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ORIGINAL CONTRIBUTION

Age-related changes in basal substrate oxidation and visceral adiposity and their association with metabolic syndrome

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Abstract
Purpose Ageing is directly associated with visceral fat (VAT) deposition and decline of metabolically active cellular mass, which may determine age-related shifts in substrate oxidation and increased cardiometabolic risk. We tested whether VAT and fasting respiratory quotient (RQ, an index of macronutrient oxidation) changed with age and if they were associated with increased risk of metabolic syndrome (MetSyn).
Methods A total of 2819 adult participants (age range: 18–81 years; men/women: 894/1925) were included; we collected history, anthropometric measures, biochemistry, smoking habits, and physical activity. The body mass index range was 18.5–60.2 kg/m^2. Gas exchanges (VO_2 and VCO_2) were measured by indirect calorimetry in fasting conditions, and RQ was calculated. Body composition was measured by bioelectrical impedance. Abdominal subcutaneous fat and VAT were measured by ultrasonography. MetSyn was diagnosed using harmonised international criteria. Multivariate linear and logistic regression models were utilised.
Results VAT increased with age in both men ($r = 0.31$, $p < 0.001$) and women ($r = 0.37$, $p < 0.001$). Basal RQ was not significantly associated with age ($p = 0.49$) and VAT ($p = 0.20$); in addition, basal RQ was not a significant predictor of MetSyn (OR 3.31, 0.57–19.08, $p = 0.27$). VAT was the primary predictor of MetSyn risk in a fully adjusted logistic model (OR 4.25, 3.01–5.99, $p < 0.001$).
Conclusions Visceral adiposity remains one of the most important risk factors for cardiometabolic risk and is a significant predictor of MetSyn. Post-absorptive substrate oxidation does not appear to play a significant role in age-related changes in body composition and cardiometabolic risk, except for a correlation with triglyceride concentration.

Keywords Indirect calorimetry · Substrate oxidation · Ageing · Body composition · Metabolic risk

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Introduction

Ageing is characterised by a decline in total energy expenditure (EE) including resting EE, dietary-induced thermogenesis and activity EE [1, 2]. These changes may predispose to weight gain and accumulation of abdominal fat [3], which are closely linked to the pathogenesis of cardiometabolic, musculoskeletal and neuro-degenerative diseases such as coronary heart disease, metabolic syndrome (MetSyn) or Alzheimer's disease [4].

A reduced post-prandial and exercise-induced fat oxidation has been proposed as a physiological mechanism contributing to age-related increase in adiposity and decline in lean body mass [5–8]. Quantitative and qualitative modifications in these tissue depots are associated with ageing and influence the oxidative efficiency of metabolically active cellular mass, which may predispose to weight gain, promote the expansion of visceral fat and ectopic fat deposition [9, 10]. The physiological basis of these hypotheses is sound and, therefore, several research groups have investigated these metabolic pathways in an attempt to identify novel mechanisms of metabolic ageing and regulation of energy balance [1, 11–13]. Human studies have produced contrasting findings and provided so far a feeble empirical support to these mechanistic hypotheses. However, the negative results could in part be explained by methodological factors rather than by intrinsic flaws of the purported hypotheses based on the existence of age-related changes in oxidative metabolism. Possible factors include small sample size, narrow and restrictive inclusion criteria, clustering of populations into non-continuous, discrete age groups (i.e. young vs old) or different methods utilised for the assessment of body composition and EE.

The scientific evidence linking basal substrate oxidation to impaired metabolic health is even more limited. The study of the association between substrate oxidation and cardiovascular risk factors [(i.e. glucose, triglycerides, insulin, free fatty acids (FFAs)] [7, 14–21] is often a secondary objective, which may impact on the statistical power and selective presentation of results. In the last 10 years, a handful of studies have explored the association of basal substrate oxidation with chronic disease risk. Outcomes included prediction of subclinical atherosclerosis [18], hypertriglyceridemia [20, 22–24], liver steatosis [15] or ventricular cardiac remodelling [19]. Results have overall indicated a potential role of basal substrate oxidation in the pathogenesis of these disorders; however, studies were characterised by important differences and limitations and, therefore, a cautious interpretation of the current evidence is needed. An appraisal of relevant human studies investigating the association between substrate oxidation with ageing, adiposity and cardiovascular risk factors is reported in Table S1 of the Online Supplementary Material.

The considerable sample size of our study and the detailed assessment of body composition and EE in an assorted, adult population has allowed a re-evaluation of these hypotheses linking the age-related decline in fat oxidation to the increase in adiposity. The primary objective of the analyses was to investigate whether ageing was correlated with adiposity indexes and fasting gas exchanges [oxygen (O2) consumption and carbon dioxide (CO2) production], respiratory quotient (RQ) and fractional, non-protein substrate oxidation (fat, carbohydrates) in male and female subjects. Subsequently, we tested whether RQ, as an integrated measure of substrate oxidation, was a significant predictor of sub-cutaneous and visceral fat deposition after adjustment for relevant covariates. Finally, we tested whether adiposity indexes and basal substrate oxidation were significant predictors of individual cardiovascular risk factors [i.e. systolic and diastolic blood pressure (BP), glucose, high density lipoproteins (HDL), triglycerides] and risk of developing MetSyn.

Methods

Study protocol and participants

The study sample included participants recruited consecutively among subjects attending the International Center for the Assessment of Nutritional Status (ICANS, University of Milan) for clinical and nutritional evaluation between February 2010 and September 2013. The sample comprised adult Caucasian men and women (age $\geq$ 18 years) with a body mass index (BMI) $\geq$ 18.5 kg/m^2. 3442 subjects (male/female: 977/2465) were recruited. We excluded subjects with medical conditions likely to influence EE (e.g. cancer, systemic inflammatory disorders, acute and chronic kidney failure, HIV, thyroid and adrenal disorders); as a result, 2819 adult participants (age range: 18–81 years; men/women: 894/1925) were included in the final analysis. All measurements were taken in the morning after an overnight fast. The higher prevalence of females is representative of the higher number of females attending our outpatient nutritional clinic. The study procedures were approved by the University of Milan Ethical Committee, and all participants gave written informed consent.

Smoking, physical activity, dietary habits and health status

Current smoking habits were recorded as current smokers, never smoked or former smokers. A detailed medical interview was conducted, and self-reported diagnosis of medical conditions was collected. Disease count including major chronic diseases, such as cancer, cardiovascular diseases, thyroid and adrenal disorders, systemic inflammatory diseases (i.e. Crohn's disease, Ulcerative Colitis, Sjögren's syndrome, systemic lupus erythematosus, systemic sclerosis), HIV, and acute and chronic kidney failure, was calculated for each subject. Physical activity level (PAL) was assessed using the short version of the International Physical Activity Questionnaire (IPAQ) [25]. A standardised 11-question dietary questionnaire was administrated to evaluate adherence to Mediterranean diet with higher score indicating greater

adherence (range 0–14). Subjects were asked whether they were following any special diet (i.e. weight loss, gluten free) and menopausal status was assessed in women.

