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BODY COMPOSITION IN 65+ EUROPEAN POPULATION: A FOCUS ON INFLAMMATORY AND METABOLIC MARKERS IN HEALTHY AND FRAIL SUBJECTS

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1. GENERAL INTRODUCTION

In 2019 the population aged 65 years or over in Europe grew up by 2.9% compared to 2009, reaching a percentage of 20.3% of the EU population. Italy and Greece were the countries with the highest percentage of elderly people, respectively 22.8% and 22.0% of the population, while Ireland and Luxembourg had the lowest percentages: 14.1% and 14.4% (Eurostat, 2020).

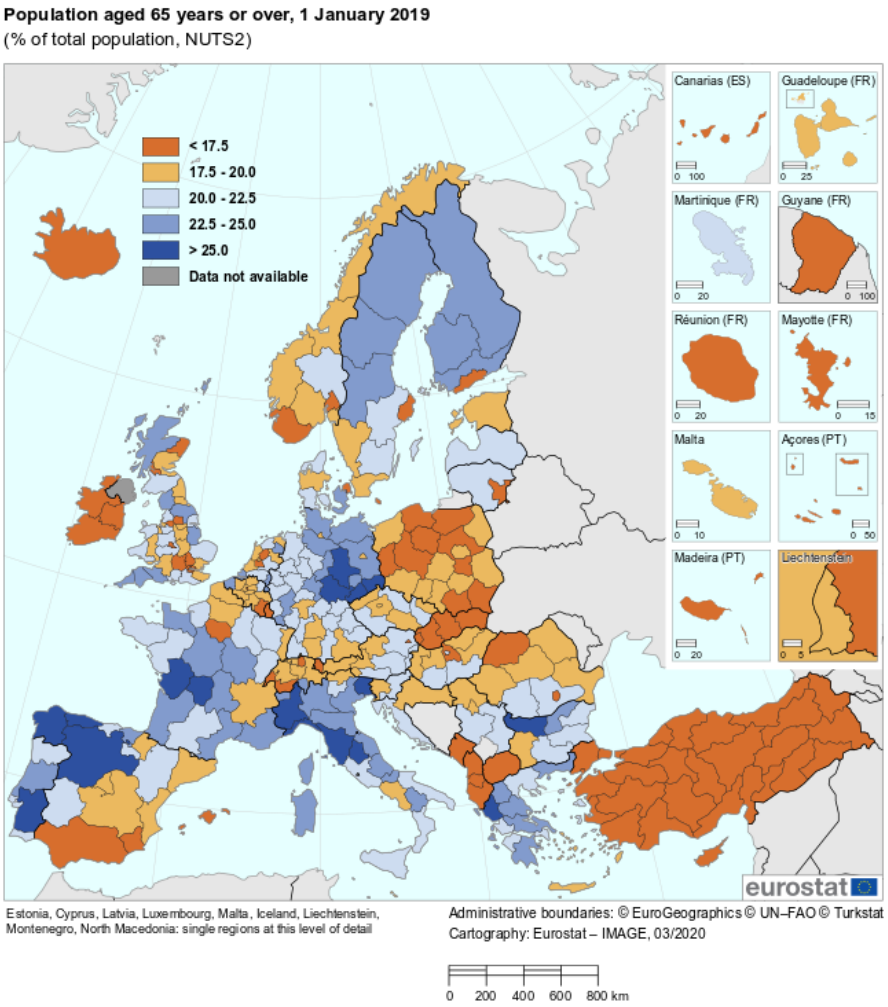


Figure 1.1 | 27 EU Population aged 65 years or over, 1 January 2019 (Eurostat, 2020).

This trend is bound to increase over time, in fact a Eurostat study shows that people aged 65 years and over will increase from 20% in 2018 to 31% in 2100 (Eurostat, 2019). However, the effects of the Covid-19 outbreak on mortality could change this trend. Indeed, if we analyzed mortality data of 2020 the number of deaths across the 31 European countries starts to rise abruptly at the beginning of March, in week 10, compared to previous years (average over 2016 to 2019). In particular, weeks 10-12 are marked by increased death for the age groups 70-79 and 80-89 and deaths of men rise faster than those of women every week (Eurostat, 2020 c).

Aging is a complex phenomenon characterized by different mechanisms. These mechanisms, called “seven pillars of ageing”, includes adaptation to stress, loss of proteostasis, stem cell exhaustion, metabolism derangement, macromolecular damage, epigenetic modifications, and inflammation (Franceschi et al., 2018; Kennedy et al., 2014).

One of the universal features of the ageing process appears to be a chronic, low-grade inflammatory state called “inflammaging” (Franceschi et al., 2007; Cevenini et al., 2013).

In particular, “inflammaging” is characterized by a complex reshape in the production of pro- and anti-inflammatory mediators, which, as a whole, tilts the balance toward an increase of the level of basal inflammation. As an example, aging is characterized by a decreased production of the anti-inflammatory interleukin 10 (IL-10) and an increase of the pro-inflammatory interleukin 6 (IL-6), (Marcos-Pérez et al., 2020), this one in particular is considered a risk factor for many of the major age-associated diseases, including obesity, cardiovascular diseases, sarcopenia and frailty (Santoro et al., 2020).

In addition, there is an association between changes in body composition (BC) and aging. In fact, aging is associated with a reduction in lean mass

(LM) that is referred to as sarcopenia, and an increase of fat mass (FM) (Zong et al., 2017). These modifications of BC have likely a large impact of the health status and inflammaging in particular, as FM, and visceral fat in particular, is an important source of pro-inflammatory cytokines, produced by both the adipose tissue itself (adipokines) and the infiltrating macrophages and lymphocytes (Mancuso et al., 2016). Though BMI has always been considered a valid tool to assess overall adiposity, when it is necessary to investigate the distribution of body fat associated with chronic diseases and mortality it doesn't provide the right support. (Prentice and Jebb, 2001; Zong et al., 2017; Carmienke et al., 2013). Therefore, it is necessary to use tools that can correctly, safely and quickly evaluate the BC. There are different methods to measure BC such as computed tomography, magnetic resonance imaging, dual-energy X-ray absorptiometry and ultrasound (Ponti et al., 2020). The reference method is Dual-energy X-ray Absorptiometry (DXA), which is considered the most developed and tested technique for the evaluation of BC bone mineral density (BMD) (Bazzocchi et al., 2013; Guglielmi et al., 2016). Moreover, DXA can assess three body-composition components at a molecular level: bone mineral content (BMC), lean mass (LM) and fat mass (FM) and it is possible to measure the amount of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) in addition of total-body and standard regional body composition measures (Ponti et al., 2020). A systemic study on the age-related changes in BC was missing, as well as the connections between these changes and parameters linked to inflammaging and overall health.

In a variable percentage of elderly people, a condition of decreased capability to cope with and recover from stresses even of mild intensity is present. This condition, indicated as frailty, is a strong predictor of disability, hospitalization and mortality and a criterion for non-eligibility for invasive treatments. Frailty has been operationalized according to two

different approaches, using either phenotypical or functional components (Morley et al., 2013). Indeed, frailty can be identified according to a standardized phenotype described by Fried and colleagues (Fried LP et al., 2001) by verifying if three or more of the following criteria are met: involuntary weight loss, exhaustion, low physical activity, low gait speed, and low grip strength. Accordingly, people can be divided in three different groups: non-frail (none of these criteria are met), pre-frail (one or two features are met) and frail (three or more criteria are met). Furthermore, it has been demonstrated that an elevated waist circumference and body fat mass are risk factors for frailty in the elderly (Xu Et al., 2020). Also in this case, the connections between BC and frail status are largely unexplored, with particular regards for parameters related to inflammaging. In order to perform large scale analyses regarding these connections, a large dataset is needed, that must be interrogated with appropriate techniques of advanced statistics in order to grasp the complexity of the phenomenon. To this aim, we exploited the dataset of two research projects run in the laboratory where I did my PhD work: the NU-AGE and the PROAGE projects.

Within the framework of the European NU-AGE project – New dietary strategies addressing the specific needs of elderly population for a healthy ageing in Europe (Grant Agreement no. 266486, Coordinator Prof. Claudio Franceschi, registered at clinicaltrials.gov as NCT01754012) - non-frail and pre-frail volunteers were selected using the standardized phenotype scale proposed by Fried et al., and a DXA scan has been carried out as well. In this project 1,250 free-living elderly people, aged between 65 and 79 y.o., free of major diseases, were enrolled within five European centers (Italy, France, United Kingdom, Netherlands and Poland). All volunteers underwent multiple specific tests and laboratory analysis to accurately assess their general health, physical and cognitive functioning and nutritional status. Each measurement was carried out before a

nutritional intervention (T0) and after 12 months (T1), allowing to collect in the project database over 2,000 parameters (Santoro et al., 2014). A large amount of data has also been collected on the composition and functionality of intestinal flora, immune system, genetic and epigenetic, transcriptomic and metabolomic.

