Clinical Nutrition 32 (2013) 569–578

Contents lists available at SciVerse ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Health and ageing: A cross-sectional study of body composition

Alberto Bazzocchi [a,b,*], Danila Diano [a], Federico Ponti [a], Andrea Andreone [a], Claudia Sassi [a], Ugo Albisinni [b], Giulio Marchesini [c], Giuseppe Battista [a]

[a] Imaging Division, Clinical Department of Radiological and Histocytopathological Sciences, University of Bologna, Sant'Orsola – Malpighi Hospital, Via G. Massarenti 9, 40138 Bologna, Italy
[b] Diagnostic and Interventional Radiology, "Rizzoli" Orthopaedic Institute, Via G. C. Pupilli 1, 40136 Bologna, Italy
[c] Unit of Metabolic Diseases & Clinical Dietetics, University of Bologna, Sant'Orsola – Malpighi Hospital, Via G. Massarenti 9, 40138 Bologna, Italy

ARTICLE INFO

Article history:
Received 23 June 2012
Accepted 2 October 2012

Keywords:
Body composition
Body fat distribution
Health
Reference standard
Absorptiometry
Photon

SUMMARY

Background & aims: The aim of this work was to provide a complete profile of body composition (BC) in healthy subjects and to investigate age and gender-related differences by dual-energy X-ray absorptiometry (DXA) and its latest developments.
Methods: Italian volunteers among blood donors were enrolled in 5 different age bands (from 18 to 70 years old) to reach the threshold of 25 males and 25 females per single band (total: 250 subjects). All non-obese subjects who satisfied selective inclusion criteria were measured for weight and height and submitted to DXA, to determine fat mass (FM), non-bone lean mass (LM), bone mineral content and density, at regional and whole-body level. Moreover, the assessment of android visceral FM was performed by a new software.
Results: A decrease in LM and increase in FM was observed with ageing, although the phenomenon was proved to be attenuated in women. The central and visceral redistribution of FM was also shown along lifetime, but women were not affected as men by this change.
Conclusions: This paper is a report on the status of healthy Italian subjects in their adulthood, to be used as a reference for future investigations on physiology, pathological human conditions, and differences between countries.
© 2012 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.

1. Introduction

The assessment of body composition (BC) is essential in the study of biology, both human and animal. In the research field, BC is increasingly considered a hot topic in the characterization of metabolic status[1,2]; nevertheless, BC clinical applications and opportunities have not been completely defined yet, thus not entirely been transferred to daily practice. Investigations on BC have involved various populations and diseases (e.g. obesity, diabetes, and endocrine diseases but also gastrointestinal, renal, and infectious diseases) as well as physiological and paraphysiological conditions such as in athletes or in growth and ageing processes.[3–6]

A gold standard technique for the assessment of human BC[7,8] is represented by dual-energy X-ray absorptiometry (DXA). DXA measurements are based on a 3-compartment model that can be simplified into fat mass (FM), non-bone lean mass (LM) and bone mineral content (BMC). This technique is able to assess the body masses (and bone mineral density–BMD) on a regional and whole-body basis. DXA is accurate, reproducible, fast, relatively inexpensive, and involves very low radiation dose to the patient. All these advantages and the predisposition of the latest DXA technologies to BC analysis make this densitometric method ideal for clinical use and longitudinal studies, in both adults and children.[9,10] Furthermore, a new software has recently been proposed to separately assess the visceral compartment of android fat by DXA, and results concerning its accuracy are very promising. Reported coefficient of determination (r^2) for regression of computed tomography (CT) on iDXA values were 0.959 for females, 0.949 for males and 0.957 combined.[11]

The development and application of all medical techniques and methods that are involved in the measurement of clinical

Abbreviations: BC, body composition; DXA, dual-energy X-ray absorptiometry; FM, fat mass; LM, lean mass; BMC, bone mineral content; BMD, bone mineral density; CT, computed tomography; BMI, body mass index; ROIs, regions of interest; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; A/G, android/gynoid; aFMR, appendicular FM ratio; aLMR, appendicular LM ratio; FMI, fat mass index; LMI, non-bone lean mass index; VMI, visceral mass index.

* Corresponding author. Imaging Division, Clinical Department of Radiological and Histocytopathological Sciences, University of Bologna, Sant'Orsola – Malpighi Hospital, Via Massarenti 9, 40138 Bologna, Italy. Tel.: +39 0516364964; fax: +39 051397738.
E-mail address: abazzo@inwind.it (A. Bazzocchi).

0261-5614/$ – see front matter © 2012 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.
http://dx.doi.org/10.1016/j.clnu.2012.10.004

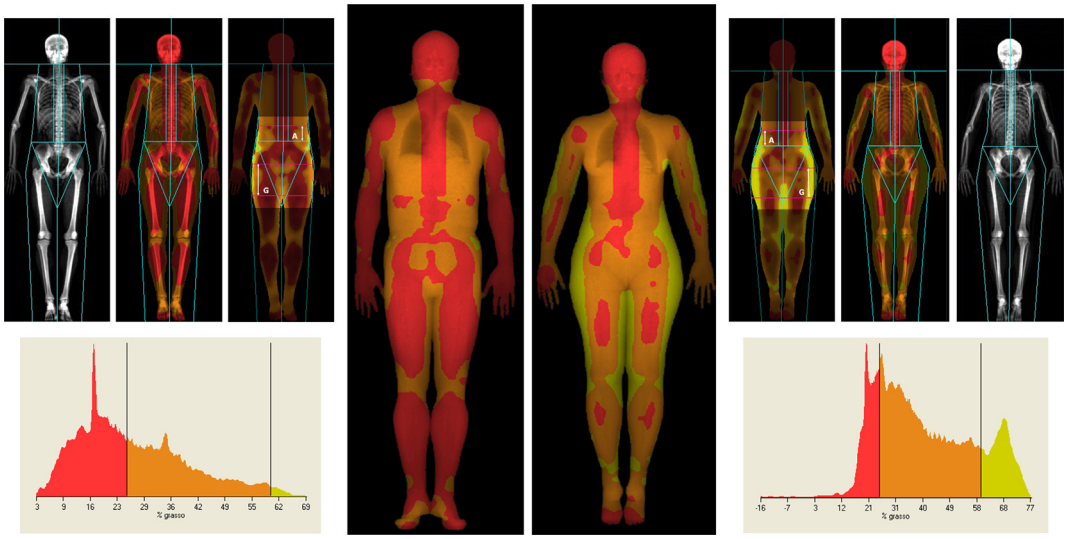

Fig. 1. Dual-energy X-ray absorptiometry (DXA) examination of body composition. The DXA scan allows to assess fat mass, non-bone lean mass, and bone mineral content and density on both whole-body and regional basis. In this picture the topography of regions of interests (ROIs) and the analysis of tissue density are enhanced, in a male (left) and female (right) sample. ROIs are automatically drawn by the software according to anatomical landmarks, and these are submitted to the corrective intervention of the imaging operator (A and G are "android" and "gynoid" regions, respectively). In the central part of the picture, the distribution of fat and lean is shown following the typical colour scale, from yellow (high fat percentage) to red (low fat percentage – lean); in the lower corners, the two histograms represent the amount of tissue mass spread by different percentage of fat content. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

parameters are based on standard reference data. BC values can be considered among the most variable to be collected and analysed, since the differences between worldwide populations and countries are remarkable.[12] However, some parameters and indexes as measured with different techniques in the analysis of BC may be proposed for collection and comparative evaluations among healthy and unhealthy populations. Today, these kinds of data on general or "normal" populations are almost completely missing or patchy.[12] Thus, efforts in the definition of a normative database should be made, even on a regional basis.

The concept of "health" is somewhat difficult to be tested, and subjects are arbitrarily considered healthy in different studies on criteria as established by authors. Although some categories of "healthy" people have been studied and analysed from a BC point of view (i.e. athletes, newborn infants, children, elderly, women in menopause, twins),[13,14] data on healthy subjects of the general population as included by homogenous and selective criteria are almost completely missing.[15–19]

The purpose of this study was to provide a complete profile of BC in healthy subjects and to investigate age and gender-related differences by the latest DXA technology.

2. Materials and methods

2.1. Study design and population

In a cross-sectional analysis of BC, we prospectively recruited subjects from 18 to 70 years old among blood donors of our hospital, in order to reach the threshold of 25 males and 25 females per 5 different age bands (A, 18–30 years old; B, 31–40 years old; C,

Table 1
Body composition, whole body data and differences by sex and age.