Anthropometry

Anthropometric measurements were collected by the same observer, according to standardised procedures. Body weight (WT, kg) and height (HT, cm) were measured to the nearest 0.1 kg and 0.5 cm, respectively. Body mass index (BMI) was calculated as (weight/height^2) and classified using the WHO criteria (18.5–24.9 kg/m^2—normal weight, 25.0–29.9 kg/m^2—overweight and ≥30 kg/m^2—obese). Waist circumference (WC) was measured in duplicate at the umbilical level with a flexible tape to the nearest 0.1 cm.

Bioelectrical impedance (BIA)

Impedance (*Z*) was measured using a tetrapolar 8-point tactile electrode system (InBody 720, Biospace, Seoul, Korea) at 1, 5, 50, 250, 500 and 1000 kHz. The system measured the impedance of the participant's right arm, left arm, trunk, right leg and left leg. Total body impedance value was calculated by summing the segmental impedance values. Participants stood on the scale platform of the instrument and grasped the handles of the device, to provide contact with a total of eight electrodes (two for each foot and for each hand). Manufacturer's equations were used to estimate body composition variables. The intra-examination coefficient of variation for BIA was 0.8 %.

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). Visceral (VAT) and subcutaneous (SAT) adipose tissue were measured one centimetre 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 convex-array probe as the distance between the anterior wall of the aorta and the posterior surface of the rectus abdominis muscle [26]. Each measurement was taken three times, and the mean was calculated. The within-day intra-operator coefficient of variation for repeated measures of VAT and SAT in our laboratory is 0.8 %.

Resting energy expenditure and respiratory quotient (RQ)

An open-circuit ventilated-hood indirect calorimetry system was used (Sensor Medics 29n, Anaheim, CA, USA). Resting VO_2 and VCO_2 measurements were measured in the early morning, after an overnight fast, under standardised conditions, with the person lying awake and emotionally undisturbed, completely at rest and comfortably supine on a bed, their head under a transparent ventilated canopy, in a thermally neutral environment (24–26 °C). When relevant, the participant was asked to abstain from smoking on the morning of the measurement. Oxygen consumption and carbon dioxide production were measured every minute for 30–40 min, and data collected during the first 5–10 min were discarded, as recommended by Isbell et al. [27]. This time period allowed the subjects to acclimatise to the canopy and instrument noise. The calorimeter was calibrated daily before starting the tests, using a two-point calibration method based on two separate mixtures of known gas content. The flow rate was calibrated with a 3-L syringe, according to the calorimeter manufacturer's instructions. The average of the last 20 min of measurements was used to determine 24 h REE according to the standard abbreviated Weir equation [28]. The molar ratio of O_2 consumed to CO_2 produced was used to derive an index (RQ) of the relative amounts of substrate that were being oxidised. The rates of carbohydrate and fat oxidation were determined with Lusk's tables [29] and the measured RQ, assuming that the variation in total RQ was not significantly affected by protein oxidation and that 12 % of REE was due to protein oxidation [30]. These assumptions will not affect the relative contributions of carbohydrate and fat oxidation to the total substrate oxidation but may only influence the absolute values of carbohydrate and fat oxidised [30].

Laboratory measurements

Fasting cholesterol, HDL cholesterol, triglycerides and glucose were measured using an enzymatic method (Cobas Integra 400 Plus, Roche Diagnostics, Rotkreuz, Switzerland). Blood pressure was measured by a physician using a random-zero mercury sphygmomanometer following JNC 7 guidelines [31].

Metabolic syndrome

The metabolic syndrome (MS) was diagnosed using the harmonised international definition [32]. Large waist was defined as WC ≥ 102 cm in men and ≥88 cm in women, low HDL cholesterol as HDL cholesterol <40 mg/dL in men and <50 mg/dL in women, high triglycerides as triglycerides ≥150 mg/dL, high BP as systolic BP ≥ 130 mm

Hg or diastolic BP ≥ 85 mm Hg, and high glucose as glucose ≥100 mg/dL. MS was defined as three or more of the above components. In addition, a continuous metabolic risk *Z* score was computed as the average of the *Z* scores for the individual metabolic traits. The risk *Z* score was calculated using the following variables: glucose, HDL, triglycerides and systolic and diastolic BP. The individual *Z* score was reversed for HDL to indicate a higher metabolic risk with decreasing values [33].

Statistical analyses

The data are reported as mean ± SD (continuous variables, normally distributed), median and quartile cut offs (continuous variables, not normally distributed) and frequency and percentage (categorical variables). Variables were checked for normality distribution using Q–Q plots and appropriate transformations were applied to skewed variables (i.e. PAL, SAT and VAT, systolic and diastolic BP, glucose, HDL, triglycerides). Groups were stratified by sex and age categories (18.0–39.9, 40.0–59.9, ≥60 years), and they were compared using univariate analysis of variance entering sex (*S*) and age categories (*A*) as between-subjects factors. A Sex–Age (*A***S*) interaction term was added to the model. Chi-square test was used to compare categorical variables. Pearson's correlation analyses were performed to evaluate the association between variables after stratification by sex. Multiple linear regression analyses were conducted to identify predictors of metabolic risk *Z* score, individual metabolic biomarkers (systolic and diastolic BP, glucose, HDL, triglycerides) and body composition measures of adiposity (FMI, SAT and VAT); each one of these variables was entered as a dependent variable in the model. Independent variables were age, sex, smoking, FFMI, disease count, IPAQ-physical activity level, Mediterranean diet score, dieting, REE and RQ. In middle-aged women, only (40–59 years) analyses were also adjusted for menopausal status. Body composition measurements (FFMI, FMI, SAT and VAT) were added to the regression models to predict metabolic outcomes. Intercepts (α) and regression coefficients ($b \pm$ SE) are reported. Beta coefficients (β, SE) and odds ratios (OR, 95 % CI), predicting MetSyn based on different body composition (FMI, FFMI, SAT, VAT) and metabolic indices (RQ, REE), were obtained from logistic regression models after controlling for potential covariates (i.e. age, sex, smoking, disease count and PAL). All data were fitted to unadjusted and adjusted logistic regression models whereby the probability of MetSyn (independent variable, *y* axis) was modelled against each metabolic and body composition indexes [(RQ, WC, FFMI, FMI, SAT, VAT), dependent variables, *x* axis] according to the *logit* model

$$logit(p) = 1/1 + \exp -[(\beta_0 + \beta_1 x)]$$

where p is the probability of presence of MetSyn, β_0 is the intercept of the logistic model, and β_1 is the regression coefficient for the predicting variable indicating the effect of a unit of change in the independent variable on the probability of presence of MetSyn. Analyses were conducted using Excel for Windows and STATISTICA v.10 for Windows. p value was set at <0.01 to account for spurious associations due to multiple comparisons and large sample size.