The presence of frailty was one of the exclusion criteria in the NU-AGE project, as the aim was to include healthy elderly (Berendsen et al., 2014).

So, in collaboration with the Nestlé Institute of Health Sciences (NIHS) from Lausanne (Switzerland), one of the partners of the NU-AGE study, a new project has been defined and funded to recruit frail subjects, named PRO-AGE: "Omics for Aging-ProAGE" (n. 14.02. NIHS Code NPDI n. DUND-100373). As reported in **Figure 1.2**, PRO-AGE uses the same protocol as NU-AGE for the recruitment of subjects, the age is between 65 and 79 years and it has been run in Italy (Bologna). Again, frailty has been assessed with the presence of at least 3, or more, of the parameters proposed by Fried et al. and the same measurements of NU-AGE were carried out at T0 and after a follow up of 12 months in each of the 23 recruited subjects with the exception of the nutritional trial that has not been administered to these subjects.

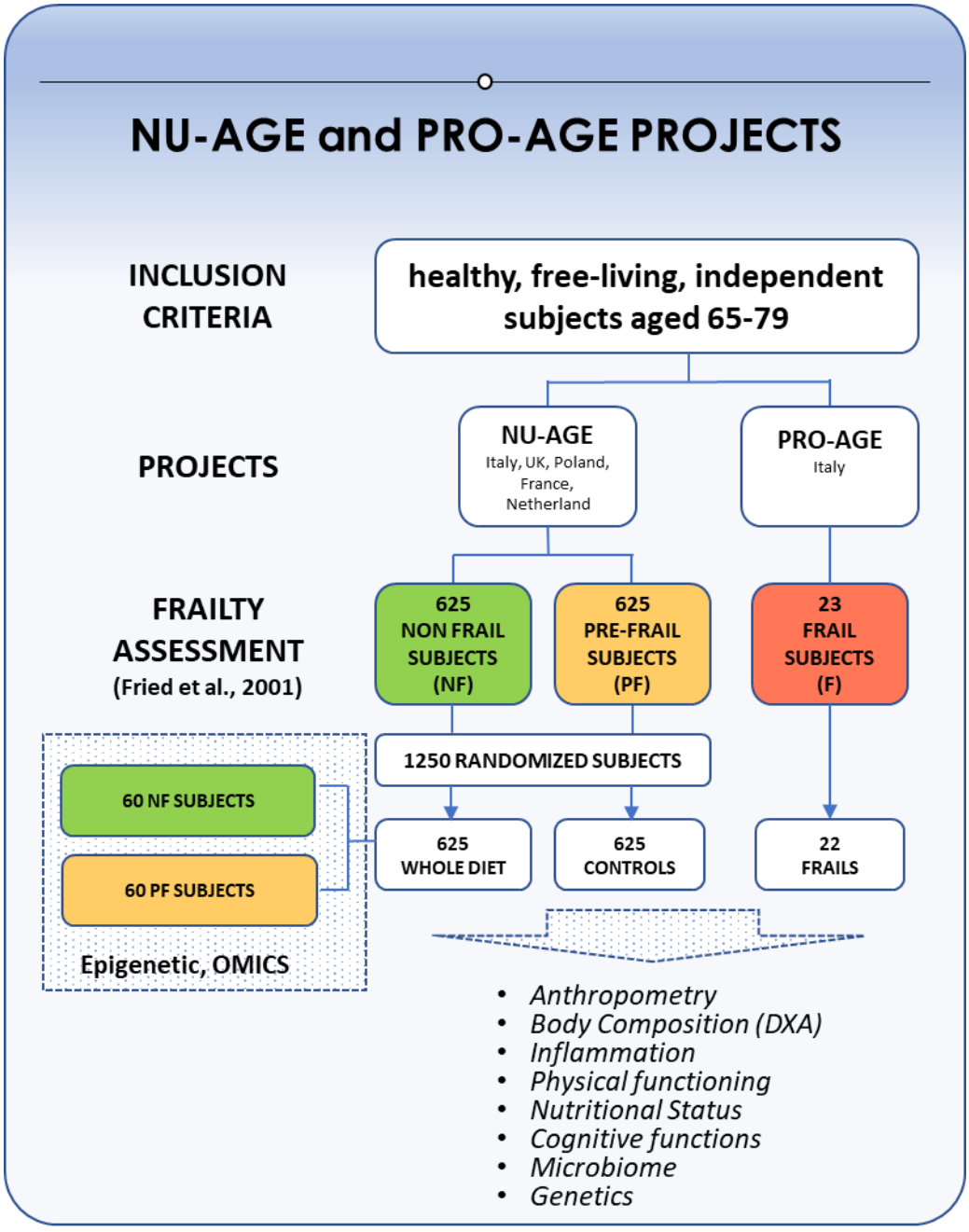


Figure 1.2 | Characteristics and criteria of NU-AGE and PRO-AGE projects.

2. AIMS OF THE STUDY

The main aim of my PhD work was to analyze the connections between BC parameters, hematochemical and biochemical parameters with a special focus on inflammaging on a large population sample of elderly people with or without frailty. To do this, I made use of innovative statistical methods to analyze the data collected in the NU-AGE and PROAGE projects.

In particular I focused on answering these three scientific questions:

- Is body composition in elderly across Europe different? Are there Body composition differences by sex?
- Which are the inflammatory and metabolic markers associated with body composition in the elderly?
- Which are the main differences of body composition and health markers that characterize frail individuals? Is there a BC "frailty signature"?

These three questions have generated the results of my PhD thesis that will be presented and discussed in the following three Chapters. The first two Chapters regard data that have already been reported in publications in international journals that I co-authored, while the latter reports on still unpublished data.

It is well known that accumulation of fat causes serious medical complications, and the prevalence of many of this were associated with obesity, such as hypertension, diabetes, cardiovascular diseases which increases with age. The aim of the first study (Santoro et al., 2018a) was to evaluate the BC assessed by DXA in 1,250 healthy elderly to investigate country- and gender-related differences. In addition, we used

Unsupervised Machine Learning technique, i.e. Hierarchical Cluster Analysis, to define specific BC profiles specific for males and females.

It has been reported that an increase in fat mass is correlated with markers of inflammation among elderly (Brinkley et al., 2018; Schrager et al., 2007). Nevertheless, not so many studies on the correlation between inflammatory parameters and the distribution of fat, lean and bone mass are available. The aim of the second study (Santoro et al., 2018 b) was to assessed the correlations of those BC parameters with several inflammatory and adipose related parameters.

As mentioned the presence of frailty has been assessed using the standard phenotype scale described by Fried et al. even if other diagnostic criteria are proposed, such as Frailty Index by Rockwood.

Despite the Fried et al. scale is the most used, the presence of some components of this phenotype, i.e. low hand grip strength or low gait speed, are more relevant than others.

This may affect the correct detection of the pre-frail subject, in fact a systematic review by Fernandez-Garrido et al. (2014) show that the prevalence of pre-frailty can change in different cohorts of people aged over 65, ranging between 35 and 60%. The aim of the third study was to detect differences of body composition and health markers that characterize pre-frail or frail individuals. In addition, through regression analysis we will try to define a “frailty signature”.

3. BODY COMPOSITION IN ELDERLY ACROSS EUROPE

This Chapter regard data that have already been reported in publication *A Cross-Sectional Analysis of Body Composition Among Healthy Elderly From the European NU-AGE Study: Sex and Country Specific Features* (Santoro et al., 2018a).

3.1 INTRODUCTION

As mentioned in the general introduction, there is an association between changes in BC and aging. In general, this association is characterized by a decrease in subcutaneous FM, while visceral fat and muscle fat infiltration tend to increase with age. Since the BC is associated with several diseases and decline in health status, monitoring weight among older adults becomes very important. In addition, weight gain, weight cycling and in particular weight loss are associated with higher mortality risk (Cheng FW et al., 2015). However, this changes in total FM and LM are often independent from changes in weight and therefore not detectable using body mass index (BMI). In fact, this tool doesn't provide the right support when it is necessary to investigate the distribution of individuals' body fat contents (Prentice AM, 2001; Zong et al., 2017). Several studies related to BC have shown that, independently of BMI levels, trunk fat has been linked to metabolic abnormalities (Bjorntorp, 1991; Bosy-Westphal et al., 2015), and visceral and neck adipose tissue are related to incidence of cardiovascular diseases (Arsenault et al., 2012; Britton et al., 2013; Torriani et al., 2014). Moreover, there is evidence that in older populations there is an association between improved physical function and the preservation of lean muscle mass with scarce muscle fat infiltration (Beavers et al., 2013; Reinders, Murphy, Koster, et al., 2015),

while increase in muscle fat infiltration is associated with higher mortality risk (Miljkovic et al., 2015; Reinders, Murphy, Brouwer, et al., 2015). Thus, the attention of clinicians to study the BC has increased. Due to its relatively low cost, fast acquisition time and low radiation exposure, as compared to other available techniques, DXA is considered the gold standard for the assessment of human BC (Alberto Bazzocchi et al., 2013; Guglielmi et al., 2016).