DXA data – total body									
Age (Group)		BMI (Kg/m^2) mean ± s.d.	FM (g) mean ± s.d.	FMI (kg/m^2)	LM (g) mean ± s.d.	LMI (kg/m^2)	BMC (g) mean ± s.d.	BMD (g/cm^3) mean ± s.d.	T-score mean ± s.d.
18–30 (A)	m	23.97 ± 2.35	17,262 ± 5459	5.48 ± 1.65	55,377 ± 5025	17.63 ± 1.48	3138 ± 335	1.259 ± 0.121	0.6 ± 1.2
	f	21.96 ± 2.43	19,005 ± 3762	7.03 ± 1.55	38,547 ± 3445	14.20 ± 1.20	2268 ± 235	1.086 ± 0.063	0.1 ± 0.6
p (sex)		0.005	NS		0.000		0.000	0.000	0.022
31–40 (B)	m	24.52 ± 2.56	18,239 ± 6439	5.6 3 ± 1.89	57,787 ± 6405	17.91 ± 1.48	3164 ± 433	1.229 ± 0.106	0.3 ± 1.0
	f	23.89 ± 3.97	22,692 ± 7735	8.53 ± 3.10	38,901 ± 3103	14.53 ± 1.26	2286 ± 167	1.098 ± 0.085	0.2 ± 0.7
p (sex)		NS	0.021		0.000		0.000	0.000	NS
41–50 (C)	m	24.81 ± 2.71	19,461 ± 6476	6.23 ± 2.09	56,357 ± 6587	17.94 ± 1.45	3142 ± 369	1.224 ± 0.082	0.3 ± 0.8
	f	23.94 ± 2.67	22,724 ± 5933	8.57 ± 2.12	38,275 ± 3069	14.45 ± 0.84	2283 ± 242	1.103 ± 0.085	0.2 ± 0.8
p (sex)		NS	0.045		0.000		0.000	0.000	NS
51–60 (D)	m	24.94 ± 2.91	21,042 ± 7068	6.75 ± 2.24	54,195 ± 5503	17.37 ± 1.65	2934 ± 324	1.160 ± 0.088	−0.4 ± 0.8
	f	23.51 ± 2.38	22,068 ± 5662	8.25 ± 1.90	38,580 ± 3878	14.45 ± 1.17	2146 ± 271	1.028 ± 0.088	−0.5 ± 0.9
p (sex)		NS	NS		0.000		0.000	0.000	NS
61–70 (E)	m	25.92 ± 2.70	23,648 ± 7111	7.73 ± 2.03	52,713 ± 5852	17.34 ± 1.29	2923 ± 421	1·148 ± 0·109	−0.5 ± 1.1
	f	24.27 ± 2.80	22,740 ± 4812	8.87 ± 1.73	37,689 ± 4470	14.69 ± 1.22	2044 ± 281	0·990 ± 0·091	−0.9 ± 0.9
p (sex)		0.039	NS		0.000		0.000	0.000	NS
p (age)	m	0.004	0.000		0.014		0.002	0.000	0.000
	f	0.001	0.021		NS		0.034	0.002	0.002

BMI, body mass index; FM, fat mass; FMI, fat mass index; LM, non-bone lean mass; LMI, non-bone lean mass index; BMC, bone mineral content; BMD, bone mineral density; NS, not statistically significant.

41–50 years old; D, 51–60 years old; E, 61–70 years old; total recruitment: 250 patients). The single-centre enrolment of volunteers was carried out within a period from June to March. All participants were Caucasians, living in Italy. The inclusion criteria were fitness for blood donation, normal glycaemic and lipid profiles, normal hepatic and renal function, the absence of relevant past or present diseases (diabetes and endocrinological/metabolic diseases; liver, kidney, neoplastic diseases as well as any other apparatus or systemic pathology), stable weight in the last year, and body mass index (BMI) between 18 and 30 kg/m^2.

Normal fitness for blood donation considered were: no drugs consumption, no recent tattoos or piercing, no unsafe intercourse, no recent long travel abroad, a part from fitting with suitable biochemical blood results and physical condition; chest radiographs and electrocardiography were performed for each donor, by default.

A deep and standardized anamnesis was collected to exclude significant pathologies or aberrant habits (i.e. smokers >20 cigarettes/die, alcoholism etc.). Other conditions that might induce an iatrogenic variation in the metabolic status, such as for transexual subjects, also determined the exclusion from the study. The potential assumption of drugs was also investigated in all patients to rule out any pharmacological treatment potentially modifying the BC, with particular reference to hormonal therapies (e.g. thyroid drugs, steroids, and estro-progestinic).

Furthermore, pregnant women and subjects with surgical hardware, implantable devices, foreign bodies, or those who were recently submitted to diagnostic tests using nuclides or barium or radio-opaque substance were excluded from the study.

Each person signed an informed consent prior to participation. The study protocol was approved by the local ethical committee and was conducted according to the Declaration of Helsinki.

Height and weight of patients were measured barefoot, with subjects wearing only underwear and a cloth gown, to the nearest 0.1 cm and 0.1 kg respectively, using a mechanical balance with altimeter (Seca 711, Seca GmBH & Co Kg, Germany). BMI was calculated as weight/area (Kg/m^2). On the same day, patients were submitted to DXA analysis.

2.2. Dual-energy X-ray absorptiometry

A whole-body DXA scan was performed to measure total and regional BC using a new fan-beam densitometer (Lunar iDXA, Madison, WI, USA; enCORE™ 2011 software version 13.6). The scanner was calibrated daily using a standard calibration block supplied by the manufacturer. All metal items were removed before densitometry. The subjects wearing only underwear and a cloth gown were placed in a supine position with arms at sides slightly separated from the trunk and correctly centred on the scanning field. The scan duration was about 7 min in standard mode for total body scan. Regions of interest (ROIs) were defined by the analytical program including six different corporeal districts: *total body*, *trunk*, *upper limbs*, *lower limbs*, *android region* (a portion of the abdomen included between the line joining the two superior iliac crests and extended cranially up to the 20% of the distance between this line and the chin) and *gynoid region* (a portion of legs leaving from the femoral great throcanter, directed caudally up to a distance double of the android region). For each region, DXA scanned the weight (in g) of total mass, FM, LM, and BMC (Fig. 1). A whole-body BMD was also assessed and the percentage of FM and whole-body T-score were calculated.

Moreover, visceral fat analysis was performed by CoreScan, a very new software option for the assessment of visceral fat (mass and volume) in the android region.[11] The detection of the layer thickness of the subcutaneous adipose tissue (SAT) at sides of the

Table 2
Body composition, regional (central) data and differences by sex and age.