Results

Sample characteristics

Older subjects (≥60 years, 13 %) were less represented compared to middle-aged (40–59 years, 52 %) and young (18–39 years, 35 %) subjects. Age was equally distributed between sex and age groups (*A***S*, $p = 0.45$). BMI was higher in older subjects and in male subjects. A similar association with age was observed for other body composition variables including WC, FM and FFM. However, the distribution of these variables across sex and age groups was similar (i.e. *A***S* interaction terms were not significant). Physical activity level appeared to be characterised by a U-shaped distribution as lower levels of physical activity were observed in middle-aged male and female subjects (*A*, $p < 0.001$; *S*, $p = 0.04$). Age and sex had a significant influence on all diagnostic criteria of MetSyn (BP, glucose, HDL, triglycerides, metabolic risk *Z* score), whereas the interaction term (*A***S*) was significant for triglycerides, systolic and diastolic BP only (*A***S*, $p < 0.001$ for all) (Table 1 and Table S2 of the Online Supplementary Material).

RQ and fuel oxidation

Both VO_2 and VCO_2 gas exchanges showed a significant decline with age ($p < 0.001$) with a rate of −0.3 and −0.2 mL/min/year for VO_2 and VCO_2, respectively. Male subjects were characterised by a greater VO_2 consumption and VCO_2 production ($p < 0.001$ for both) with a per cent difference in +23 and +25 % compared to female subjects, respectively (Fig. 1a). Basal RQ values were not associated with age ($p = 0.49$), but a greater RQ ($p < 0.001$) was observed in male (0.84 ± 0.05) than female (0.81 ± 0.05) subjects (Fig. 1b). These differences in RQ are reflected in the observed sex differences in basal non-protein macronutrient oxidation (Fig. 1c). Female subjects were characterised by a greater basal fat oxidation (64 ± 19 %) compared to males (56 ± 18 %), whereas reciprocal values were observed for basal carbohydrate oxidation (females: 36 ± 19 %; males: 44 ± 36 %) ($p < 0.001$). Resting EE declined with age (*A*, $p < 0.001$) and a significant difference between males and females was observed (*S*, $p < 0.001$) (Fig. 1d).

Table 1 Description of the main characteristics of the study population stratified by sex (male, female) and age (18–39, 40–59 and ≥60 years)

	All (years)			Female (years)			Male (years)			*p* value
	18–39	40–59	≥60	18–39	40–59	≥60	18–39	40–59	≥60	
N	992	1463	364	733	963	229	259	500	135	–
Age (years)	31.2 (6.1)	48.3 (5.5)	65.3 (4.4)	30.9 (6.1)	48.2 (5.5)	65.0 (4.5)	31.9 (5.8)	48.6 (5.4)	65.8 (43.5)	*A*: $p < 0.001$ *S*: 0.004 *A*S*: 0.45
Weight (kg)	78.8 (18.2)	81.7 (18.4)	83.4 (17.5)	73.4 (14.9)	74.5 (14.7)	76.2 (14.5)	94.3 (18.1)	95.6 (16.8)	95.4 (15.4)	*A*: 0.08 *S*: $p < 0.001$ *A*S*: 0.50
BMI (kg/m^2)	28.3 (5.4)	29.4 (5.4)	31.0 (5.5)	27.6 (5.4)	28.5 (5.4)	30.5 (5.7)	30.1 (5.0)	31.0 (4.9)	32.0 (5.1)	*A*: $p < 0.001$ *S*: $p < 0.001$ *A*S*: 0.32
WC (cm)	91.7 (14.6)	97.5 (14.6)	104.1 (13.9)	87.8 (12.6)	92.3 (12.7)	98.6 (12.3)	102.6 (14.3)	107.3 (12.7)	112.9 (11.8)	*A*: $p < 0.001$ *S*: 0.11 *A*S*: 0.32
SAT (cm)*	2.92 (1.98, 3.96)	2.78 (2.04, 3.57)	2.49 (1.80, 3.22)	2.85 (1.92, 3.94)	2.83 (2.08, 3.60)	2.79 (2.12, 3.41)	3.09 (2.11, 4.08)	2.63 (1.97, 3.54)	2.05 (1.52, 2.78)	*A*: $p < 0.001$ *S*: $p < 0.001$ *A*S*: $p < 0.001$
VAT (cm)*	3.90 (2.79, 5.39)	5.18 (3.52, 7.49)	7.02 (4.87, 9.18)	3.39 (2.50, 4.52)	4.21 (3.02, 5.79)	5.93 (4.40, 7.56)	6.12 (4.65, 7.89)	7.49 (5.52, 9.33)	9.02 (6.96, 10.63)	*A*: $p < 0.001$ *S*: $p < 0.001$ *A*S*: 0.005
FMI (kg/m^2)	9.82 (4.32)	10.56 (4.20)	12.49 (4.58)	10.30 (4.41)	11.03 (4.36)	13.23 (4.60)	8.73 (3.91)	9.61 (3.90)	11.12 (4.01)	*A*: $p < 0.001$ *S*: $p < 0.001$ *A*S*: 0.41
FFMI (kg/m^2)	18.44 (2.45)	18.86 (2.53)	18.69 (2.48)	17.37 (1.56)	17.54 (1.64)	17.27 (1.60)	21.40 (1.83)	21.46 (1.84)	20.92 (1.93)	*A*: $p < 0.001$ *S*: $p < 0.001$ *A*S*: 0.25
Systolic BP (mmHg)*	120.0 (110.0, 125.0)	120.0 (120.0, 130.0)	130.0 (120.0, 140.0)	115.0 (110.0, 120.0)	120.0 (110.0, 130.0)	130.0 (120.0, 140.0)	130.0 (120.0, 130.0)	130.0 (120.0, 140.0)	140.0 (130.0, 140.0)	*A*: $p < 0.001$ *S*: $p < 0.001$ *A*S*: $p < 0.001$
Diastolic BP (mmHg)*	70.0 (70.0, 80.0)	80.0 (80.0, 85.0)	80.0 (77.5, 85.0)	70.0 (70.0, 80.0)	80.0 (70.0, 80.0)	80.0 (70.0, 85.0)	80.0 (70.0, 85.0)	80.0 (80.0, 90.0)	80.0 (80.0, 90.0)	*A*: $p < 0.001$ *S*: $p < 0.001$ *A*S*: $p < 0.001$
Glucose (mg/dL)*	89.0 (84.0, 94.0)	94.0 (88.0, 101.0)	99.0 (91.0, 109.5)	88.0 (83.0, 93.0)	92.0 (86.0, 98.0)	96.0 (89.0, 107.0)	93.0 (88.0, 98.0)	98.0 (92.0, 106.0)	104.0 (95.0, 118.0)	*A*: $p < 0.001$ *S*: $p < 0.001$ *A*S*: 0.21
HDL (mg/dL)*	59.0 (47.0, 70.0)	58.0 (47.0, 69.0)	57.5 (47.0, 70.5)	63.0 (54.0, 74.0)	64.0 (55.0, 74.0)	63.0 (54.0, 77.0)	45.0 (39.0, 54.0)	47.0 (40.0, 54.0)	48.0 (41.0, 57.0)	*A*: $p < 0.001$ *S*: $p < 0.001$ *A*S*: 0.31
Triglycerides (mg/dL)*	82.0 (57.5, 115.0)	92.0 (67.0, 134.0)	104.5 (78.0, 149.0)	72.0 (53.0, 100.0)	82.0 (62.0, 112.0)	100.0 (77.0, 138.0)	108.0 (80.0, 160.0)	123.0 (86.0, 170.0)	118.0 (82.0, 176.0)	*A*: $p < 0.001$ *S*: $p < 0.001$ *A*S*: $p < 0.001$