As mentioned, DXA can assess three body-composition components at a molecular level: BMC, LM, and FM in addition of BMD. Moreover, DXA allows to measure total-body and standard regional body composition, arms, legs, android and gynoid regions (**Figure 3.1**).

Figure 3.1 | A. Skeletal map of whole-body scan by Dual-energy X-Ray absorptiometry (DXA) (head—H, trunk—T, upper limbs—U, lower limbs—L, gynoid—G, and android—A). **B.** Represents the soft tissue maps of whole body DXA scan from fat mass, yellow, to bone mass, blue in old females. (Modified from Ponti et al., 2020)

There are significant differences in BC between countries, genders and human populations (Hinton et al., 2017; Kelly et al., 2009) and since there are several methods to assess BC, indexes and measures have been proposed to evaluate differences among healthy and unhealthy populations (A Bazzocchi et al., 2016). DXA measures of adiposity and muscle mass include fat mass index (FMI: total FM/height2); visceral adipose tissue (VAT); subcutaneous adipose tissue (SAT); android to

gynoid FM ratio (A/G FM), trunk to leg fat mass ratio (T/L FM); lean mass index (LMI: total LM/height2); appendicular lean mass (ALM: arms LM + legs LM) and the corresponding indexes standardized to height and weight called appendicular lean mass index (ALMI: ALM/ height2) and skeletal muscle mass index (SMI: ALM/total weight) respectively (Petak et al., 2013).

A systemic study on the age-related changes in BC was missing and, to the best of our knowledge, no study to date has evaluated BC parameters by DXA scan among elderly populations in Europe.

Within the framework of the NU-AGE project a whole-body DXA scan has been carried out in 1121 gender-balanced free-living, apparently healthy older adults aged 65 to 79 years enrolled in 5 European Countries (Italy, France, United Kingdom, the Netherlands, and Poland) (Santoro et al., 2014). As reported in the "Aims of the studies" section, in the current study we evaluate the BC assessed by DXA to investigate country- and gender-related differences. In addition, we used Unsupervised Machine Learning technique, i.e. Hierarchical Cluster Analysis, to define specific BC profiles.

3.2 METHODS

Study design and population

NU-AGE (http://www.nu-age.eu/) is a one-year, multicenter, randomized, single-blind, controlled trial (registered with clinicaltrials.gov, NCT01754012) carried out in five European centers located in France (Clermont-Ferrand), Italy (Bologna), the Netherlands (Wageningen), Poland (Warsaw), and the United Kingdom (UK, Norwich) (Santoro et al., 2014). The recruitment of participants has been described in detail previously (Berendsen et al., 2014; Santoro et al., 2014). Originally, 2668 man and women volunteers from the community aged 65–79 years, free of major overt chronic diseases compromising 2-year survival (i.e., cancer, dementia), free and independent living, and competent to make own decisions, were recruited from July 2012 to January 2014 to participate in the baseline assessment. After testing the exclusion criteria, i.e. included severe heart diseases, type 1 and insulin-treated type 2 diabetes, chronic use of corticosteroids, recent use of antibiotics or vaccinations, change in habitual medication use, presence of frailty (Fried et al., 2001), malnutrition (body mass index <18.5 kg/m2 or 10% weight loss within 6 months), or food allergy/intolerance requiring special diets, 1296 were eligible to participate in the NU-AGE trial. Complete DXA scan was performed in 1121 participants, at baseline, and were included from the NU-AGE study cohort. According to the Declaration of Helsinki, all participants signed the informed consent before their inclusion in the study. NU-AGE was approved by the Ethics Committee of the coordinator center: the Independent Ethics Committee of the S. Orsola-Malpighi Hospital Bologna (Italy), and by the local/national Ethics Committees of all the other four recruiting centers: the South-East 6 Person Protection Committee (France), the Wageningen University Medical Ethics Committee (Netherlands), the National

Research Ethics Committee–East of England (UK), and the Bioethics Committee of the Polish National Food and Nutrition Institute (Poland).

Assessment of Body Composition

A whole-body DXA scan has been carried out to measure total and regional BC using the following fan-beam densitometers in each of the five recruiting centre: Discovery QDR, Hologic Inc., Bedford, MA, USA – software version 3 (Clermont-Ferrand, France); Lunar iDXA, GE Healthcare, Madison, WI, USA – enCORETM 2011 software version 13.6 (Bologna, Italy); Lunar Prodigy, GE Healthcare, Madison, WI, USA – enCORETM 2011 software version 13.6 (Wageningen, the Netherlands and Warsaw, Poland); and Discovery Wi, Hologic Inc., Bedford, MA, USA (Norwich, UK). The scanners were calibrated daily using a standard calibration block supplied by the manufacturers following standard Quality Control procedures. DXA scans were performed by trained technicians according to state-of-the-art technique and manufacturers recommendation. No metal items were present during densitometry. Participants were positioned in the center of the scanning field in a supine position with the arms at sides and separated from the trunk. As mentioned, measurements of total-body and standard regional body composition, such as trunk, upper limbs, android and gynoid region were defined by DXA. In the UK, android and gynoid regions were not detected by the densitometer. The weight (in g) of total mass, whole body fat mass (FM), non-bone whole body lean mass (LM), and bone mineral content (BMC), was scanned. In order to reduce the possible error generated by the use of different DXA machines, specific indices have been used (**Table 3.1**).

	Index	Calculation
a	total body FM/ LM	Whole body fat mass/ whole body lean mass
b	fat mass index (FMI)	Whole body fat mass/ heigth2
c	Lean Mass index (LMI)	Whole body lean mass/ heigth2
d	android/gynoid FM	Android fat mass/ gynoid fat mass
e	android FM/LM	Android fat mass/ android lean mass
f	Appendicular Lean Mass index (ALMI)	Lean mass from arms plus legs/height2
g	skeletal mass index (SMI)	Lean mass from arms plus legs/weight

Table 3.1 | Pivotal markers of BC

The indexes of total body FM/LM, FMI, and LMI are considered markers of general mass, android/gynoid FM is related of central/peripheral distribution of FM, while the FM/LM android, and ALMI and SMI indices are markers of central abdominal distribution, low muscle mass respectively. Moreover, bone mineral density (BMD) and T-score were also considered as markers of bone health.

Data collection

An adherence scoring on the NU-AGE Diet was created with cut-off values based on the NU-AGE Food Based Dietary guidelines (Berendsen et al., 2018). The NU-AGE index is a 160-point scale that includes minimum consumption recommendations for fruits, vegetables, legumes, low-fat dairy products, low-fat cheese, fish, low-fat meat and poultry, nuts, olive oil, liquids and vitamin D3 (from a supplement), minimum and maximum intake frequencies, for whole grains and eggs, and recommendations to limit, alcohol, salt and confectionery. In this paper we have standardized

the NU-AGE index in a percentage scale ranged between 0, no adherence, and 100, fully adherent.

Data on educational level (level and years), physical activity (Physical activity scale for the elderly, PASE (Washburn et al., 1993), and medical history (use of drugs for hypertension [yes/no], use of drugs for diabetes [yes/no], use of drugs for hypercholesterolemia [yes/no], use of vitamin D supplementation [yes/no], use of calcium supplementation [yes/no]) were obtained by means of questionnaires. Height was measured with a stadiometer to the nearest 0.1 cm. Weight was measured to the nearest 0.1 kg with a calibrated scale while wearing light clothes. Body Mass Index (BMI) was calculated as weight [kg]/height[m]2. Calorie intake was calculated by mean of the 7days food record completed by the participants at baseline. Handgrip strength test was performed by standardized procedures using Jamar handheld dynamometer. Blood pressure was measured using automated and calibrated electronic blood pressure monitors. All measures were taken by trained research assistants.

Glycated haemoglobin was measured on fresh blood in each recruiting centres by standard methods. Plasma total, HDL and LDL cholesterol (mg/dL) and triglycerides (mg/dL) were measured on a konelab system and reagents were from Thermo Scientific (Asnières sur Seine, France).

Concentrations of total 25-hydroxyvitamin D (25(OH)D) [i.e. 25(OH)D2 plus 25(OH)D3] and parathyroid hormone (PTH) in all serum samples were measured at the laboratory of the Cork Centre for Vitamin D and Nutrition Research. 25(OH)D was measured by a modified version of the LC-MS/MS method that has been described in detail elsewhere (Cashman et al., 2013) and is certified by the Centers for Disease Control and Prevention's (CDC) Vitamin D Standardization Certification Program (*VDSCP: List of Certified Participants*, 2018). PTH was measured with an ELISA kit (intact PTH; MD Biosciences Inc.). Intra-assay and inter- assay

CVs were 3.0% and 5.1%, respectively (at a concentration of 47.7 and 52.6 pg/ml, respectively). All the other biochemical analyses glucose (mmol/L), insulin (mcU/mL), albumin (g/L), and creatinine (mmol/L), were measured on frozen blood and frozen urine (urea) in a centralized centre with standard methodologies.