DXA data – regional analysis																					
Age (group)		Android					Gynoid					Trunk									
		FM (g) mean ± s.d.		FMI (kg/m^2)	LM (g) mean ± s.d.		LMI (kg/m^2)	BMC (g) mean ± s.d.		FM (g) mean ± s.d.		FMI (kg/m^2)	LM (g) mean ± s.d.		LMI (kg/m^2)	BMC mean ± s.d.		FM (g) mean ± s.d.		FMI (kg/m^2)	LM (g) mean ± s.d.		LMI (kg/m^2)	BMC mean ± s.d.
18–30 (A)	m	1388 ± 666		0.44 ± 0.21	3853 ± 665		1.22 ± 0.12	59 ± 9		3628 ± 1138		1.15 ± 0.34	8316 ± 844		2.65 ± 0.26	328 ± 42		8455 ± 3452		2.68 ± 1.05	25,424 ± 2624		8.69 ± 0.72	922 ± 146
	f	1127 ± 500		0.42 ± 0.20	2668 ± 306		0.98 ± 0.10	43 ± 7		4598 ± 1141		1.71 ± 0.43	5041 ± 1150		1.87 ± 0.42	207 ± 51		8008 ± 2717		2.97 ± 1.11	18,591 ± 2003		6.85 ± 0.89	655 ± 85
p (sex)		NS			0.000			0.000		0.000			0.000			0.000		NS			0.000			0.000
31–40 (B)	m	1554 ± 805		0.48 ± 0.24	4115 ± 573		1.27 ± 0.15	57 ± 11		3372 ± 1210		1.04 ± 0.35	8606 ± 1481		2.66 ± 0.41	323 ± 49		9720 ± 3994		3.00 ± 1.19	26,798 ± 2743		8.31 ± 0.63	911 ± 161
	f	1608 ± 874		0.61 ± 0.35	2826 ± 255		1.06 ± 0.11	44 ± 6		5500 ± 1479		2.06 ± 0.58	5285 ± 601		1.97 ± 0.21	220 ± 19		9987 ± 4449		3.77 ± 1.77	18,627 ± 1524		6.96 ± 0.61	669 ± 81
p (sex)		NS			0.000			0.000		0.000			0.000			0.000		NS			0.000			0.000
41–50 (C)	m	1818 ± 841		0.59 ± 0.28	4052 ± 474		1.29 ± 0.12	57 ± 9		3560 ± 1041		1.14 ± 0.32	8510 ± 1049		2.71 ± 0.26	336 ± 52		10,454 ± 4377		3.35 ± 1.43	25,878 ± 3160		8.24 ± 0.74	893 ± 137
	f	1627 ± 855		0.62 ± 0.33	2930 ± 401		1.11 ± 0.15	44 ± 7		4999 ± 1021		1.88 ± 0.35	5150 ± 530		1.94 ± 0.16	214 ± 25		10,599 ± 4020		4.00 ± 1.50	18,371 ± 1431		6.94 ± 0.39	654 ± 98
p (sex)		NS			0.000			0.000		0.000			0.000			0.000		NS			0.000			0.000
51–60 (D)	m	2067 ± 938		0.66 ± 0.30	3955 ± 439		1.27 ± 0.14	51 ± 9		3637 ± 1246		1.17 ± 0.39	8046 ± 994		2.58 ± 0.28	304 ± 50		11,913 ± 4684		3.83 ± 1.49	25,333 ± 2596		8.12 ± 0.79	808 ± 149
	f	1642 ± 831		0.61 ± 0.28	2890 ± 382		1.08 ± 0.11	42 ± 9		4927 ± 1037		1.84 ± 0.35	5286 ± 620		1.98 ± 0.16	212 ± 31		10,201 ± 3949		3.81 ± 1.35	17,970 ± 3878		6.72 ± 1.38	585 ± 105
p (sex)		NS			0.000			0.000		0.000			0.000			0.000		NS			0.000			0.000
61–70 (E)	m	2542 ± 991		0.83 ± 0.28	3948 ± 508		1.30 ± 0.13	53 ± 14		3566 ± 980		1.17 ± 0.29	7827 ± 1148		2.57 ± 0.28	300 ± 49		14,071 ± 4912		4.59 ± 1.41	24,792 ± 4912		8.16 ± 0.64	840 ± 167
	f	1644 ± 693		0.65 ± 0.28	2715 ± 344		1.06 ± 0.11	37 ± 7		4838 ± 1178		1.88 ± 0.39	5051 ± 690		1.97 ± 0.21	199 ± 35		10,797 ± 3303		4.24 ± 1.34	18,206 ± 2013		7.06 ± 0.59	562 ± 117
p (sex)		0.000			0.000			0.000		0.001			0.000			0.000		NS			0.000			0.000
p (age)	m	0.000			NS			0.001		NS			0.019			0.007		0.000			NS			0.001
	f	0.008			NS			NS		NS			NS			NS		0.005			NS			0.016

FM, fat mass; FMI, fat mass index; LM, non-bone lean mass; LMI, non-bone lean mass index; BMC, bone mineral content; NS, not statistically significant.

Table 3
Body composition, regional (appendicular) data and differences by sex and age.

DXA data – regional analysis											
Age (group)		Upper limb					Lower limb				
		FM (g) mean ± s.d.	FMI (kg/m^2)	LM (g) mean ± s.d.	LMI (kg/m^2)	BMC (g) mean ± s.d..	FM (g) mean ± s.d.	FMI (kg/m^2)	LM (g) mean ± s.d.	LMI (kg/m^2)	BMC (g) mean ± s.d.
18–30 (A)	m	1850 ± 623	0.59 ± 0.20	7408 ± 839	2.36 ± 0.27	449 ± 45	6088 ± 1735	1.93 ± 0.52	19,224 ± 1942	6.12 ± 0.59	1207 ± 142
	f	2191 ± 414	0.81 ± 0.16	4037 ± 538	1.49 ± 0.19	297 ± 43	8039 ± 1181	2.96 ± 0.44	13,164 ± 406	4.85 ± 0.44	816 ± 123
p (sex)		NS		0.000		NS	0.000		0.000		0.000
31–40 (B)	m	1967 ± 761	0.61 ± 0.22	7912 ± 1130	2.45 ± 0.30	756 ± 1378	5662 ± 1847	1.75 ± 0.54	19,790 ± 2812	6.12 ± 0.66	1208 ± 174
	f	2609 ± 1067	0.98 ± 0.42	4127 ± 399	1.54 ± 0.17	298 ± 24	9324 ± 2401	3.49 ± 0.95	13,281 ± 1380	4.96 ± 0.51	809 ± 66
p (sex)		0.004		0.000		0.001	0.000		0.000		0.000
41–50 (C)	m	2143 ± 647	0.69 ± 0.21	7744 ± 1124	2.47 ± 0.33	491 ± 74	5989 ± 1692	1.91 ± 0.52	19,453 ± 2544	6.18 ± 0.52	1220 ± 163
	f	2773 ± 700	1.05 ± 0.25	4171 ± 474	1.58 ± 0.17	311 ± 35	8599 ± 2079	3.24 ± 0.71	12,986 ± 1462	4.90 ± 0.42	812 ± 88
p (sex)		0.001		0.000		NS	0.000		0.000		0.000
51–60 (D)	m	2206 ± 654	0.71 ± 0.21	7196 ± 974	2.31 ± 0.34	460 ± 60	6039 ± 2018	1.93 ± 0.63	17,906 ± 2896	5.73 ± 0.85	1154 ± 154
	f	2674 ± 735	1.00 ± 0.25	4211 ± 538	1.58 ± 0.17	299 ± 40	8434 ± 1628	3.15 ± 0.55	12,877 ± 1471	4.82 ± 0.44	792 ± 101
p (sex)		0.011		0.000		NS	0.000		0.000		0.000
61–70 (E)	m	2488 ± 737	0.81 ± 0.21	6806 ± 923	2.24 ± 0.28	450 ± 62	6184 ± 1706	2.03 ± 0.52	17,819 ± 2401	5.86 ± 0.55	1113 ± 171
	f	2734 ± 526	1.07 ± 0.20	4195 ± 664	1.64 ± 0.23	278 ± 46	8422 ± 2093	3.26 ± 0.58	12,498 ± 1940	4.86 ± 0.53	738 ± 112
p (sex)		NS		0.000		NS	0.000		0.000		0.000
p (age)	m	0.000		0.002		NS	NS		0.002		0.011
	f	0.002		NS		NS	NS		NS		NS

FM, fat mass; FMI, fat mass index; LM, non-bone lean mass; LMI, non-bone lean mass index; BMC, bone mineral content; NS, not statistically significant.

android region allowed the software to map and to estimate the total SAT compartment. Afterwards, the amount of android visceral adipose tissue (VAT) was indirectly derived by subtracting SAT from the total android FM (VAT = android FM – SAT).

The total and regional (android) ratios between FM and LM were involved in the description and in the analysis of results, and FM ratios between different anatomic regions were considered to examine the central/peripheral distribution: android/gynoid FM (A/G FM), body weight/(upper + lower) limbs FM (*appendicular FM ratio – aFMR*), and body weight/(upper + lower) limbs LM (*appendicular LM ratio – aLMR*); VAT/SAT ratio was also represented. In addition, *fat mass index* (*FMI*) as total FM/squared height (Kg/m^2),[20] *non-bone lean mass index* (*LMI*) as total LM/squared height (Kg/m^2) and the corresponding indexes for regional compartments were calculated to normalize BC parameters per squared height (area).

2.3. Statistical methods

The normal distribution of our sample population was tested by skewness and Kurtosis test. Normal ranges were considered for values between −2 and +2. Data were analysed by ANOVA and Mann–Whitney test to determine differences between males and females. Multivariate analysis of variance (MANOVA) was also performed to evaluate differences in values and trends of parameters and indexes as measured for males and females of the same age groups. Two-tailed p was considered significant for values less than 0.05. Statview statistical package (version 5.0.1 for Windows – SAS Inc., Chicago, IL, USA) was used for the analysis. Results are reported as frequencies or mean and standard deviation (±s.d.). The distribution of BC was also represented with percentiles (5th, 10th, 25th, 50th, 75th, 90th and 95th) and reported in graphs.

Table 4
Main indexes for body composition assessment by DXA.