Table 1 Continued

	All (years)			Female (years)			Male (years)			*p* value
	18–39	40–59	≥60	18–39	40–59	≥60	18–39	40–59	≥60	
Metabolic risk *Z* score	−1.19 (3.00)	0.37 (3.32)	1.73 (3.12)	−2.17 (2.38)	−0.86 (2.81)	0.66 (2.57)	1.56 (2.86)	2.76 (2.91)	3.55 (3.13)	*A*: $p < 0.001$ *S*: $p < 0.001$ *A*S*: 0.04
Metabolic Syndrome (%)	15.6	34.1	56.5	9.2	24.4	46.7	33.5	52.8	73.3	$p < 0.001$

Mean (SD) presented for normally distributed variables. Median (upper and lower quartiles) presented from not normally distributed variables

N number of subjects, *BMI* body mass index, *WC* waist circumference, *PAL* physical activity level, *MET* metabolic equivalent time. *FMI* fat mass index, *FFMI* fat free mass index, *HDL* high density lipoproteins, *SAT* sub-cutaneous adipose tissue, *VAT* visceral adipose tissue, *A* Age, *S* Sex, *A*S* Age*Sex interaction term

* Log transformed before analyses

Abdominal subcutaneous and visceral fat (SAT and VAT)

SAT was thicker in young males [3.09 cm (2.11 4.08)] compared to the other age groups. However, a significant age–sex interaction was found as thickness increased across the three age groups in females, whereas a significant decline was observed in males (*A*S*, $p < 0.001$). VAT thickness was greater in males [7.25 (5.40 9.27) vs 3.95 cm (2.91 5.53), $p < 0.001$]. VAT was associated with age ($p < 0.001$) in both sex groups but females were characterised by a greater increase in visceral fat (+1.4 % per year) compared to males (+1.0 % per year) (*A*S*, $p = 0.005$) (Table 1).

Correlation analyses

The correlation matrix reporting the association between age, metabolic and body composition variables and cardiovascular risk factors is reported in Table 2. RQ was not significantly associated with any variables in males and females. Age was significantly associated with FMI in both groups, whereas a weak, inverse association with FFMI was only found in males ($r = -0.10$, $p = 0.004$). An inverse, significant correlation of age with SAT was found in males only ($r = -0.23$, $p < 0.001$), whereas a significant, direct association with VAT was observed in both males ($r = 0.31$, $p < 0.001$) and females ($r = 0.37$, $p < 0.001$) (Table 2). Waist circumference showed a strong association with VAT (males, $r = 0.71$, $p < 0.001$; females, $r = 0.71$, $p < 0.001$) and a significant, inverse association of PAL with WC ($r = -0.11$, $p = 0.001$) and VAT ($r = -0.15$, $p < 0.001$) was only observed in males (Table 2). Age, FMI and VAT were associated with an overall increase in cardiometabolic risk factors in both males and females. SAT was instead associated with all cardiovascular risk factors in females, whereas, in males, a weak association was only found with systolic and diastolic BP (Table 2).

Multiple linear regression

Basal RQ was not associated with adiposity indexes. FMI was directly associated with age, sex (i.e. greater adiposity in female subjects), disease count and FFMI and inversely with PAL. Age and sex showed a direct association with VAT but they showed an inverse association with SAT. However, both adiposity layers were associated with disease count, FFMI and PAL (Table S3 of the Online Supplementary Material). VAT was a significant predictor of blood biomarkers of cardiovascular risk (i.e. glucose, HDL, triglycerides) and overall metabolic risk *Z* score but it was not associated with systolic and diastolic BP (Table 3). Age was a significant, independent predictor of all cardiovascular risk factors. An increase in basal RQ values was