Statistical Methods

After testing the data distribution, according to Shapiro-Wilk test for Normality ($p < 0.01$) we decided to use non-parametric statistical tests. R studio (Version '1.0.136' for Windows) was used as analysis' tool and results are reported as mean and standard deviation (± SD). Data were analyzed by non-parametric statistical tests, i.e. Mann - Whitney and Kruskal - Wallis tests, to determinate differences between males and females and between the five countries, respectively. To test differences between all pairs of country a pairwise comparison was used. A type I error of 0.05 (p-value) in two-tailed tests was considered significant. The Benjamini–Hochberg correction was applied (q-value) in order to reduce the error due to multiple testing of the variables. Furthermore, we decided to perform an unsupervised machine learning technique, i.e. hierarchical cluster analysis, to detect different groups based on Body Composition's parameters. We used this method instead of k-means analysis because the desired number of groups was not defined a priori. Indeed, this method constructs a hierarchy of nested clusters which does not require cluster number specification (Chalise et al., 2014). Several studies have shown differences between Males and females in BC (Hinton et al., 2017; Kelly et al., 2009), so were separately investigated in this analysis.

3.3 RESULTS

Descriptive Statistics

Table 3.2 shows anthropometric, physical, nutritional and body composition characteristics of NU-AGE study participants by gender. Differences among 620 Female (55%) and 501 Males (45%) are significant for anthropometric measures, diet assessment, physical functioning and BC parameters while there are no differences in education. So, males and female were separately investigated in this analysis. Regarding anthropometrics characteristics males are taller than female, and have higher weight, BMI, waist circumference and waist to hip ratio. On the other hand, males show grater physical activity (PASE score) and strength (handgrip strength) together with higher calorie intake. BC parameters highlight a higher presence of fat mass markers in female with significant differences compared to males in terms of FM, FMI, FM/LM and android FM/LM but lower android/gynoid FM, males have significantly higher lean mass markers than females in terms of LM, ALMI, LMI, SMI and also higher bone content markers in terms of BMC, BMD, T-score, L1-L4 T-score, L1—L4 BMD, neck T-score and neck BMD than females.

1121 NU-AGE participants' characteristics were separately investigated according to country of origin: Italy (*n = 236*), Poland (*n = 222*), UK (*n = 246*), France (*n = 184*) and The Netherlands (*n = 233*). **Table 3.3** shows anthropometric, physical, nutritional and body composition characteristics of NU-AGE study participants by country. Significant differences among country emerged, in particular French subjects have the lowest values in terms of weight, BMI, hip and waist circumference then other countries, while Polish subjects have the highest ones, with the exception of hip circumference that is higher in English subjects. Moreover, French subjects have the highest adherence to the NU-AGE diet at baseline and the highest calorie intake, while the lowest adherence and the lowest calorie intake are reported in Dutch subjects

and Italian subjects, respectively. English subjects have the highest handgrip strength value, in both Males and Females, and PASE score, while Polish subjects and Italian subjects have the lowest handgrip strength and the lowest PASE score, respectively. BC parameters highlight significant differences in terms of FM, FMI, FM/LM and android FM/LM with highest values reported in Polish subjects and lowest values in French subjects. In fact, French subjects have higher lean mass markers, i.e. LM, ALMI, LMI and SMI, unlike the Italian and Polish subjects who have the lowest ones. Regarding bone mass, the highest values are reported in Dutch subjects (BMC, T-score and BMD) and the lowest ones in English subjects (L1-L4 BMD and neck BMD). Given the differences between Female and Male (**Table 3.2**) we have analyzed the differences between countries by gender

Cluster Analysis

In order to answer the question defined in the section "aims of the studies" and define a specific BC profiles among the participants a cluster analysis was performed. Males (n = 501) and females (n = 620) profiles were separately investigated using the following ten BC markers: FM, FMI, LM, LMI, ALMI, FM/LM, SMI, t-score, BMC and BMD in addition to BMI. The hierarchical cluster analysis identified five clusters for females and six clusters for males. According to the mean value of BMI we named these clusters as: Normal Weight (NW; BMI= 21.4 kg/m2; N=89), Overweight A (OWA; BMI= 25.1 kg/m2; N=251), Overweight B (OWB; BMI= 26.6 kg/m2; N=137), Low Obesity A (LOA; BMI= 31.5 kg/m2; N=61), and Low Obesity B (LOB; BMI= 31.9 kg/m2; N=82) in females (**Table 3.4 A**) and Normal Weight (NW; BMI= 24.0 kg/m2; N=122), Overweight A (OWA; BMI= 25.7 kg/m2; N=20), Overweight B (OWB; BMI= 26.3 kg/m2; N=233), Low Obesity A (LOA; BMI= 30.1 kg/m2; N=34), Low Obesity B

(LOB; BMI= 30.4 kg/m2; N=80) and Moderate Obesity (MO; BMI= 35.5 kg/m2; N=12) in males (**Table 3.4 B**).

The distribution of female within the five cluster is divided as follow: 40.5% in OWA and 22,1% in OWB, NW represents the 14.4%, LOA 9.8% and LOB 13.2 %. In these groups the increase of BMI coincides with an increase in fat mass, in terms of FM, FMI and FM/LM markers, on the contrary no correlation with lean and bone masses emerged. Comparing clusters with similar BMI, such as OWA (BMI= 25.1 kg/m2) and OWB (BMI= 26.6 kg/m2) and LOA (BMI= 31.5 kg/m2) and LOB (BMI= 31.9 kg/m2), a different distribution of BC emerged. Particularly, evaluating differences among the two overweight groups, OWA with respect to OWB have lower fat mass in terms of FM (23.8 vs 26.9 kg; p=3.1e-08), FMI (9.2 vs 10.9 kg/m2; p=4.0e-14) and FM/LM (0.6 vs 0.7; p<2e-16), but higher lean mass in terms of LM (40.4 vs 37.2 kg; p=2.7e-11), LMI (15.5 vs 15.1 kg/m2, p=0.00075) and SMI (0.3 vs 0.2; p=4.0e-11) and bone mass in terms of T score (-0,4 vs -1.9; p<2e-16), BMC (2190.4 vs 1804.1 g; p<2e-16) and BMD (1.1 vs 0.9 g/cm2; p<2e-16). Comparing the two low obesity groups, LOA with respect to LOB have lower fat mass in terms of FM (32.7 vs 38.5; p=7.7e-08), FMI (12.9 vs 14.9; p=1.5e-06) and FM/LM (0.7 vs 0.9; p<5.8e-16) and bone mass in terms of T score (-0.6 vs 0.2; p=1.3e-05), BMC (2133.4 vs 2454.4; p=3.2e-08) and BMD (1.05 vs 1.13; p=4.7e-07), but higher lean mass in terms of LM (47.1 vs 42.1; p=4.4e-07), LMI (18.6 vs 16.2; p=2.3e-16), ALMI (8.0 vs 6.8; p<2e-16) and SMI (0.25 vs 0.21; p<2e-16) (**Table 3.4 A**).

The distribution of males within the six cluster is divided as follow: the 46.5% in OWB and 24.3 % in NW, while OWA represents the 4.0%, LOA 6.8%, LOB 16.0 % and MO 2.4%.

As shown for female, also in male subjects the increase of BMI coincides with an increase in fat mass, in terms of FM, FMI and FM/LM markers, and no correlation with lean and bone masses emerged. (**Table 3.4 B**).

Moreover, clusters with similar BMI such as OWA (BMI= 25.7 kg/m2) and OWB (BMI=26.3 kg/m2) and LOA (BMI= 30.1 kg/m2) and LOB (BMI= 30.4 kg/m2) have a very different distribution of BC. Comparing the two overweight groups, OWA with respect to OWB have lower fat mass in terms of FM (15.6 vs 22.2 kg; p=7.6e-06), FMI (5.1 vs 7.5 kg/m2; p=1.3e-06) and FM/LM (0.2 vs 0.4; p=2.7e-10), but higher lean mass in terms of LM (61.8 vs 54.0 kg; p=3.9e-06), LMI (20.3 vs 18.2 kg/m2; p=8.2e-08), ALMI (9.1 vs 8.1 kg/m2; p=1.1e-07) and SMI (0.4 vs 0.3; p=4.0e-09) and bone mass in terms of T score (1.9 vs -0.4; p=4.1e-13), BMC (3576.2 vs 2891.4 g; p=4.4e-09) and BMD (1.4 vs 1.2 g/cm2; p=4.7e-13). Comparing the two low obesity groups, LOA with respect to LOB have lower fat mass in terms of FM (23.3 vs 31.5 kg; p=3.0e-09), FMI (7.8 vs 10.3 kg/m2; p=2.2e-07) and FM/LM (0.4 vs 0.5; p=9.8e-14) and bone mass in terms of T-score (-0.5 vs 0.7; p=1.4e-07) and BMC (2791.9 vs 3391.6 g; p=1.0e-09) but higher and BMD (1.6 vs 1.3; p=8.2e-07) and lean mass in terms of LM (65.8 vs 59.5 kg; p=5.2e-07), LMI (22.0 vs 19.3 kg/m2; p=6.3e-12), ALMI (9.8 vs 8.5 kg/m2; p=1.8e-10) and SMI (0.32 vs 0.28; p=1.8e-11) (**Table 3.3 B**).