DXA indexes										
Age (group)		TFM/TLM	AFM/ALM	A/G FM	aFMR	aLMR	VAT	VMI (kg/m^2)	SAT	VAT/SAT
		mean ± s.d.	mean ± s.d.	mean ± s.d.	mean ± s.d.	mean ± s.d.	mean ± s.d.		mean ± s.d.	mean ± s.d.
18–30 (A)	m	0.31 ± 0.09	0.37 ± 0.19	0.42 ± 0.12	10.14 ± 2.38	2.85 ± 0.20	444 ± 335	0.1 ± 0.1	944 ± 443	0.52 ± 0.35
	f	0.49 ± 0.09	0.43 ± 0.19	0.34 ± 0.57	5.90 ± 0.58	3.49 ± 0.26	149 ± 156	0.1 ± 0.1	978 ± 366	0.14 ± 0.10
p (sex)		0.000	NS	NS	0.000	0.000	0.000			NS
31–40 (B)	m	0.31 ± 0.10	0.37 ± 0.18	0.52 ± 0.51	11.13 ± 2.63	2.86 ± 0.19	767 ± 500	0.2 ± 0.1	787 ± 393	1.10 ± 0.79
	f	0.58 ± 0.18	0.56 ± 0.30	0.28 ± 0.12	5.60 ± 0.92	3.67 ± 0.38	358 ± 326	0.1 ± 0.1	1250 ± 639	0.27 ± 0.18
p (sex)		0.000	0.004	NS	0.000	0.000	0.000			0.000
41–50 (C)	m	0.35 ± 0.12	0.46 ± 0.22	0.54 ± 0.16	10.23 ± 2.34	2.91 ± 0.23	899 ± 607	0.3 ± 0.2	919 ± 345	0.98 ± 0.60
	f	0.59 ± 0.13	0.56 ± 0.29	0.34 ± 0.19	5.69 ± 0.72	3.75 ± 0.27	520 ± 515	0.2 ± 0.2	1106 ± 619	0.33 ± 0.49
p (sex)		0.000	0.001	NS	0.000	0.000	0.000			NS
51–60 (D)	m	0.39 ± 0.13	0.53 ± 0.24	0.61 ± 0.13	10.25 ± 2.96	3.14 ± 0.40	1060 ± 616	0.3 ± 0.2	1051 ± 662	1.10 ± 0.60
	f	0.57 ± 0.14	0.56 ± 0.24	0.33 ± 0.13	5.77 ± 0.83	3.68 ± 0.35	526 ± 469	0.2 ± 0.2	1116 ± 444	0.45 ± 0.26
p (sex)		0.000	NS	0.000	0.000	0.000	0.000			NS
61–70 (E)	m	0.45 ± 0.12	0.64 ± 0.23	0.75 ± 0.17	9.57 ± 1.91	3.22 ± 0.23	1713 ± 850	0.6 ± 0.2	828 ± 288	2.19 ± 1.01
	f	0.60 ± 0.09	0.60 ± 0.21	0.34 ± 0.11	5.70 ± 0.65	3.75 ± 0.27	650 ± 412	0.3 ± 0.2	994 ± 363	0.65 ± 0.29
p (sex)		0.000	NS	0.000	0.000	0.000	0.000		0.000	0.003
p (age)	m	0.000	0.000	0.000	0.000	0.000	0.000		NS	0.000
	f	0.002	0.005	0.000	0.002	0.002	0.000		NS	0.000

TFM, total fat mass; TLM, total non-bone lean mass; AFM, android fat mass; ALM, android non-bone lean mass; A/G FM android/gynoid fat mass; aFMR, appendicular fat mass ratio; aLMR, appendicular non-bone lean mass ratio; VAT, visceral adipose tissue; VMI, visceral mass index; SAT, subcutaneous adipose tissue; NS, not statistically significant.

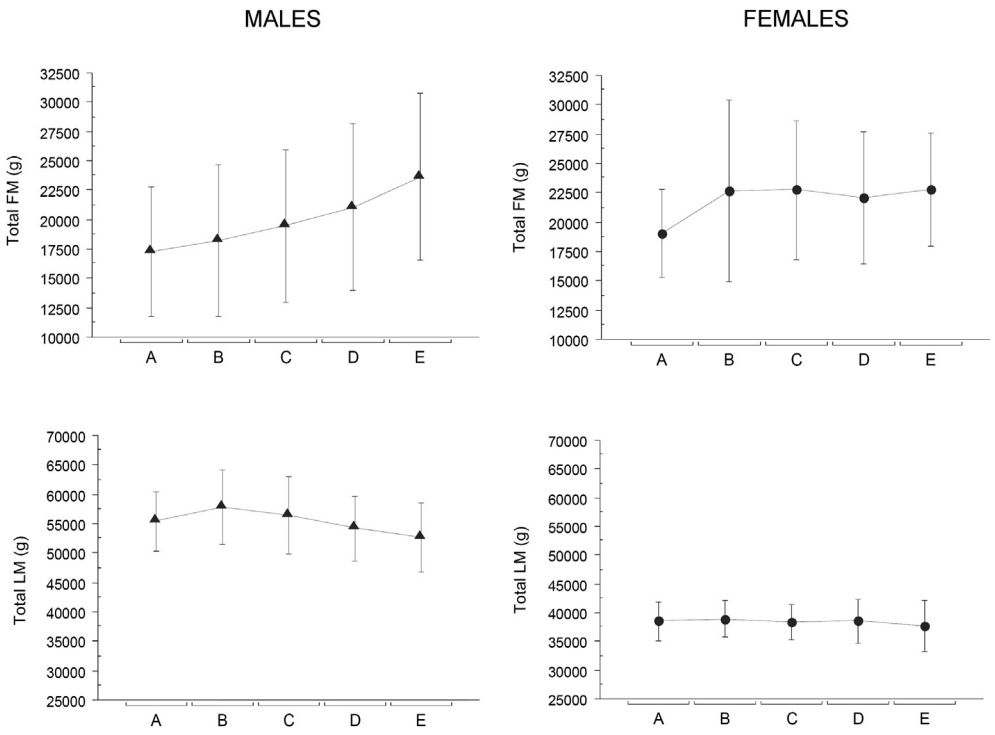

Fig. 2. Parameters of body composition at whole-body level (fat mass – FM, non-bone lean mass – LM).

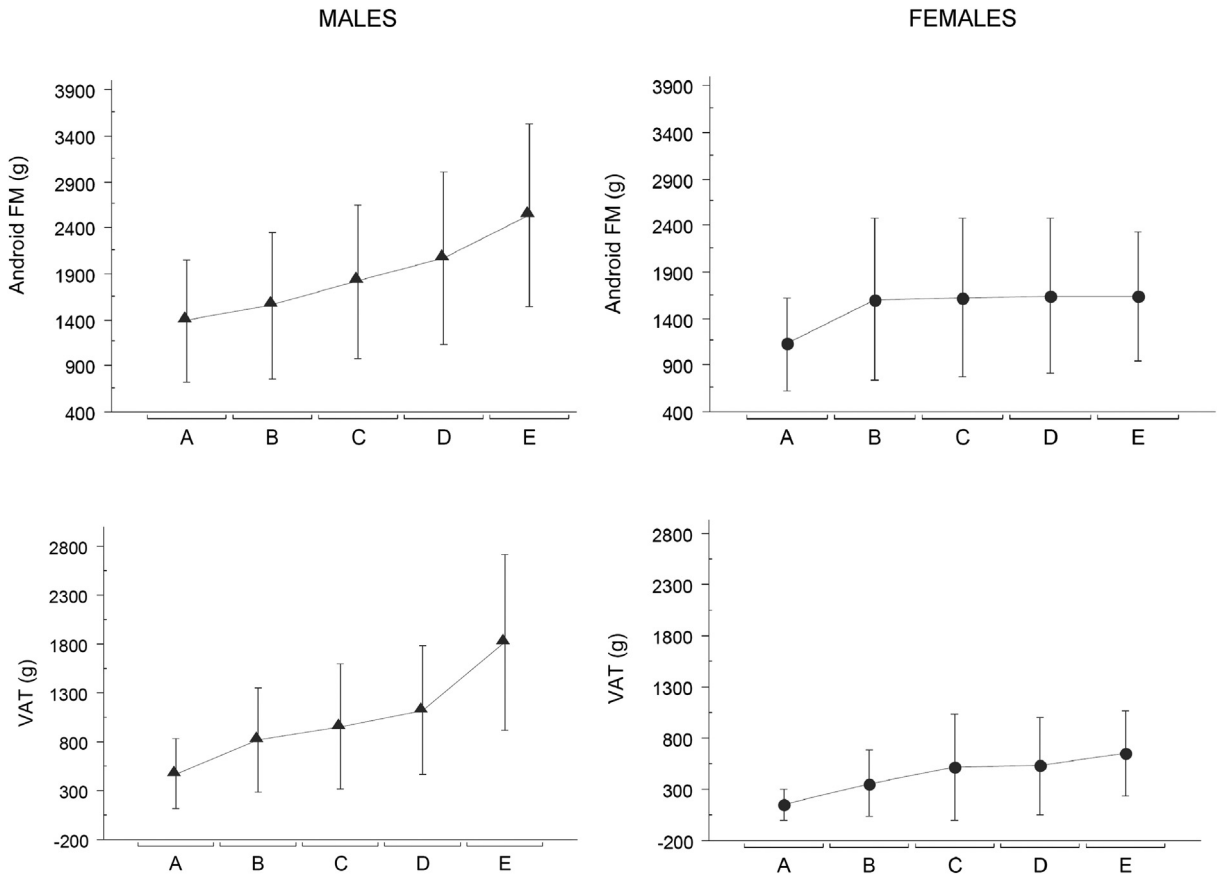

Fig. 3. Parameters of body composition at central level (fat mass – FM, android visceral fat – VAT).