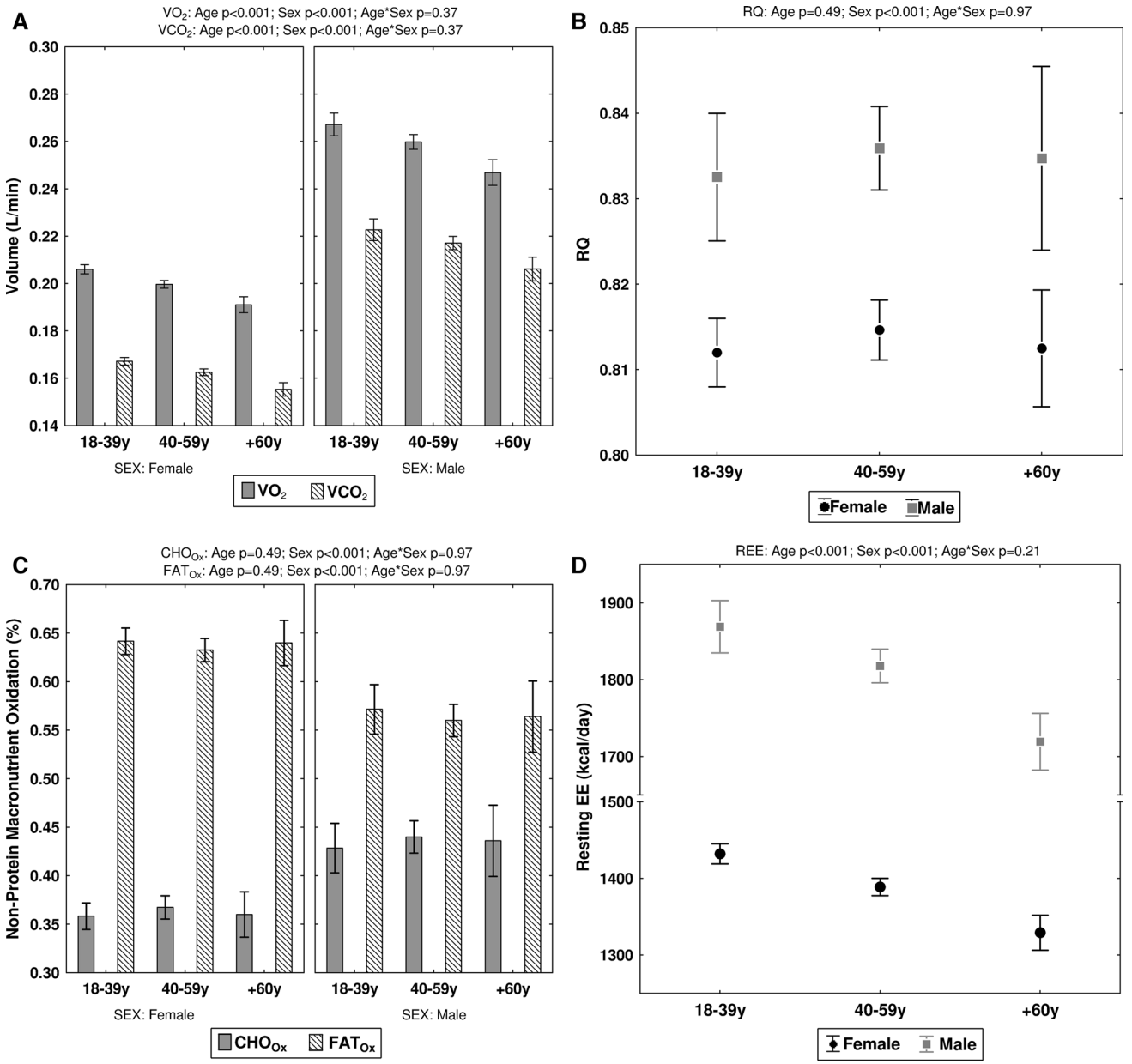

Fig. 1 Age-related mean changes in gaseous exchanges (VO_2 and VCO_2), respiratory quotient (RQ), resting energy expenditure (EE) and non-protein fat and carbohydrate (CHO) oxidation in male and female subjects. *Error bars* are 95 % CI

associated with greater triglyceride levels ($b = 0.43 \pm 0.15$, $p = 0.005$) and PAL was an independent predictor of plasma glucose levels only ($b = -0.0002 \pm 0.00009$, $p = 0.01$) (Table 3). The adjustment for dieting and Mediterranean diet score did not modify the results (Table S4 of the Online Supplementary Material).

Logistic regression

Basal RQ was not a significant predictor of MetSyn in both unadjusted and adjusted models (Table 4). This is clearly showed in the *logit* probability plot (Fig. 2a) since no detectable trends in the probability of having a diagnosis of MetSyn were observed across the range of basal RQ values. FMI showed a progressive, increased risk of MetSyn in the unadjusted and partially adjusted models but its role was significantly attenuated in the fully adjusted model (Fig. 2b). The increase in WC was a strong determinant of the probability of MetSyn in models with lower degree of adjustment; however, the predictive accuracy of this index was completely removed in the fully adjusted model (i.e. VAT was the only significant predictor of the

Table 2 Correlation matrix to evaluate the association between age, metabolic and body composition outcomes and cardiovascular risk factors in male (lower diagonal) and female (upper diagonal) subjects

	Age	BMI	WC	RQ	REE	SAT*	VAT*	FMI	FFMI	SBP*	DBP*	GLU*	HDL*	TRY*	Metabolic risk *Z* score	PAL*
Age (years)	–	**0.17**	**0.29**	0.003	**−0.20**	−0.05	**0.37**	**0.20**	0.01	**0.38**	**0.31**	**0.31**	0.03	**0.23**	**0.35**	0.02
BMI (kg/m^2)	**0.11**	–	**0.91**	0.001	**0.64**	**0.63**	**0.64**	**0.97**	**0.74**	**0.42**	**0.39**	**0.32**	**−0.32**	**0.30**	**0.54**	−0.05
WC (cm)	**0.25**	**0.91**	–	0.03	**0.60**	**0.64**	**0.71**	**0.89**	**0.66**	**0.45**	**0.41**	**0.36**	**−0.33**	**0.35**	**0.58**	−0.07
RQ	−0.01	0.06	0.07	–	−0.05	0.02	0.02	−0.01	0.03	−0.01	0.01	0.009	0.02	0.03	0.01	−0.01
REE	**−0.23**	**0.69**	**0.64**	0.03	–	**0.41**	**0.35**	**0.55**	**0.66**	**0.25**	**0.24**	**0.19**	**−0.25**	**0.16**	**0.35**	−0.02
SAT (cm)*	**−0.23**	**0.40**	**0.38**	0.04	**0.35**	–	**0.35**	**0.61**	**0.35**	**0.25**	**0.28**	**0.14**	**−0.20**	**0.21**	**0.32**	−0.08
VAT (cm)*	**0.31**	**0.65**	**0.71**	0.02	**0.39**	0.02	–	**0.64**	**0.43**	**0.38**	**0.35**	**0.37**	**−0.27**	**0.38**	**0.53**	−0.06
FMI (kg/m^2)	**0.18**	**0.94**	**0.89**	0.04	**0.56**	**0.40**	**0.64**	–	**0.55**	**0.42**	**0.44**	**0.32**	**−0.29**	**0.30**	**0.53**	−0.07
FFMI (kg/m^2)	−0.10	**0.70**	**0.57**	0.09	**0.68**	**0.21**	**0.38**	**0.42**	–	**0.27**	**0.25**	**0.22**	**−0.30**	**0.18**	**0.38**	0.02
SBP*	**0.24**	**0.45**	**0.45**	0.04	**0.37**	**0.11**	**0.37**	**0.43**	**0.30**	–	**0.75**	**0.28**	−0.07	**0.25**	**0.74**	−0.02
DBP*	**0.15**	**0.43**	**0.42**	−0.004	**0.35**	**0.18**	**0.31**	**0.40**	**0.31**	**0.71**	–	**0.22**	−0.04	**0.23**	**0.70**	−0.02
GLU*	**0.33**	**0.17**	**0.21**	−0.002	0.07	−0.07	**0.26**	**0.16**	**0.12**	**0.20**	**0.14**	–	**−0.17**	**0.24**	**0.59**	−0.01
HDL*	0.10	**−0.23**	**−0.21**	−0.007	**−0.26**	−0.06	**−0.21**	**−0.16**	**−0.29**	−0.10	−0.10	−0.08	–	**−0.32**	**−0.51**	−0.01
TRY*	0.07	**0.15**	**0.17**	0.08	**0.18**	−0.004	**0.24**	**0.12**	**0.15**	**0.14**	**0.14**	**0.12**	**−0.45**	–	**0.58**	−0.01
Metabolic risk *Z* score	**0.22**	**0.43**	**0.44**	0.03	**0.38**	0.06	**0.43**	**0.39**	**0.35**	**0.64**	**0.64**	**0.54**	**−0.50**	**0.63**	–	−0.04
PAL (METs/wk)*	−0.09	−0.09	**−0.11**	−0.05	0.02	−0.01	**−0.15**	**−0.12**	0.02	0.02	0.004	−0.05	−0.05	−0.05	−0.09	–