Among the six clusters identified in males the MO group (BMI= 35.5 kg/m2) have the highest values for fat mass comparing to other five clusters (FM= 42.4 kg; FMI= 13.9 kg/m2; FM/LM=0.6) but also the highest values for some lean and bone mass markers (LM=67.3 kg; and BMC= 3667.6 g) (**Table 3.3 B**).

SMI and BMD do not discriminate very much among the clusters both in males and females compared to the other BC markers.

Metabolic profile across the BC clusters

Several metabolic parameters were identified and compared among female and males' clusters to better understand their characteristics (**Table 3.5**).

In females, significant differences emerged among the five clusters. In particular, the highest levels of HDL cholesterol, and the lowest ones of triglycerides, glycated haemoglobin, glucose, insulin, HOMA (IR and β), urea and diastolic pressure were reported in NW cluster.

The female cluster with the highest BMI (LOB) shows the lowest level of calorie intake in comparison of LOA that has the highest one, in addition, LOB cluster have the highest values of triglycerides, glycated haemoglobin, glucose, insulin, HOMA (IR and β), urea and diastolic pressure. Instead, no significant differences among clusters emerged for adherence to the NU-AGE diet and the circulating levels of total cholesterol and LDL, albumin, creatinine, 25(OH)D, PTH and systolic blood pressure among the five clusters (**Table 3.4 A**).

In addition to the metabolic parameters, other variables that may impact the metabolic profile were analyzed, such as the number of subjects using drugs for the control of cholesterol, glucose, blood pressure and supplementation of calcium and vitamin. Significant differences emerged in in the percentage of females taking hypertensive drugs (62.2% in LOB), this may explain the similar values in systolic blood pressure across the five clusters. Moreover, differences emerged in clusters with similar BMI, LOA and LOB with 34.4% and 62.2% of subjects taking anti-hypertensive drugs, respectively. Despite the similar BMI, the percentage of females taking anti-hypertensive drugs is higher in the cluster with higher fat markers. The LOA cluster shows the lowest percentage of female taking vitamin D3 supplementation (10 mg/day) (13.11%) and calcium

supplementation (3.3%) while the highest values were reported in OWB cluster (25.5%) and LOB cluster (18.3%), respectively (**Table 3.4 A**).

Handgrip strength and PASE score were used to evaluate differences in the physical functioning. LOA cluster shows the highest values for the handgrip strength test (**Table 3.4 A**). This cluster is indeed characterized by the highest values for lean mass markers (FMI, LMI, ALMI) (**Table 3.3 A**). The PASE score is highest in the NW and lowest one in the LOB cluster (**Table 3.4 A**).

While in female clusters no significant difference emerged for adherence to the NU-AGE diet, the NW male cluster shows the highest adherence in comparison to others. In addition, the NW cluster have the highest levels of HDL cholesterol and the lowest ones of triglycerides, glucose, insulin, HOMA IR and urea. As the LOB female cluster, males within the cluster with the highest BMI (MO) showed the lowest levels of calorie intake. Moreover, MO cluster have the lowest levels of HDL cholesterol and the highest ones of triglycerides, glucose, insulin, HOMA IR, HOMA β and urea. Males in the OWA cluster have the lowest levels of HOMA β. The highest and the lowest levels of albumin are found within the LOA and LOB clusters respectively, while the highest and the lowest levels of PTH are found within the OWB and OWA clusters, respectively.

Instead, no significant differences among clusters emerged for the circulating levels of total cholesterol and LDL, glycate haemoglobin, creatinine, 25(OH)D, diastolic and systolic blood pressure (**Table 3.4 B**).

Like for female clusters, also among the six clusters no significant differences emerged in the percentage of elderly taking statins and drugs for the reduction of glycemia, while all the males within the MO cluster were using anti-hypertensive drugs. This could explain the similar values in systolic and diastolic blood pressure across the six clusters. In addition, clusters with similar BMI have different percentage of males taking anti-

hypertensive drugs: LOA cluster have 46.7% instead of LOB cluster with 63.7%, while OWA and OWB shows 35.0% and 51.9% respectively. Also in this case, the percentage of mlaes taking anti-hypertensive drugs is higher in the cluster with higher fat markers. None of the males in MO cluster was taking vitamin D supplementation but no significant difference emerged among clusters. Moreover, no males within the LOB and MO clusters was taking calcium supplementation, while the higher percentage (15%) of subjects used calcium supplementation was in OWA cluster (**Table 3.4 B**). Despite no differences emerged among the six clusters for the levels of vitamin D (supplementation and also serum 25(OH)D level), it is interesting to note that OWA, LOB and MO have the highest values for bone mass markers (T-score and BMC) (**Table 3.3 B**). Males within the LOA cluster have the highest values for the handgrip strength test (**Table 3.4 A**). Indeed, this cluster is characterized by the highest values for some lean mass markers (LMI and ALMI) (**Table 3.3 B**). While the PASE score is highest in the OWA and lowest in the LOB cluster (**Table 3.4 B**).

Figure 3.2 shows the comparison of each metabolic parameter between clusters. When comparing cluster with similar BMI (OWA vs OWB, LOA vs LOB) no significant difference emerged for all the metabolic parameters, with the exception of values for triglyceride (**Figure 3.2 A, panel B**) that resulted higher in female LOB with respect to LOA; and values for albumin (**Figure 3.2 B, panel H**), that resulted higher in male LOA with respect to LOB. As expected, all the metabolic parameters analyzed are within normal range (see footnotes in **Table 3.4**), since in the NU-AGE study all participants were healthy elderly, except triglycerides and urea levels within the MO cluster and glucose and systolic blood pressure in the LOA, LOB and MO clusters in males (**Table 3.4 A and B**).

Distribution of the BC clusters per Country

Figure 3.3 shows the percentage of subjects of each country within each BC cluster for female and males.

The NW and OWA female clusters are mainly composed of Dutch (32% and 28% respectively) and English subjects (29% and 24% respectively). While the highest percentage of Italians (38%) is in OWB cluster followed by the English subjects (26%); in the LOA cluster the majority of subjects comes from UK (39%) and France (30%) while the LOB cluster is represented for the 51% by Polish subjects followed by a 20% of Dutch subjects (**Figure 3.3 A**).

The NW male cluster is mainly composed by English (33%) and French (30%) subjects while the OWA cluster is represented by Dutch (44%) Polish (21%) and French (21%) subjects, within the OWB cluster the majority of subjects are Italians (37%) and Polish (24%), the majority of English (42%) and French (46%) subjects belong to the LOA cluster, the LOB cluster is mainly composed by Italians (31%), Dutch (25%) and Polish (25%), the MO cluster is mainly composed by Dutch (33%) and Polish (33%) subjects equally (**Figure 3.3 B**).

3.4 CONCLUSIONS

The aim of this study is to define a BC profile in the elderly across Europe. The 1121 elderly participants to the European project NU-AGE have been thoroughly studied for their dietary intake (Berendsen et al., 2014) and their anthropometric, metabolic, physical and cognitive status (Santoro et al., 2014), in particular DXA scan was assessed to evaluate their BC in terms of fat, lean and bone mass.

Due to significant aging-related depletion of sex hormones such as rapid loss of estradiol and progesterone in women after menopause, it has been thought that in elderly BC of women would become more similar to men. On the contrary, many results (Lauretta et al., 2017), including ours, demonstrate that there is a great difference among BC in elderly women and men. In fact, female aged 65 or more years old tend to have higher fat mass, in particular in the gynoid region, but lower lean and bone mass than males aged same. As anticipated, what makes women different from men is represented by sex hormones, i.e. estrogen, progesterone and testosterone, and given the increase in older people, there is a great interest in understanding the complex interrelationships between increasing age and hormonal regulation.

Age-associated endocrine changes comprise the decline of basal hormonal levels, pulsatile hormone distribution, and activity of hormonal axis, which result in changes in body composition. Men and women experience different age-associated alterations of the hormonal system: significant decrease in testosterone and loss of estradiol and progesterone, respectively. As mentioned in introduction, there is a strong association between Aging and several diseases like osteoporosis, diabetes mellitus type 2, frailty, and sarcopenia (Lauretta et al., 2017) and gender-specific differences with respect to symptoms, interactions, diagnosis, and therapy must be taken into consideration.