3. Results

The total recruitment of 250 patients was reached with an initial screening of 307 subjects; among the blood donors, 57 candidates were not allowed to proceed with BC analysis because they did not fit with all inclusion criteria.

All patients were within the normal width of the whole-body scan DXA field of view, thus no "half-body" analysis needed to be performed.

The normal distribution of the population was proved for all parameters. Due to the large amount of information available from the collected measurements, results are better displayed in tables and graphs. The descriptive statistics for all DXA parameters and indexes are shown in Tables 1–4.

BMI was higher for males, but this increased with age for both men and women ($p = 0.004$ and 0.001, respectively) (Table 1).

In men, total FM constantly increased from the youth to the older age. In women there was a sensitive increase of FM in group B; after group B decade, mean values of total FM remained steady, while consistently decreased in group D. However, in all groups but the group E, total FM was higher in females than in males (Fig. 2). On the other hand, android FM was constantly higher in men with progressive increase through different ages, while this compartment increased during the first decade in women (up to similar amount of android FM found for the two sexes in group B), and it remained unchanged afterwards (Fig. 3). A slow growth of VAT, which was higher in males of all age groups, was recorded until 51 years old in both sexes; it subsequently showed an important increase in males, while in women remained steady ($p < 0.0001$ for both sexes – Fig. 3, Table 4). SAT compartment was nearly the same in males and females in their 20s; after opposite progressions in the following decade (↓ in men, ↑ in women), at 60s–70s SAT finally got lower values than women in males and equal values to the first decade in females (Table 4). Android FM/LM ratio and total FM showed similar trends in both sexes with the tendency towards similar values in the elderly ($p = 0.190$–0.997 and $p = 0.369$–0.604, respectively – Fig. 4, Tables 1 and 4); moreover, android FM/LM ratio was constantly higher in females of all ages, with the exception of group E (Fig. 4).

Total LM was higher in men, although this seemed to decrease during lifetime; on the contrary, women did not show a decrease in their total LM ($p = 0.014$ and 0.784 – Fig. 2, Table 1). However, total FM/LM ratio in women was constantly higher than in men ($p < 0.0001$ – Fig. 4, Table 4).

Gynoid FM was always higher in women, especially in group B ($p < 0.0001$ – Table 2). However, the A/G FM ratio of this population was substantially steady in women, while increased in men (from 0.34 to 0.34 – $p = 0.006$ and from 0.42 to 0.75 – $p < 0.0001$ – Fig. 4, Table 4).

FM regionally increased in upper limbs but not in lower limbs, in both sexes. LM tended to decrease in both upper and lower limbs, although this was not for upper limbs of women (Fig. 5). The aFMR and aLMR were in line with the analysis of the limbs as a whole, with a more significant increase of aLMR in males with ageing due to the upper and lower limb LM cumulative decrease (Table 4).

The BC was also represented by graphs in percentiles for FMI, LMI, android FM/squared height (android FMI), VAT/squared height (VMI), upper limbs FM/squared height (upper FMI), upper limbs LM/squared height (upper LMI), lower limbs FM/squared height (lower FMI), lower limbs LM/squared height (lower LMI), aFMR, aLMR, A/G ratio, and total FM/LM ratio (Figs. 6 and 7).

4. Discussion

Evidence of the importance in studying BC has grown rapidly in the last few years.[1,2]

As previously stated, the collection of BC data as reference standard for different populations worldwide may prove to be a very hard work. However, reference values of BC of healthy people may be fundamental in order to evaluate and to compare patients affected by different pathological conditions. A regional-basis approach is required in the collection of such kind of parameters and a comparison among normal populations coming from all over the world would also offer special insights into the prevalence of diseases and risks for health.

For instance, bone densitometric values (BMD) have been collected for DXA and these are basic in the definition and management of osteoporosis.[21]

Thus, this work provides important data on BC of the "healthy" phenotype of Italian people and represents one of the first to consider the need for building a normative database of BC on national-basis.

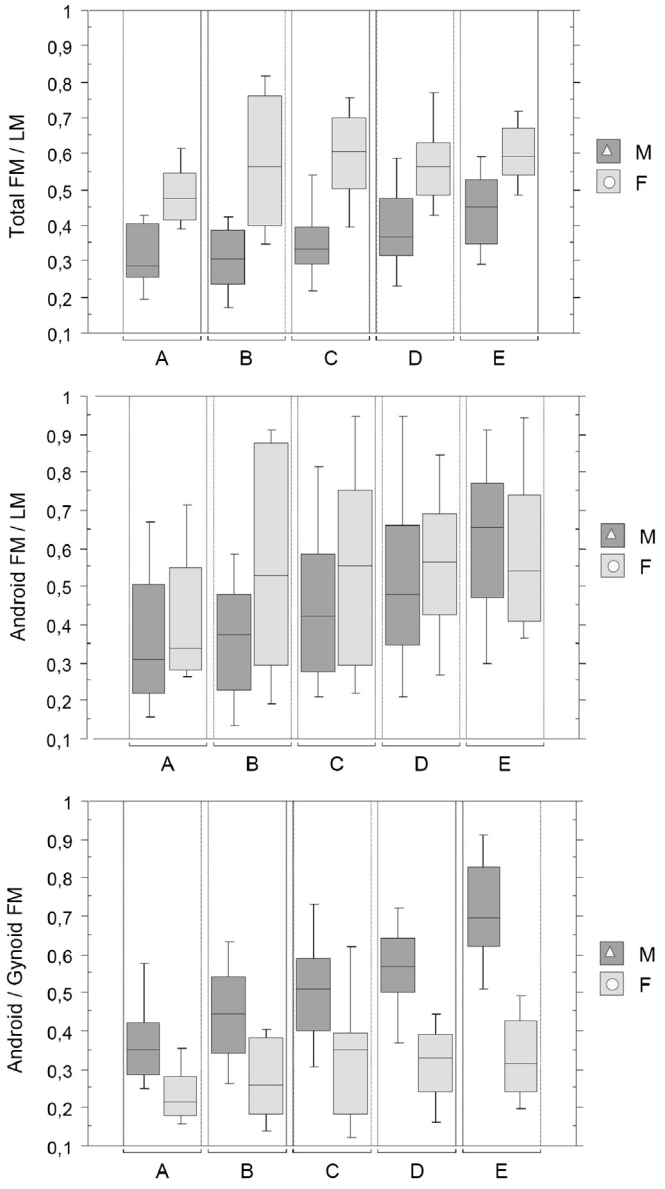

Fig. 4. Box plots show the different trends of total fat mass/lean mass (total FM/LM), android fat mass/lean mass (android FM/LM) and android/gynoid fat mass (android/gynoid FM) ratios in males and females of the five age decades (males – M, females – F; A, 18–30 years old; B, 31–40 years old; C, 41–50 years old; D, 51–60 years old; E, 61–70 years old).

