1.7)	6 (2.0)	25 (8.3)	106 (35.5)	*< 0.001*
Diabetes or fasting glucose ≥ 5.6 mmol/l	12 (4.0)	21 (7.0)	12 (4.0)	31 (10.3)	60 (20.1)	*< 0.001*
High Blood pressure[§]	23 (7.7)	44 (14.7)	46 (15.3)	90 (30.0)	135 (45.2)	*< 0.001*
Low HDL cholesterol[§]	13 (4.3)	39 (13.0)	73 (24.3)	80 (26.7)	183 (61.2)	*< 0.001*
High triglycerides[§]	-	-	-	18 (6.0)	183 (61.2)	*< 0.001*
LDL Cholesterol ≥ 3.37 mmol/l	55 (18.4)	74 (24.7)	93 (31.0)	86 (28.7)	117 (39.1)	*< 0.001*
CHD or MI	1 (0.3)	-	4 (1.3)	5 (1.7)	22 (7.4)	*< 0.001*
TIA or IS	1 (0.3)	-	3 (1.0)	10 (3.3)	30 (10.0)	*< 0.001*
Current or former smoker	111 (37.2)	90 (30.1)	100 (33.4)	94 (31.3)	99 (33.3)	*0.436*

Baseline characteristics were presented as mean ± SD for continuous variables. Rates and proportions were calculated for categorical data.

[†] Chi-squared test for trend. [††] Anova Trend Analysis (Significant values when $P < 0.05$).

[§] According to criteria established by ATP III

Supplemental Figure 1. (A) Correlation between VAI and glucose disposal rate (M-value) in 24 Type 1 diabetic patients (DM1 Group), 29 Type 2 diabetic patients (DM2 Group) and 21 non-alcoholic fatty liver disease and polycystic ovary syndrome (non-diabetic group). **(B)** Correlation between WC and M-value in the same groups. **(C)** Correlation between BMI and M-value in the same groups. Total patients: 30 males and 44 females, age 31.71 ± 13.15 years, BMI 30.10 ± 8.32 Kg/m^2, WC 97.04 ± 19.13 cm.



Univariate correlations were performed using non-parametric test (Spearman, Rs).

Supplemental figure 2. Comparison of ROC Curves for Coronary Heart Disease (CHD) and/or Myocardial Infarction (MI) **(A)** and Transient ischemic attack (TIA) and/or Ischemic Stroke (IS) **(B)**.