As mentioned, sarcopenia is closely related to age, and is defined by loss of muscle mass and strength, and associated with chronic disease, sarcopenic obesity, and prolonged immobilization (Rosenberg, 1997). In addition, a reduction in anabolic hormones plays a key role in the development and maintenance of sarcopenia. In particular, it has been shown that testosterone reduction in older men plays a key role, consequently it is useful to administer testosterone in hypogonadal men in order to reduce muscle strength loss (Lauretta et al., 2017). In contrast, there is no evidence of an association between decreased estrogen and loss of muscle mass in women. Another gender difference can be found in the pathophysiology of osteoporosis. In fact, women tend to have a very rapid reduction in bone content at a younger age than men, who have higher bone density and content and develop principles of osteoporosis at a later age. In addition, women have a more pronounced decrease in hormone production than men, as estrogen plays an important role in bone health this aspect may be linked to the presence of fractures 5-10 years earlier in women than men (Elmer and al., 2017).

Our results showed also geographic differences in BC across the 5 countries of NU-AGE project. Overall, French participants have the highest values of lean mass markers and the lower of fat mass compared with participants from the other countries (Italy, the Netherlands, Poland, and the United Kingdom). In contrast, Polish participants have the higher values of fat mass markers while the highest value of bone mass markers are reported for Dutch elderly. These differences among the 5 countries could be attributed to genetic predisposition, dietary habits, lifestyle or physical activity, stress or education, because the criteria for exclusion and inclusion in the study were the same for all countries (Santoro et al., 2014). The French elderly are found to be the most adherent to the NU-AGE diet, although they have a higher caloric intake than other countries. However, there is evidence that French subjects consume more fish and

low-fat meat than others (Berendsen et al., 2018), which may contribute to higher values of lean mass than other countries. Despite the higher presence of lean mass value, the French subjects do not show the highest values for physical activity, PASE score and handgrip strength. In contrast, Polish, who have the highest levels of fat mass, have higher intakes of whole grains, eggs, vegetable and low-fat cheese (Berendsen et al., 2018), also show the lowest values for handgrip strength. Obesity rates in the European adult population (18-75 years old) varies by country; Romania, Italy Netherlands, Belgium, and Sweden have the lowest rates of obesity (9.4%, 10.7%, 13.3%, and 14%, respectively) while the highest rates are in Malta (26%), Latvia (21.3%), Hungary (21.2%), Estonia (20.4%), and the United Kingdom (20.1%) (Eurostat, 2016). In addition, an increase in obesity rates has been shown in European countries between 2010 and 2014, with the exception of Italy (Blundell et al., 2017) and it is affected by aging. In fact, a 2016 Eurostat study shows that the obesity rate in those aged 65-74 is 22.5% for France, 15.7% for Italy, 17.7% for the Netherlands, 28.4% for Poland, and 20.7% for the United Kingdom. These data are partially confirmed by our results, in fact Polish subjects are found to have more fat mass than other countries considered in the study, as opposed to French subjects who have more lean mass. This could be explained by the type of measurement, self-reported BMI in the Eurostat study and standardized and accurate measures such as DXA in the NU-AGE project. Though BMI has always been considered a valid tool to assess overall adiposity, it fails to distinguish between the relative contribution of fat mass and lean mass (Blundell et al., 2014). Moreover, different metabolic parameters were compared across BC clusters identified in elderly males and females. It is well known that an increase in fat mass, together with aging, can cause medical complications, such as hypertension, diabetes and cardiovascular disease. The inclusion and exclusion criteria in the NU-AGE project allowed to select healthy

subjects, as a result all metabolic parameters are within the range, although, as shown in the results, there are differences between clusters. In particular, subjects within the cluster with lowest BMI have the highest HDL cholesterol levels but the lowest triglycerides, glucose, insulin, HOMA, urea compared with the other clusters and females have the lowest level of diastolic pressure. While the clusters with higher BMI have the lowest HDL cholesterol levels but the highest triglycerides, glucose, insulin, HOMA (IR and β) and urea and diastolic pressure (only females) levels compared with the other clusters. The similar levels of cholesterol and LDL among the clusters may be explained by the fact that the number of subjects taking statins does not change among clusters. In addition, considering groups with similar BMI the percentage of subjects taking antihypertensive drugs is higher in the group with higher fat mass markers. The percentage of elderly taking vitamin D and calcium supplements is higher in the clusters with higher BMI who also have higher BMC and T-score values. Additionally, subjects with highest values of lean mass markers have also highest values of handgrip strength.

In conclusion, the results presented in this paper provide a synthesis of the health status of elderly subjects in Europe that can be used as a reference for studies related to gender differences in body composition, disease conditions, and differences between European countries. The study has some weaknesses, such as the fact that the subjects are healthy volunteers, highly educated and interested in nutrition and health issues and therefore may not be representative of the population of the same age. Although the weaknesses, there are also strengths. Indeed, the fact of using standardized and accurate tools to study body composition, i.e. DXA, at the European level is certainly important.

Table 3.2 | Characteristics of the NU-AGE participants by sex (N=1,121)

Characteristics	**Women** ***n = 620***	**Men** ***n = 501***	***p-value***	***q-value***
Age (*years*)	70.7 ± 3.9	71.0 ± 4.1	NS	NS
Weight *(kg)*	67.7 ± 11.2	80.6 ± 12.6	<2.2e-16	<2.2e-16
Height *(cm)*	160.0 ± 6.7	173.0 ± 6.4	<2.2e-16	<2.2e-16
BMI (kg/m^2)	26.5 ± 4.1	26.9 ± 3.7	1.16e-02	2.73e-02
Waist circumference (*cm*)	86.9 ± 10.8	96.7 ± 11.1	<2.2e-16	<2.2e-16
Hip circumference (*cm*)	103.3 ± 9.1	101.5 ± 7.6	1.32e-03	3.54e-03
Waist to Hip circumference ratio	0.85 ± 0.31	0.95 ± 0.06	<2.2e-16	<2.2e-16
Education				
Primary school, N (%)	25 (4.0)	12 (2.4)	NS	NS
Low Secondary school, N (%)	71 (11.5)	72 (14.4)		
Up Secondary school, N (%)	238 (38.4)	195(38.9)		
College, N (%)	286 (46.1)	222 (44.3)		
Education (years)	12.4 ± 3.4	13.0 ± 3.8	2.15e-02	NS
Diet Assessment				
Adherence to NU-AGE diet	52.5 ± 10.3	50.0 ± 9.3	6.80e-05	2.16e-04
Calorie Intake (*kcal*)	1680.9 ±327.8	2123.3 ± 445.0	<2.2e-16	<2.2e-16
Physical Functioning				
Hand grip strength (*kg*)	25.2 ± 5.5	39.6 ± 7.0	<2.2e-16	<2.2e-16
PASE Score	127.8 ± 48.9	140.9 ± 59.5	3.53e-04	1.01e-03

Body Composition parameters	**Women** *n = 620*	**Men** *n = 501*	*p-value*	*q-value*
FM (*kg*)	26.2 ± 8.06	22.0 ± 8.37	<2.2e-16	<2.2e-16
FMI (*kg/m²*)	10.3 ± 3.16	7.35 ± 2.74	<2.2e-16	<2.2e-16
FM/LM	0.65 ± 0.19	0.39 ± 0.14	<2.2e-16	<2.2e-16
LM (*kg*)	40.3 ± 4.97	57.1 ± 6.71	<2.2e-16	<2.2e-16
LMI (*kg/m²*)	15.7 ± 1.53	19.1 ± 1.80	<2.2e-16	<2.2e-16
ALMI (*kg/m²*)	6.56 ± 0.77	8.47 ± 0.87	<2.2e-16	<2.2e-16
SMI	0.25 ± 0.03	0.32 ± 0.04	<2.2e-16	<2.2e-16
T-score	-0.82 ± 1.2	-0.19 ± 1.2	<2.2e-16	<2.2e-16
BMC (*g*)	2092.5 ± 357	2947.8 ± 483	<2.2e-16	<2.2e-16
BMD (g/cm^2)	1.03 ± 0.11	1.19 ± 0.11	<2.2e-16	<2.2e-16
Android/Gynoid FM*	0.50 ± 0.15	0.78 ± 0.21	<2.2e-16	<2.2e-16
Android FM/LM*	0.79 ± 0.30	0.61 ± 0.25	2.70e-16	2.82e-15
l1 l4 BMD (g/cm^2) $^{\Delta}$	1.0 ± 0.17	1.17 ± 0.2	<2.2e-16	<2.2e-16
l1 l4 T-score $^{\Delta}$	-1.0 ± 1.4	-0.11 ± 1.65	2.74e-05	9.53e-05
Neck BMD (g/cm^2) $^{\Delta}$	0.78 ± 0.12	0.88 ± 0.14	<2.2e-16	<2.2e-16
Neck T-score$^{\Delta}$	-1.36 ± 0.93	-1.07 ± 0.9	<2.2e-16	<2.2e-16

BMI, body mass index; FM, fat mass; FMI, fat mass index; LM, lean mass; LMI, non-bone lean mass index; ALMI, non-bone appendicular lean mass index; SMI, skeletal mass index; BMC, bone mineral content; BMD, bone mineral density; Values are means ± SDs, unless otherwise stated. NS, not statistically significant. * (F=474, M=416); $^{\Delta}$ (F=387, M=298); p-value (Mann - Whitney and Kruskal - Wallis tests); q-value (Benjamini–Hochberg multiple testing correction).