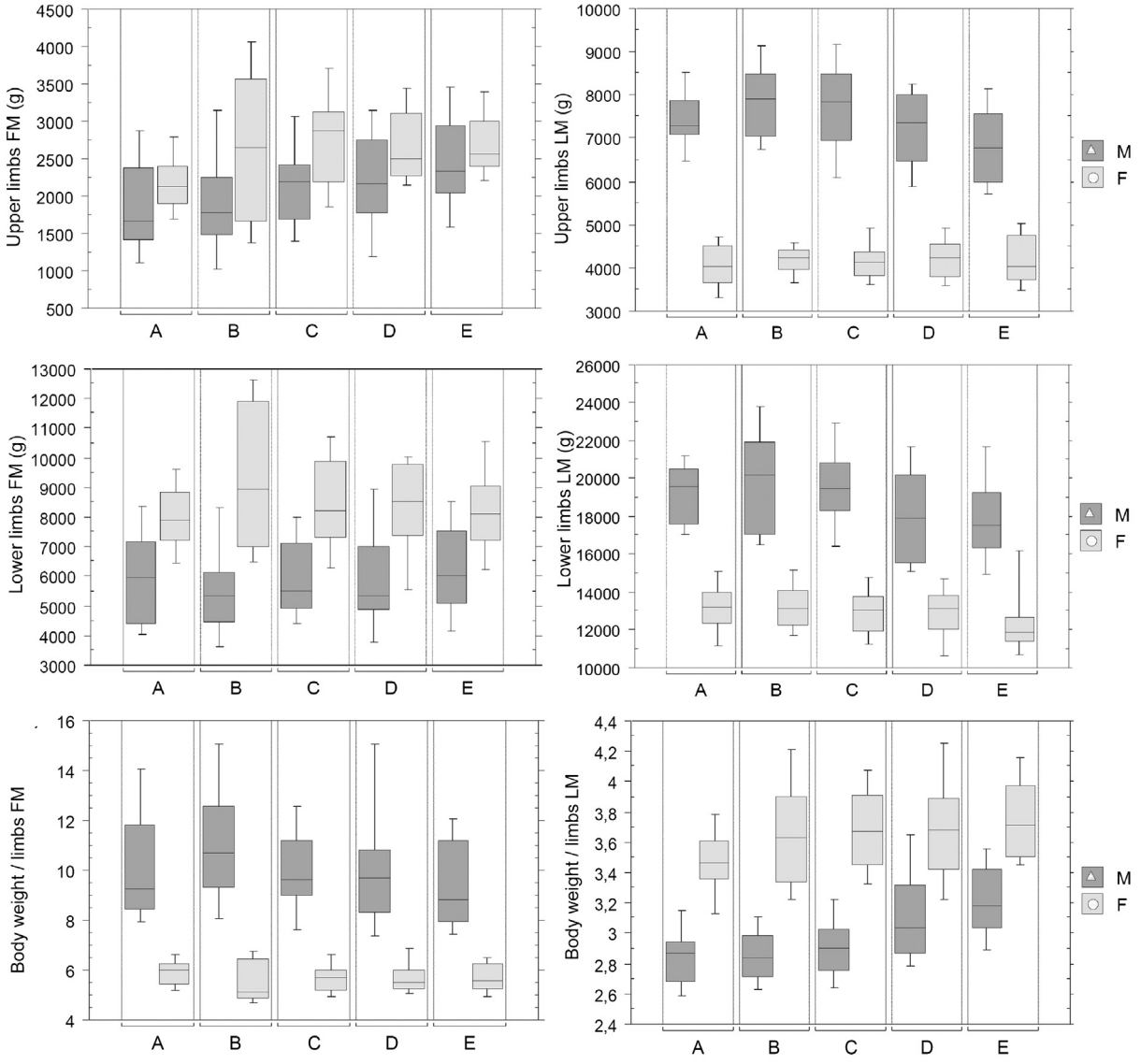

Fig. 5. Parameters of body composition at appendicular level (fat mass – FM, non-bone lean mass – LM; males – M, females – F).

The assessment of BC changes with ageing is essential as such variations are related to health status and physical function.[13,22]

Even if a few authors collected subjects to get normal or control references to be compared with those of patients affected by the selected disease under investigation very few researches focused their attention on the study of "healthy" BC.[15–19] The ageing evolution of BC should be analysed at the light of three different processes: (a) reduction in height and BMD (osteopenia and osteoporosis); (b) progressive decrease in LM and increase in FM (sarcopenia, and sarcopenic obesity); (c) redistribution of FM, central and visceral (VAT).[23,24] All these can be observed and monitored by DXA.

In the healthy population of this study BMI increased with ageing, mainly as the result of FM increase (Table 1). However, FM in women increased up to 40s and remained steady afterwards, differently from the evolution of FM in men (remarkable increase, especially after 60s). On the other hand, LM was higher in men but decreased from the age of 40 years old, while the amount of LM was constant from 20s to 70s in healthy women (Fig. 2). Jackson et al.[19] have recently analysed the evolution of BC in men along their lifetime, and they found the same increasing trend for BMI and FM (up to 80s), with LM decreasing after 47 years old (marked decrease after 60s). Our data partially confirms the results coming from previous experience, like those of Aguado and Kyle by DXA; the studies of the two authors and respective colleagues achieved results similar to ours concerning FM and LM in men, but not in women.[15,18] The only remarkable difference is represented by the earliest peak of LM for what concerns men of our population (30–40 versus 35–59 of Kyle) (Fig. 2). In the analysis performed by Kyle and colleagues FM increased in women until the age of 60–74 years old, while it significantly decreased after 75 years (as comparable to Aguado).[15,18] On the other side, women LM was highest in the 18–34 year-old patients, with a gradual progression to lower values afterwards.[15] Furthermore, the study by Aguado and colleagues described a continuous decrease in LM% with ageing in both women and men (although more striking for the latter).[18] The authors cited above aimed at investigating healthy people, even though with different criteria to define the "healthy" condition. Finally, in our population the results suggest a more stable evolution of LM in healthy women, while the starting point for LM

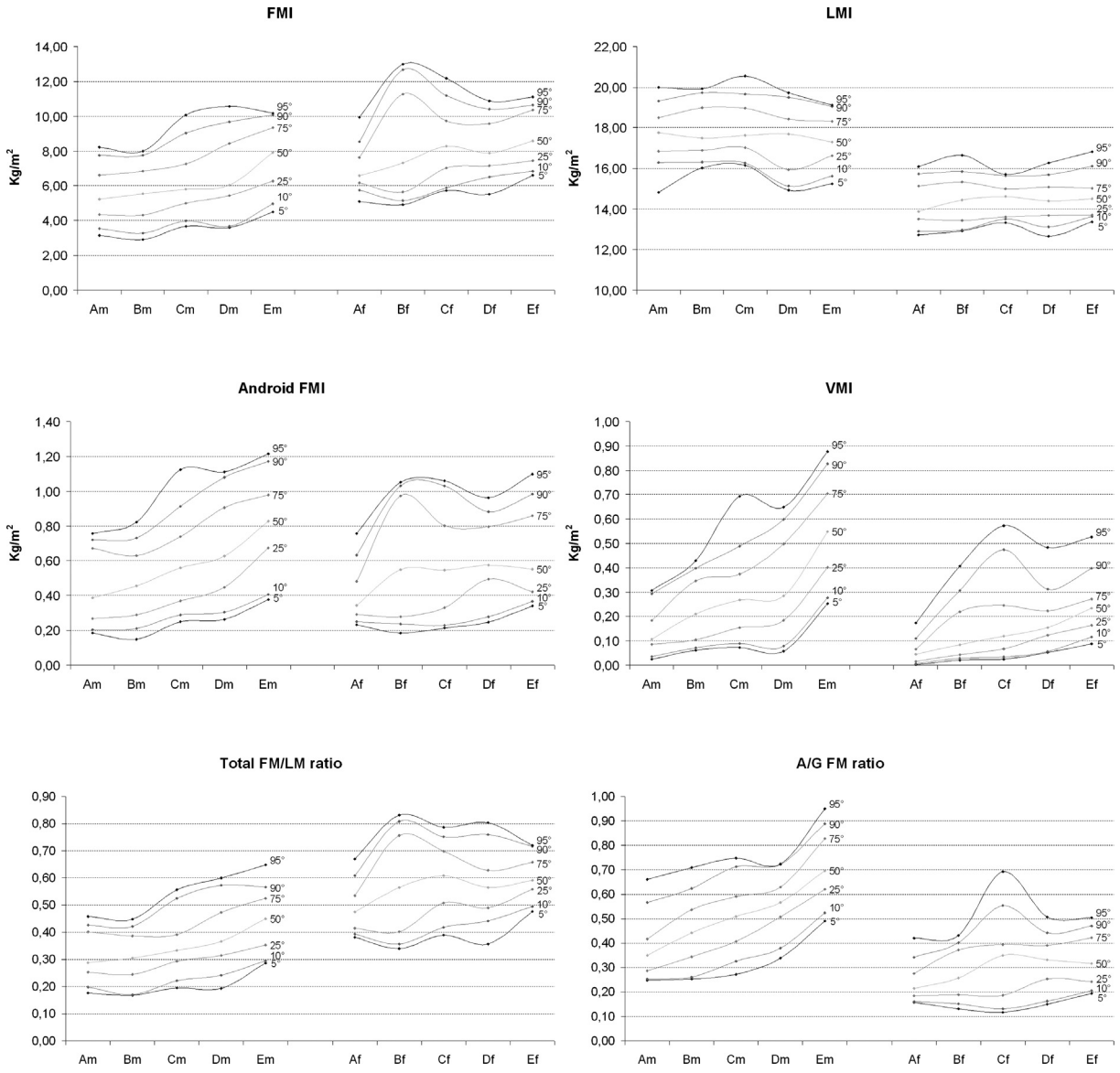

Fig. 6. The distribution of body composition represented with percentiles (5th, 10th, 25th, 50th, 75th, 90th and 95th) in graphs for different parameters (fat mass index – FMI, non-bone lean mass index – LMI, android fat mass/squared height – android FMI, android visceral fat/squared height – VMI, total fat mass/non-bone lean mass ratio – total FM/LM, and android/gynoid fat mass ratio – A/G FM). The suffixes –m and –f are for males and females (i.e. Am, Af etc.), respectively.