Pearson product-moment correlation coefficients (r) are presented. Significant results are highlighted in bold. Significance level for this analysis has been set at $p < 0.001$ to account for multiple testing and large sample size

BMI body mass index, *WC* waist circumference, *Pre-PF* pre-peritoneal fat, *SAT* sub-cutaneous adipose tissue, *VAT* visceral adipose tissue, *FMI* fat mass index, *FFMI* fat free mass index, *PAL* physical activity level, *CHO* carbohydrate, *MET* metabolic equivalent time

* Log transformed before analyses

Table 3 Multiple linear regression to identify lifestyle and body composition predictors of cardiovascular risk factors and cumulative metabolic risk

	Glucose (mg/dL)*			Systolic BP (mmHg)*			Diastolic BP (mmHg)*			HDL (mg/dL)*		
	b	SE	*p*	*b*	SE	*p*	*b*	SE	*p*	*b*	SE	*p*
R^2	0.23			0.38			0.32			0.35		
Intercept	4.19	0.04	**<0.001**	4.39	0.03	**<0.001**	3.92	0.03	**<0.001**	4.66	0.08	**<0.001**
Age (years)	0.002	0.0002	**<0.001**	0.002	0.0001	**<0.001**	0.002	0.0002	**<0.001**	0.002	0.0004	**<0.001**
Sex (male, female)	0.009	0.009	0.34	0.02	0.007	**0.003**	0.03	0.008	**<0.001**	−0.16	0.02	**<0.001**
Smoking (yes, no)	0.003	0.003	0.27	−0.004	0.002	0.08	−0.002	0.003	0.50	0.03	0.006	**<0.001**
RQ	0.04	0.04	0.38	−0.007	0.03	0.81	0.01	0.03	0.77	−0.02	0.07	0.73
REE (kcal/day)	0.0005	0.00001	**0.001**	0.0001	0.00001	**<0.001**	0.00009	0.00001	**<0.001**	−0.000005	0.00003	0.88
Disease count (*n*)	0.006	0.001	**<0.001**	0.003	0.001	**<0.001**	0.001	0.001	0.11	−0.006	0.002	0.02
PAL (METs/week)*	−0.0002	0.00009	**0.01**	0.0001	0.00007	0.02	0.00004	0.00008	0.55	0.0002	0.0002	0.38
FFMI (kg/m^2)	0.005	0.001	**0.007**	0.001	0.001	**<0.001**	0.002	0.0001	0.14	−0.03	0.003	**<0.001**
FMI (kg/m^2)	0.001	0.008	0.05	0.003	0.0006	0.23	0.004	0.0007	**<0.001**	−0.003	0.002	0.05
VAT (cm)*	0.04	0.007	**<0.001**	0.01	0.02	0.03	0.01	0.006	0.04	−0.09	0.01	**<0.001**
SAT (cm)*	−0.01	0.005	0.04	0.009	0.004	0.03	0.02	0.005	**<0.001**	−0.006	0.01	0.60

	Triglycerides (mg/dL)*			Metabolic risk *Z* score		
	b	SE	*p*	*b*	SE	*p*
R^2	0.24			**0.54**		
Intercept	3.18	0.16	**<0.001**	−13.67	0.80	**<0.001**
Age (years)	0.003	0.001	**<0.001**	0.05	0.004	**<0.001**
Sex (male, female)	0.10	0.04	**0.003**	1.20	0.18	**<0.001**
Smoking (yes, no)	−0.04	0.01	**0.002**	−0.22	0.07	**<0.001**
RQ	0.43	0.15	**0.005**	0.86	0.77	0.26
REE (kcal/day)	0.0002	0.0001	**<0.001**	0.003	0.0003	**<0.001**
Disease count (*n*)	0.03	0.01	**<0.001**	0.16	0.03	**<0.001**
PAL (METs/week)*	0.0004	0.0004	0.29	0.00	0.00	0.95
FFMI (kg/m^2)	−0.001	0.01	0.87	0.14	0.04	**<0.001**
FMI (kg/m^2)	−0.005	0.003	0.16	0.08	0.02	**<0.001**
VAT (cm)*	0.28	0.03	**<0.001**	1.22	0.13	**<0.001**
SAT (cm)*	0.06	0.02	**0.01**	0.16	0.11	0.15

Significant results are highlighted in bold

b raw regression coefficient, *SE* standard error, R^2 coefficient of determination, *REE* resting energy expenditure, *FMI* fat mass index, *FFMI* fat free mass index, *RQ* respiratory quotient, *BP* blood pressure, *PAL* physical activity level, *MET* metabolic equivalent time, *SAT* sub-cutaneous adipose tissue, *VAT* visceral adipose tissue

* Log transformed before analyses

model) (Fig. 2c). VAT was the individual predictor with the greatest odds of developing MetSyn (OR 4.25, 3.01 5.99, $p < 0.001$) (Table 4). The *logit* probability plot showed a small attenuation of the probability of MetSyn in the fully adjusted model confirming the primary role of VAT in the pathogenesis of MetSyn (Fig. 2d). FFMI was not a significant predictor of MetSyn once lifestyle factors and adiposity indexes were entered into the logistic model (Table 4, Figure S2 of the Online Supplementary Material). The adjustment for Mediterranean diet score, dieting and menopausal status (women only, 40–59 years) did not modify the results with RQ being not significant, whereas VAT remained the strongest predictor of MetSyn (Table S5 and S6 of the Online Supplementary Material).