Table 3.3 | Characteristics of the NU-AGE participants by country of origin (N=1,121)

Characteristics	**Italy** ***n = 236***	**Poland** ***n = 222***	**UK** ***n = 246***	**France** ***n = 184***	**The Netherlands** ***n = 233***	***p-value***	***q-value***
Age (*years*)	71.7 ± 3.8	71.3 ± 3.8	70.1 ± 3.9	70.1 ± 3.8	71.0 ± 4.1	1.31e-06	5.33e-06
Female sex	119 (50.4)	127 (57.2)	154(62.6)	91 (49.5)	129(55.1)	3.35e-02	NS
Weight (*kg*)	72.7 ± 12.7	75.7 ± 14.5	73.5 ± 13.5	70.0 ± 12.7	74.7 ± 13.4	1.65e-03	4.34e-03
Height (*cm*)	163.9 ± 9.4	163.9 ± 9.3	166.0 ± 9.0	166.0 ± 9.0	169.2 ± 8.2	8.59e-10	2.86e-09
BMI (kg/m^2)	27.0 ± 3.8	28.0 ± 4.1	26.6 ± 3.9	25.4 ± 3.4	26.0 ± 3.6	1.45e-11	1.05e-10
Waist circumference (*cm*)	92.8 ± 11.4	93.3 ± 11.8	91.4 ± 12.0	86.3 ± 11.4	91.6 ± 11.9	1.98e-08	1.03e-07
Hip circumference (*cm*)	101.4 ± 7.4	103.6 ± 8.7	104.7 ± 9.1	99.1 ± 8.5	103.2 ± 7.9	2.45e-12	1.10e-11
Waist to Hip circumference ratio	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	4.75e-09	
Education							
Primary school N, (%)	25 (10.6)	0 (0.0)	1 (0.4)	4 (2.2)	7 (3.0)		
Low Secondary school N, (%)	61 (25.9)	8 (3.6)	0 (0.0)	46 (25.0)	28 (12.0)		
Up Secondary school N, (%)	94 (39.8)	41 (18.5)	88 (35.8)	60 (32.6)	149 (64.0)	<2.2e-16	<2.2e-16
College N, (%)	56 (23.7)	172 (77.5)	157 (63.8)	74 (40.2)	49 (21.0)		
Education (years)	11.2 ± 4.2	15.4 ± 2.7	11.8 ± 1.7	12.5 ± 3.7	12.3 ± 3.7	<2.2e-16	<2.2e-16
Diet Assessment							
Adherence to NU-AGE diet	52.8 ± 9.5	52.7 ± 10.1	50.5 ± 8.8	55.9 ± 9.1	46.3 ± 9.8	<2.2e-16	<2.2e-16
Calorie Intake (*kcal*)	1733 ± 376	1850 ± 518	1903 ± 389	2024 ± 482	1912 ± 405	1.60e-09	9.62e-09
Physical Functioning							
Handgrip strength (*kg*)	31.1 ± 9.7	30.4 ± 9.9	34.8 ± 9.1	31.1 ± 8.8	30.5 ± 9.3	3.53e-07	1.52e-06
Women	23.5 ± 5.3	23.7 ± 4.6	29.7 ± 5.4	23.9 ± 4.1	23.8 ± 4.8	<2.2e-16	<2.2e-16
Men	38.9 ± 6.6	39.3 ± 7.7	43.4 ± 7.3	38.2 ± 5.8	38.6 ± 6.6	1.00e-05	3.56e-05
PASE Score	114.5± 50.9	131.7 ± 63.6	151.2 ± 53.0	134.9 ± 50.6	137.2 ± 52.6	2.52e-13	2.31e-12

Body Composition parameters	**Italy** *n = 236*	**Poland** *n = 222*	**UK** *n = 246*	**France** *n = 184*	**The Netherlands** *n = 233*	*p-value*	*q-value*
FM (*kg*)	26.2 ± 7.4	28.0 ± 9.1	23.3 ± 7.9	20.4 ± 7.4	23.2 ± 8.5	<2.2e-16	<2.2e-16
FMI (*kg/m²*)	9.9 ± 3.0	10.5 ± 3.5	8.5 ± 3.1	7.5 ± 2.9	8.2 ± 3.1	<2.2e-16	<2.2e-16
FM/LM	0.60 ± 0.19	0.64 ± 0.24	0.50 ± 0.18	0.42 ± 0.17	0.49 ± 0.20	<2.2e-16	<2.2e-16
LM (*kg*)	45.0 ± 8.7	45.6 ± 10.3	48.8 ± 10.4	50.8 ± 10.6	49.2 ± 9.9	7.45e-11	4.96e-10
LMI (*kg/m²*)	16.6 ± 2.0	16.7 ± 2.3	17.6 ± 2.5	18.3 ± 2.3	17.0 ± 2.2	1.68e-12	1.39e-11
ALMI (*kg/m²*)	7.4 ± 1.2	7.1 ± 1.1	7.5 ± 1.3	7.9 ± 1.3	7.2 ± 1.2	3.79e-09	2.13e-08
SMI	0.28 ± 0.03	0.26 ± 0.04	0.28 ± 0.05	0.32 ± 0.05	0.28 ± 0.05	<2.2e-16	<2.2e-16
T-score	-0.69 ± 1.15	-0.19 ± 1.25	-0.92 ± 1.23	-0.58 ± 1.23	-0.29 ± 1.21	3.90e-12	3.17e-11
BMC (*g*)	2463 ± 597	2610 ± 615	2233 ± 494	2327 ± 489	2729 ± 621	<2.2e-16	<2.2e-16
BMD (*g/cm²*)	1.07 ± 0.15	1.15 ± 0.12	1.06 ± 0.13	1.11 ± 0.12	1.14 ± 0.12	<2.2e-16	<2.2e-16
Android/Gynoid FM*	0.65 ± 0.22	0.63 ± 0.21	-	0.54 ± 0.20	0.67 ± 0.24	9.06e-08	4.25e-07
Android FM/LM*	0.77 ± 0.28	0.85 ± 0.28	-	0.45 ± 0.18	0.70 ± 0.25	<2.2e-16	<2.2e-16
l1 l4 T-score$^{\Delta}$	-0.84 ± 1.38	-0.36 ± 1.78	-0.63 ± 1.53	-	-	4.14e-02	NS
l1 l4 BMD (g/cm^2)$^{\Delta}$	1.10 ± 0.17	1.15 ± 0.22	0.97 ± 0.18	-	-	<2.2e-16	<2.2e-16
Neck T-score$^{\Delta}$	-1.39 ± 0.95	-1.19± 0.92	-1.12 ± 0.96	-	-	2.92e-02	NS
Neck BMD (g/cm^2)$^{\Delta}$	0.85 ± 0.13	0.89 ± 0.13	0.75 ± 0.12	-	-	<2.2e-16	<2.2e-16

BMI, body mass index; FM, fat mass;FMI, fat mass index; LM, lean mass;LMI, non-bone lean mass index; ALMI, non-bone appendicular lean mass index; SMI, skeletal mass index; BMC, bone mineral content; BMD, bone mineral density; Values are means ± SDs, unless otherwise stated. NS, not statistically significant. * (N=875); $^{\Delta}$ (N=704); p-value (Mann - Whitney and Kruskal - Wallis tests); q-value (Benjamini–Hochberg multiple testing correction).