decrease in men is positioned at 30s–40s, in an earlier stage than experience of past literature (Fig. 2).[15,18,19]

Since the dawn of BC investigations, the importance of fat distribution has been clear and supported by extensive scientific evidence.[25] A "central" (or "abdominal" or "android") distribution of FM was associated with the development of higher risk profile for cardiovascular and metabolic diseases.[26]

The burden of such risk is attributed to VAT compartment, while SAT plays a controversial role.[25,27] Moreover, the two fat depots are distinct in their endocrine and paracrine secretion profiles with different impacts on glucose homeostasis.[27] Hence, the differential assessment of VAT and SAT has always been considered one of the most important targets of BC analysis. On the other hand, the accurate measurement of VAT compartment represents one of the most challenging goals for the techniques available in clinical practice. DXA android region was designed to be as representative as possible of abdominal VAT, thus to predict metabolic risk of patients. As mentioned above, the new advancement in DXA data analysis has recently allowed an even more accurate estimation of this "hot" fat depot, and our study is one of the first to explore this potential.[11]

In this study, the main parameters advocated as predictors of "central" pattern of FM distribution and visceral fat (i.e. android FM and VAT) showed a rising distancing of women from men along lifetime (and especially after 30s–40s). Males progressively grew in android and visceral fat, while female population of the healthy sample seemed to go towards a less pronounced android or visceral distribution of fat in older decades (Fig. 3). The stability of A/G FM ratio in females was also expression of avoided central distribution in healthy female with ageing (Fig. 4). In males, on the contrary, our results showed a linear progression to "abdominal" redistribution of FM over the time (Figs. 3 and 4). Notably, SAT depot was significantly higher in females and nearly superimposable in males and females of the two latest decades. However, the results from the previously mentioned studies by Aguado and Kyle, and by our team show some differences. In the population of Aguado central

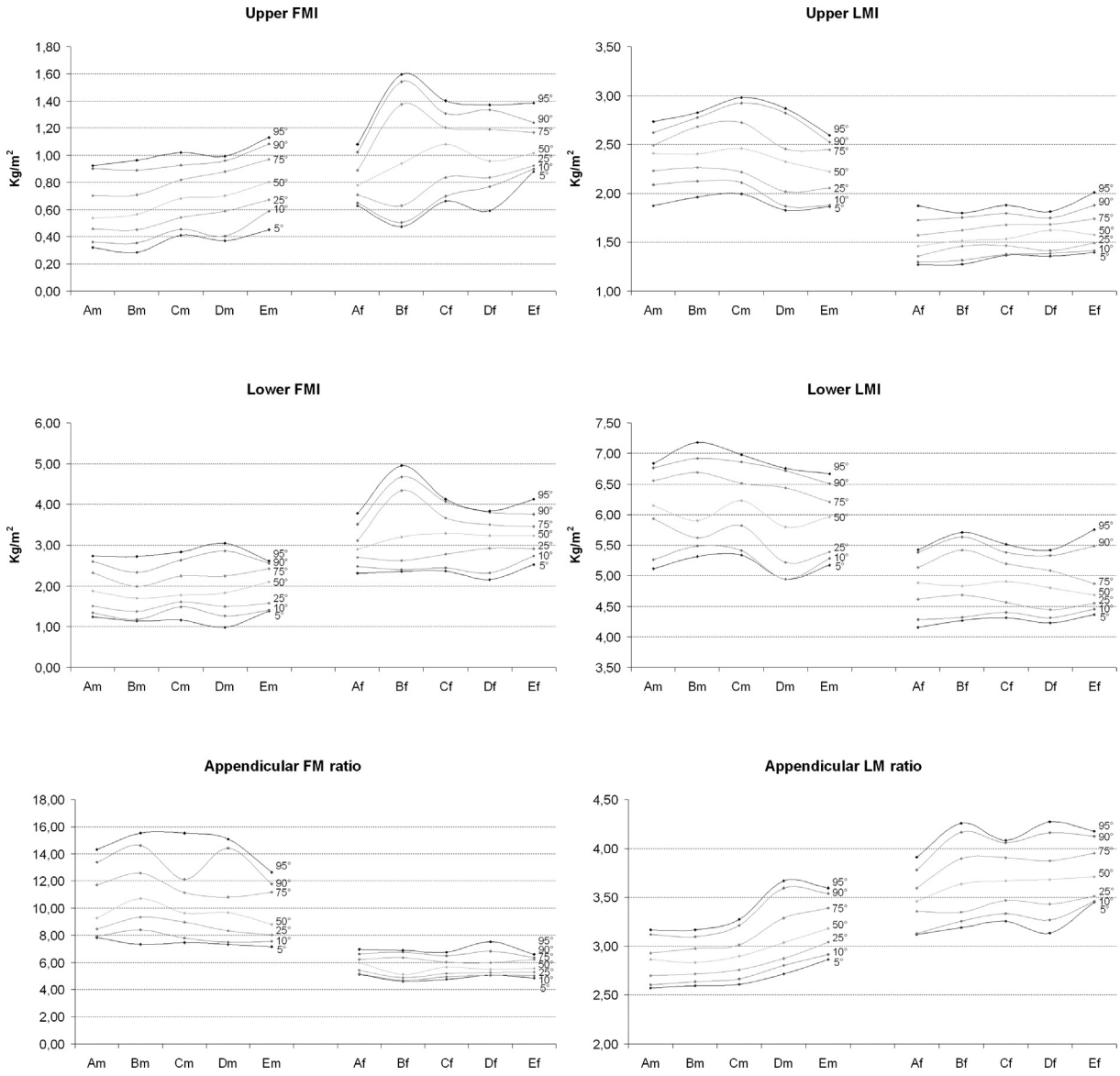

Fig. 7. The distribution of body composition represented with percentiles (5th, 10th, 25th, 50th, 75th, 90th and 95th) in graphs for different parameters (upper limbs fat mass/squared height – upper FMI, upper limbs non-bone lean mass/squared height – upper LMI, lower limbs fat mass/squared height – lower FMI, lower limbs non-bone lean mass/squared height – lower LMI, appendicular fat mass ratio – aFMR, appendicular lean mass ratio – aLMR). The suffixes –m and –f are for males and females (i.e. Am, Af etc.), respectively.

FM increased up to 55 years old, and remained steady thereafter in males, while it constantly increased in females up to 70s; in Kyle both sexes grew in central FM until they reached 60–74 years old. The central distribution of FM in the previously cited studies, however, was focused on the trunk region and no one considered and provided data on android region (particularly VAT, and A/G FM) to focus on the very central distribution of FM.[15,18]

The FM/LM distribution at the upper and lower limbs showed a general decrease of LM, but women seemed to maintain a more favourable mass ratio (stable LM) for arms. In the appendicular body, the decrease of LM was associated to increase of FM, and LM impoverishment started after 40s in men (remarkable after 50s), and later, in the 50s, in female (Fig. 5). Kyle et al. found that appendicular FM became progressively higher until 60–74 years old, where peak FM was noted in men and women, and was lower thereafter; LM of the limbs was observed stable up to 35–59, with a subsequent decrease in the two sexes.[15] A very recent study by Coin et al. was focused on limbs BC by DXA. In a cohort of patients from 20 to 80 years old, the authors described a progressive increase of FM% in males, while an increase with subsequent stability of FM% in females after 60–69 years old was observed.[28]

The main differences when comparing this study to ours were, once again, the relatively stability of LM in the women of our population.