Discussion

Summary of main findings

Contrary to the hypothesised relationship between ageing and basal substrate oxidation, our results did not document an age-related decline in RQ. However, we observed a lower basal RQ (i.e. greater basal fat oxidation) in females,

Table 4 Logistic regression to evaluate the risk for MS (dependent variable, binary) associated with metabolic and body composition measurements

	β (SE)	OR (95 % CI)	p value
Model 1[a]			
RQ	0.95 (0.85)	2.59 (0.48–13.86)	0.26
REE (kcal/day)	0.004 (0.003)	1.00 (0.99–1.00)	0.21
FMI (kg/m^2)	0.07 (0.01)	1.08 (1.05–1.11)	**<0.001**
FFMI (kg/m^2)	0.10 (0.03)	1.10 (1.03–1.19)	**0.004**
VAT (cm)*	2.15 (0.15)	8.61 (6.32–11.74)	**<0.001**
SAT (cm)*	−0.04 (0.13)	0.95 (0.73–1.25)	0.75
Model 2[b]			
RQ	1.19 (0.89)	3.31 (0.57–19.08)	0.17
REE (kcal/day)	0.001 (0.003)	1.00 (1.00–1.01)	**0.001**
FMI (kg/m^2)	0.05 (0.01)	1.06 (1.02–1.10)	**0.002**
FFMI (kg/m^2)	0.08 (0.04)	1.09 (1.00–1.18)	0.03
VAT (cm)*	1.44 (0.17)	4.25 (3.01–5.99)	**<0.001**
SAT (cm)*	0.27 (0.14)	1.31 (0.73–1.25)	0.89

Significant results are highlighted in bold

β regression coefficient, *SE* standard error, *OR* odds ratio (95 % CI), *FMI* fat mass index, *FFMI* fat free mass index, *RQ* respiratory quotient, *REE* resting energy expenditure, *SAT* sub-cutaneous adipose tissue, *VAT* visceral adipose tissue

* Log transformed before analyses

[a] Unadjusted model

[b] Model adjusted for age, sex, smoking, disease count and physical activity level

which also appeared to influence fasting triglyceride concentrations. Ageing was associated with typical trajectories for SAT and VAT. Specifically, a strong association between age and VAT thickness was found in both males and females, whereas a significant, negative association with SAT was only found in male subjects. Age and VAT were strongly associated with cardiovascular risk factors and risk for MetSyn. Surprisingly, VAT was primarily associated with metabolic (i.e. glucose, HDL and triglycerides) and not with vascular (systolic and diastolic BP) outcomes, which appeared to have a strong relationship with ageing, FFMI (systolic BP), FMI and SAT (diastolic BP). Waist circumference is a proxy measure of abdominal adiposity and strongly correlated with VAT enlargement, increased release of FFA, adipokines and pro-inflammatory cytokines, insulin resistance, and development of MS [34]. Nevertheless, WC is affected also by subcutaneous adiposity that, as clearly emerges from our results, is not strongly correlated to metabolic imbalances. As a confirmation, WC lost its predictive capacity when VAT was added to the regression models. Ultrasonographic assessment of VAT thus appears to be a better risk predictor of MetSyn than WC.

Comparison with body of evidence

We investigated the role played by physiological factors in the pathogenetic trajectory of MetSyn (ageing → substrate oxidative capacity → visceral adiposity → metabolic health). Previous studies has only explored segments of this trajectory and, therefore, this analysis provides, for the first time, an integrated snapshot of the interactive connections between these physiological factors in relation to the risk of developing MetSyn. The large sample size, the use of ventilated-hood indirect calorimetry to estimate fuel oxidation and VAT (ultrasonography) are important strengths of our analyses. Previous studies have investigated the association of either basal RQ or fat oxidation with body weight, BMI or FM [5, 14, 23, 35–41]. The majority of these studies were cross-sectional [5, 14, 23, 35, 36, 38–40, 42]. The two most relevant longitudinal studies on this topic were conducted by Zurlo et al. [41] and Seidell et al. [37]. The former evaluated the association of 24-h RQ with prospective changes in body weight and FM in 152 Pima Indians; a higher 24-h RQ was associated with greater weight and FM gain after two years and a strong heritability of the ratio of fat to carbohydrate oxidation was observed. The latter followed 775 men for approximately 10 years to observe that a higher basal RQ was a weak but significant predictor of weight gain. Cross-sectional studies testing the association between basal substrate oxidation and adiposity have instead reported contrasting results. Nagy et al. [23] measured basal substrate oxidation in 720 adults and found a significant negative correlation of basal fat oxidation with FM, insulin and triglycerides. The only study to test the association between basal RQ and VAT has been recently conducted by Croci et al. [15]. The authors found a significant association of basal RQ with liver steatosis and triglyceride levels but not with VAT, FM or BMI. These results are in line with our observations. However, a direct comparison of the results is complicated by the differences in the methodological, phenotypic characteristics of the populations or statistical approaches, which require a careful interpretation of the results. We have performed a comprehensive review of the topic to highlight similarities and differences of the current body of evidence (Table S1 of the Online Supplementary Material).

The impact of the ageing process on metabolic flexibility is a recognised physiological phenomenon [12]. Mechanisms include a decline in the amount of lean body mass (quantitative modification) as well as qualitative changes in mitochondrial efficiency, switch in muscular fibre type, capillary density, anabolic resistance to endocrine signals, reduced regenerative capacity or increased oxi-inflammatory load [1, 43, 44]. These mechanisms form the theoretical foundations for a projected decline in fat oxidation with