BMC was reported as reference (Table 1) but it was not taken into consideration in the analysis of bone metabolic status because of its poor value if compared with BMD. Moreover, whole-body BMD has notoriously been attributed a poor value in the definition of the bone status, if compared to BMD measurement in the most critic sites of fractures (i.e. lumbar spine, femur). Thus, bone parameters have not been discussed not to drive misleading conclusions.

Although large populations have been considered in different studies of the literature aiming at normal or “healthy” populations, inclusion criteria were often variable and loose. Besides, DXA has been very seldom used to study BC in healthy people, and analysis

of data was nearly always retrospective or multi-centric.[14,16,28] Other studies tried to focus on healthy BC, by other clinically available techniques.[15,19]

Despite the inclusion criteria of our study are strictly selective, the relatively low number of patient and the cross-sectional fashion remain a major limitation for the analysis to drive conclusions about the longitudinal evolution of BC in healthy people. The younger "healthy" won't be sure to become an older "healthy". Moreover, volunteers were recruited in a period going from early summer to late winter, and this is another important element to be taken into consideration.

An extension to older ages would also be recommended, although the criteria assumed in this study for patients' recruitment would be hardly respected.

Efforts were made in the exclusion of most relevant metabolic disorders and implications in the recruitment criteria of this study. However, overweight patients were included to make the "healthy" population as close as possible to the general population of the country. Even if BMI cannot distinguish between FM and LM – higher BMI could be also due to higher value of LM – especially in "healthy" people, this point deserves special consideration.

Our healthy sample provides interesting insights into the physiology of BC. The phenomenon of progressive decrease in LM and increase in FM with ageing was proved in the healthy population, but this was more attenuated in women. The central and visceral redistribution of FM was also observed along lifetime, although women were less affected by this change.

The results of this study are not truly representative of the entire Italian population, because the enrolment was centred in Emilia Romagna. However, Emilia Romagna is one of the most populated regions of Italy. Moreover, it is possible to state, with due reservation, that this region has a "well-balanced" population concerning phenotype, life-style (and immigration policy), if compared with northern or southern Italian populations, or to other isolated populations.

In conclusion, this is a report on the BC status of healthy Italian subjects in their adulthood, to be used as a reference for future investigations on pathological human conditions and differences between countries.

Statement of authorship

Study concept and design: Alberto Bazzocchi and Danila Diano. Acquisition of data: Danila Diano, Andrea Andreone and Alberto Bazzocchi. Analysis and interpretation of data: All authors. Drafting of the manuscript: Alberto Bazzocchi, Danila Diano and Federico Ponti. Critical revision of the manuscript for important intellectual content: All authors. Study supervision: Alberto Bazzocchi, Giulio Marchesini and Giuseppe Battista.

Conflict of interest

No funding or conflict of interest to declare.

Acknowledgements

The authors would like to thank Dr. Monica Benni and Dr. Pasqualepaolo Pagliaro for their help in the organization of patients' enrolment.

References

1. Thibault R, Genton L, Pichard C. Body composition: why, when and for who? *Clin Nutr* 2012;**31**:435–47.
2. Muller MJ, Bosy-Westphal A, Later W, Haas V, Heller M. Functional body composition: insights into the regulation of energy metabolism and some clinical applications. *Eur J Clin Nutr* 2009;**63**:1045–56.
3. de Aquino LA, Pereira SE, de Souza Silva J, Sobrinho CJ, Ramalho A. Bariatric surgery: impact on body composition after Roux-en-Y gastric bypass. *Obes Surg* 2012;**22**:195–200.
4. Wiskin AE, Wootton SA, Hunt TM, Cornelius VR, Afzal NA, Jackson AA, et al. Body composition in childhood inflammatory bowel disease. *Clin Nutr* 2011;**30**:112–5.
5. St-Onge MP. Relationship between body composition changes and changes in physical function and metabolic risk factors in aging. *Curr Opin Clin Nutr Metab Care* 2005;**8**:523–8.
6. Malina RM. Body composition in athletes: assessment and estimated fatness. *Clin Sports Med* 2007;**26**:37–68.
7. Lee SY, Gallagher D. Assessment methods in human body composition. *Curr Opin Clin Nutr Metab Care* 2008;**11**:566–72.
8. Andreoli A, Scalzo G, Masala S, Tarantino U, Guglielmi G. Body composition assessment by dual-energy X-ray absorptiometry (DXA). *Radiol Med* 2009;**114**:286–300.
9. Toombs RJ, Ducher G, Shepherd JA, De Souza MJ. The impact of recent technological advances on the trueness and precision of DXA to assess body composition. *Obesity (Silver Spring)* 2012;**20**:30–9.
10. Leonard CM, Roza MA, Barr RD, Webber CE. Reproducibility of DXA measurements of bone mineral density and body composition in children. *Pediatr Radiol* 2009;**39**:148–54.
11. Kaul S, Rothney MP, Peters DM, Wacker WK, Davis CE, Shapiro MD, et al. Dual-energy X-ray absorptiometry for quantification of visceral fat. *Obesity (Silver Spring)* 2012;**20**:1313–8.
12. Kelly TL, Wilson KE, Heymsfield SB. Dual energy X-Ray absorptiometry body composition reference values from NHANES. *PLoS One* 2009;**4**:e7038.
13. Genton L, Karsegard VL, Chevalley T, Kossovsky MP, Darmon P, Pichard C. Body composition changes over 9 years in healthy elderly subjects and impact of physical activity. *Clin Nutr* 2011;**30**:436–42.
14. Shaw NJ, Crabtree NJ, Kibirige MS, Fordham JN. Ethnic and gender differences in body fat in British schoolchildren as measured by DXA. *Arch Dis Child* 2007;**92**:872–5.
15. Kyle UG, Genton L, Slosman DO, Pichard C. Fat-free and fat mass percentiles in 5225 healthy subjects aged 15 to 98 years. *Nutrition* 2001;**17**:534–41.
16. Boorsma W, Snijder MB, Nijpels G, Guidone C, Favuzzi AM, Mingrone G, et al. Body composition, insulin sensitivity, and cardiovascular disease profile in healthy Europeans. *Obesity (Silver Spring)* 2008;**16**:2696–701.
17. Kyle UG, Genton L, Hans D, Karsegard L, Slosman DO, Pichard C. Age-related differences in fat-free mass, skeletal muscle, body cell mass and fat mass between 18 and 94 years. *Eur J Clin Nutr* 2001;**55**:663–72.
18. Henche SA, Torres RR, Pellico LG. An evaluation of patterns of change in total and regional body fat mass in healthy Spanish subjects using dual-energy X-ray absorptiometry (DXA). *Eur J Clin Nutr* 2008;**62**:1440–8.
19. Jackson AS, Janssen I, Sui X, Church TS, Blair SN. Longitudinal changes in body composition associated with healthy ageing: men, aged 20–96 years. *Br J Nutr* 2012;**107**:1085–91.
20. Peltz G, Aguirre MT, Sanderson M, Fadden MK. The role of fat mass index in determining obesity. *Am J Hum Biol* 2010;**22**:639–47.
21. Prevention and management of osteoporosis. *World Health Organ Tech Rep Ser* 2003;**921**:1–164.
22. Guo SS, Zeller C, Chumlea WC, Siervogel RM. Aging, body composition, and lifestyle: the Fels Longitudinal Study. *Am J Clin Nutr* 1999;**70**:405–11.
23. Gomez-Cabello A, Vicente Rodriguez G, Vila-Maldonado S, Casajus JA, Ara I. Aging and body composition: the sarcopenic obesity in Spain. *Nutr Hosp* 2012;**27**:22–30.
24. Zamboni M, Mazzali G, Fantin F, Rossi A, Di Francesco V. Sarcopenic obesity: a new category of obesity in the elderly. *Nutr Metab Cardiovasc Dis* 2008;**18**:388–95.
25. Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation* 2007;**116**:39–48.
26. Misra A, Vikram NK. Clinical and pathophysiological consequences of abdominal adiposity and abdominal adipose tissue depots. *Nutrition* 2003;**19**:457–66.
27. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev* 2000;**21**:697–738.
28. Coin A, Giannini S, Minicuci N, Rinaldi G, Pedrazzoni M, Minisola S, et al. Limb fat-free mass and fat mass reference values by dual-energy X-ray absorptiometry (DEXA) in a 20–80 year-old Italian population. *Clin Nutr* 2012;**31**:506–11.