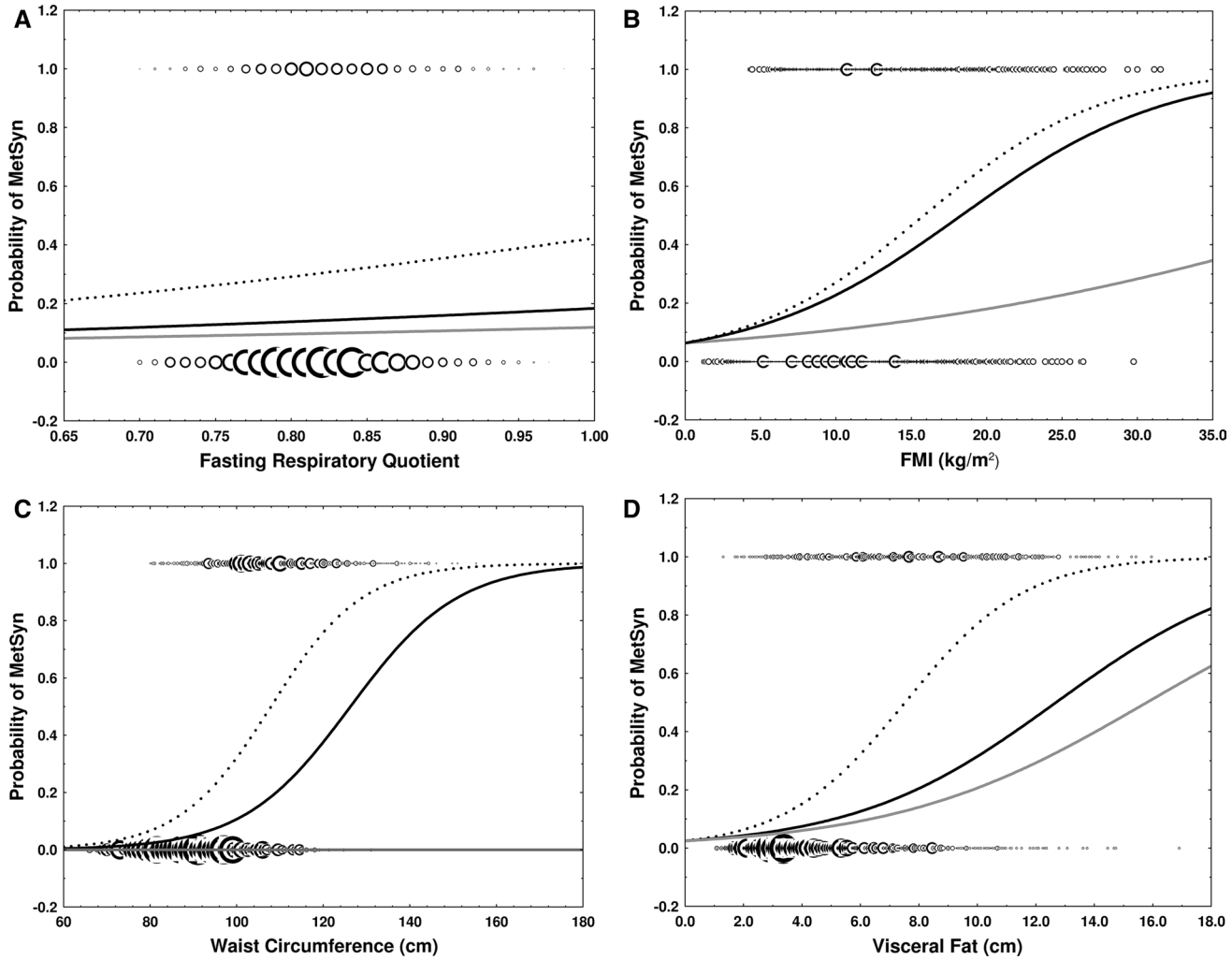

Fig. 2 Probability of MS (MetSyn) with age, visceral fat, fasting respiratory quotient and fat mass index (FMI). Logistic models were represented unadjusted (*dotted line*), partially adjusted for lifestyle factors and health status [age, smoking, disease count and physical activity (*solid black like*)] and fully adjusted for lifestyle factors, health status, metabolic (RQ, REE) and body composition variables [fat free mass index (FFMI), FMI, visceral and subcutaneous adiposity (*solid grey line*)]. *Bubble plots* were used to indicate the frequency of distribution of each independent variable in the two MS groups (0 = no MS; 1 = MS)

ageing and contribute to explain the age-related changes in body composition. However, the contrasting results in the literature do not currently support this hypothesis. Our study aimed to provide more robust evidence on the association between ageing, basal substrate oxidation and adiposity by modelling life course trends of the selected outcomes in a large population ($N = 2819$, age range = 18–81 years). Even if our results may point to a lack of association between ageing and substrate oxidation in basal condition, age-related changes in body composition and oxidative metabolism could be more evident in post-prandial states [45], when the substrate partitioning and oxidative capacity of AT becomes crucial in regulation metabolic homoeostasis.

The association of ageing and VAT with an increased risk of cardiovascular and metabolic diseases is widely recognised [4, 46]; however, the dynamics of the intricate pathophysiological events connecting these physiological factors requires further research. Our intent was to evaluate whether differences in basal substrate oxidation may have contributed to VAT accumulation and, as a result, play a role in the pathogenesis of MetSyn. However, the role of basal substrate oxidation appears to be marginal but it is not completely dismissed since it may influence the regulation of triglyceride metabolism. A non-significant association between basal substrate oxidation and MetSyn was also reported by Ferro et al. [16]. A similar association between basal substrate oxidation and triglyceride concentrations has been previously

observed [20, 23]. This is not surprising considering that after an overnight fast fat oxidation is predominant and, therefore, the high basal RQ may represent a proxy measure for the identification of subjects with an impaired fat oxidation during fasting conditions. Our study did not find additional significant associations of basal substrate oxidation with other cardiovascular risk factors, whereas previous studies have found significant associations with systolic BP [16], subclinical atherosclerosis (carotid intima media thickness, left ventricular concentric remodelling) [18, 19], liver steatosis [15] and insulin function [8, 17].

Limitations

The cross-sectional design is the main limitation of the study which impacts on the ascertainment of the causality of associations. However, the large sample size, the utilisation of ultrasonography for the assessment of abdominal fat layers and opportunity to control for several confounding factors allowed the conduction of detailed and focussed analyses. Although bowel distention could have interfered with measurement of VAT diameter, previous studies demonstrated a high correlation of VAT area determined by CT scan and sonographically determined VAT diameter [47]. We conclude that ultrasound measurement is a quick and reliable method of low costs and without radiation exposure to identify patients with increased cardiovascular risk. An additional limitation is the lack of standardisation of the dietary and PAL prior to the study visit which requires a cautious interpretation of the results. Previous research has reported a day-to-day coefficient of variation for fasting RQ of approximately 2.1 %. This value is significantly less than variations in free-living energy and macronutrient intakes, which show a CV greater than 25 %. Hence, the likelihood that acute day-to-day variations in food quotient (FQ) may be accompanied by equivalent excursions in day-to-day RQ is minimal [42]. In addition, we have no reason to suspect that participants had changed their habitual macronutrient intake or altered their level of activity prior to their participation in this cross-sectional study. This appears to be supported by the lack of influence of physical activity level, dieting and Mediterranean diet score adjustment on the prediction of MetSyn in the multivariate linear and logistic regression models.

Conclusions

In summary, ageing is associated with an increased visceral adiposity in males and females, which appears to be the most important marker for MetSyn. Basal substrate oxidation appears to have a peripheral role in visceral adipogenesis and pathogenesis of MetSyn, but the significant association with triglyceride concentrations warrants investigation in future studies. Our study has provided novel findings from a large and heterogeneous adult population, but the limitations of a cross-sectional study design remain; therefore, longitudinal studies are needed to substantiate the physiological relevance of basal and post-prandial RQ as a predictor of impaired metabolic health as well as of the between-subject variability in response to energy and dietary manipulations.

Statement of authorship The manuscript was conceived by MS who analysed the data and wrote the first draft of the manuscript. Data were collected by AL, SB, AB and AT. All authors contributed to critical interpretation subsequent of results. All authors contributed to the final revision of the manuscript. The corresponding author (MS) is the guarantor for the manuscript and had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis reported in the manuscript.

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Compliance with ethical standards

Conflict of interest All authors have no conflicts of interest to declare.

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