Table 3.4 A | Five body composition groups identified by a cluster analysis performed on ten BC parameters and BMI in women (N=620)

Clusters	BMI (kg/m²)	FM (kg)	FMI (kg/m²)	LM (kg)	LMI (kg/m²)	ALMI (kg/m²)	FM/LM	SMI	T score	BMC (g)	BMD (g/cm²)
Normal weight (NW; n = 89; 14.4%)	21.4± 1.7	15.9± 3.4	6.1± 1.3	38.4± 3.1	14.9± 1.1	6.2± 0.6	0.4± 0.1	0.29± 0.03	-1.4± 1.0	1905.1± 230.9	1.0± 0.1
Overweight A (OWA; n = 251; 40.5%)	25.1± 1.9	23.8± 4.0[1]	9.2± 1.5[1]	40.4± 4.1[1]	15.5± 1.1[1]	6.4± 0.5	0.6± 0.1[1]	0.26± 0.02[1]	-0.4± 1.0[1]	2190.4± 294.9[1]	1.1± 0.1[1]
Overweight B (OWB; n = 137; 22.1%)	26.6± 2.7	26.9± 5.9	10.9± 2.3	37.2± 3.7	15.1± 1.0	6.3± 0.6	0.7± 0.2	0.24± 0.02	-1.9± 0.8	1804.1± 249.5	0.9± 0.1
Low Obesity A (LOA; n = 61; 9.8%)	31.5± 4.1	32.7 ±6.4[a]	12.9 ± 2.6[a]	47.1± 5.8 [a]	18.6 ±1.7[a]	8.0± 0.8 [a]	0.7± 0.1[a]	0.25± 0.02 [a]	-0.6± 1.5[a]	2133.4± 378.2[a]	1.1± 0.1 [a]
Low Obesity B (LOB; n = 82; 13.2%)	31.9± 2.4	38.5± 5.4	14.9± 2.0	42.1± 4.4	16.2± 1.1	6.8± 0.6	0.9± 0.1	0.21± 0.02	0.2± 0.8	2454.4± 297.3	1.1± 0.1

Table 3.4 B| Six body composition groups identified by a cluster analysis performed on ten BC parameters and BMI in men (N=501)

Clusters	BMI (kg/m²)	FM (kg)	FMI (kg/m²)	LM (kg)	LMI (kg/m²)	ALMI (kg/m²)	FM/LM	SMI	T score	BMC (g)	BMD (g/cm²)
Normal weight (NW; n = 122; 24.4%)	24.0± 2.1	13.7± 4.2	4.6± 1.4	57.0± 5.5	19.2± 1.4	8.5± 0.7	0.2± 0.1	0.36± 0.03	-0.9± 1.0	2631.9±4 18.8	1.1± 0.1
Overweight A (OWA; n = 20; 4.0%)	25.7± 2.8	15.6± 5.3[1]	5.1± 1.6[1]	61.8± 6.6[1]	20.3± 1.3[1]	9.1± 0.6[1]	0.2± 0.1[1]	0.36± 0.03[1]	1.9± 0.6[1]	3576.2±4 01.1[1]	1.4± 0.1[1]
Overweight B (OWB; n = 233; 46.5%)	26.3± 2.3	22.2± 5.3	7.5± 1.8	54.0± 5.3	18.2± 1.3	8.1± 0.7	0.4± 0.1	0.31± 0.02	-0.4± 0.9	2891.4±3 31.9	1.2± 0.1
Low Obesity A (LOA; n = 34; 6.8%)	30.1± 1.6	23.3± 4.8[a]	7.8 ± 1.5[a]	65.8± 5.2 [a]	22.0± 1.1 [a]	9.8± 0.6 [a]	0.4± 0.1 [a]	0.32± 0.03 [a]	-0.5± 0.9 [a]	2791.9±3 47.2 [a]	1.6± 0.1 [a]
Low Obesity B (LOB; n = 80; 16.0%)	30.4± 2.9	31.5± 5.4	10.3± 1.9	59.5± 5.8	19.3± 1.6	8.5± 0.8	0.5± 0.1	0.28± 0.02	0.7± 1.1	3391.6±4 32.8	1.3± 0.1
Moderate Obesity (MO; n = 12; 2,3%)	36.6± 2.9	42.4± 5.1	13.9± 1.5	67.3± 7.8	21.9± 2.0	8.5± 1.1	0.6± 0.1	0.26± 0.03	1.6± 1.2	3667.6±6 37.3	1.3± 0.1

Values are expressed as mean values ± SD, unless otherwise indicated.

[1,] Significant difference between OWA and OWB ([1]: p<0.0001)

[a,] Significant difference between LOA and LOB ([a]: p<0.0001)

Table 3.5 A | Metabolic profile across the five body composition clusters in women.

	Normal weight (n = 89)	Overweight A (n = 251)	Overweight B (n = 137)	Low Obesity A (n = 61)	Low Obesity B (n = 82)	p-value
Adherence to NU-AGE diet	54.9 ± 11.0	52.1 ± 10.3	51.6 ± 9.0	54.1 ± 9.6	51.7 ± 11.7	NS
Calorie Intake (kcal)	1723.6 ± 286.6	1722.8 ± 332.2	1608.1 ± 308.1	1736.5 ± 358.1	1587.7±330.8	2.38E-04
Total Cholesterol (mg/dL)	230.9 ± 40.3	220.5 ± 39.8	222.5 ± 37.2	217.3 ± 40.6	214.3 ± 39.9	NS
HDL (mg/dL)	76.3 ± 19.8	66.9 ± 39.8	66.1 ± 16.5	60.1 ± 14.8	59.6 ± 16.9	4.72e-09
LDL (mg/dL)	136.8 ± 39.6	132.7 ± 36.4	136.2 ± 33.7	137.3 ± 38.9	129.9 ± 35.0	NS
Triglycerides (mg/dL)	89.1 ± 31.9	104.4 ± 45.7	100.9 ± 36.3	99.5 ± 33.1	123.7 ± 55.0	3.02e-05
Glycated Haemoglobin (%)	5.7 ± 0.3	5.7 ± 0.3	5.7 ± 0.4	5.8 ± 0.4	5.9 ± 0.4	7.117e-03
Glucose (mmol/L)	5.2 ± 0.7	5.4 ± 0.6	5.5 ± 0.7	5.8 ± 1.1	5.9 ± 0.9	7.27e-09
Insulin (mcU/mL)	6.1 ± 3.4	8.4 ± 5.7	7.8 ± 3.8	11.3 ± 6.4	12.3 ± 6.3	2.95e-16
HOMA IR	1.5 ± 0.9	2.1 ± 1.6	1.9 ± 1.0	3.0 ± 2.0	3.3 ± 1.8	< 2.2e-16
HOMA β (%)	74.3 ± 37.1	90.9 ± 56.5	84.3 ± 46.6	100.3 ± 49.8	109.1 ± 61.2	6.58e-05
Urinary Urea (g/24h)	16.9 ± 4.5	17.9 ± 5.1	16.8 ± 4.7	20.0 ± 4.9	20.1 ± 5.2	9.56e-08
Albumin (g/L)	45.7 ± 4.4	45.5 ± 4.2	44.7 ± 3.6	45.9 ± 4.1	44.9 ± 3.2	NS
Creatinine (mmol/L)	68.6 ± 11.9	69.8 ± 12.5	67.8 ± 11.7	71.4 ± 12.5	70.5 ± 10.3	NS
25(OH)D (ng/mL)	26.8 ± 10.2	25.7 ± 8.9	24.9 ± 10.6	23.8 ± 9.4	23.3 ± 7.5	NS
PTH (pg/mL)	46.7 ± 32.1	40.3 ± 24.0	47.4 ± 28.9	41.9 ± 22.2	43.9 ± 22.8	NS
Diastolic pressure (mmHg)	70.6 ±8.9	72.9 ± 10.0	75.3 ± 10.4	77.2±9.9	75.7 ± 8.1	5.418e-05
Systolic pressure (mmHg)	132.7 ±21.3	136.3± 19.9	138.3 ±21.8	138.9±20.5	139.9± 20.6	NS
Use of medicines/ supplements						
Statins (n=155; %)	18.0	25.9	20.4	29.5	34.1	NS
Diabetics (n=16; %)	1.1	2.4	0.7	4.9	6.1	NS
Hypertension (n=265; %)	23.6	43.4	46.0	34.4	62.2	8.035e-06
Vitamin D (n=139; %)	22.5	23.5	25.5	13.11	20.7	< 2.2e-16
Calcium (n= 78; %)	11.2	12.7	13.9	3.3	18.3	< 2.2e-16
Physical Functioning						
Handgrip strength (kg)	25.0 ± 5.7	25.4 ± 5.4	24.1 ± 5.9	26.7 ± 6.1	25.4 ± 4.7	2.529e-02
PASE score	141.2 ± 43.7	132.2 ± 48.6	125.3 ±46.4	127.9 ± 52.6	104.2 ± 48.7	4.588e-06

Table 3.5 B | Metabolic profile across the five body composition clusters in men.

	Normal weight (n = 122)	Overweight A (n = 20)	Overweight B (n = 233)	Low Obesity A (n = 34)	Low Obesity B (n = 80)	Moderate Obesity (n = 12)	p-value
Adherence to NU-AGE diet	51.7 ± 9.2	50.9 ± 12.1	50.3 ± 9.1	49.2 ± 8.0	47.2 ± 9.7	49.5 ± 5.9	2.39e-02
Calorie Intake (kcal)	2329.0 ± 425.3	2502.5 ± 390.2	2009.2 ± 408.9	2207.1 ± 458.7	2040.8 ±446.3	1958.1 ± 244.4	7.54e-12
Cholesterol (mg/dL)	194.4 ± 33.6	195.2 ± 40.1	194.5 ± 39.3	189.5 ± 36.0	193.5 ± 41.8	199.8 ± 42.1	NS
HDL (mg/dL)	57.9 ± 16.1	53.3 ± 14.4	50.9 ± 13.7	50.1 ± 13.9	44.8 ± 12.1	40.6 ± 10.2	4.32e-09
LDL (mg/dL)	119.2 ± 30.3	121.6 ± 36.4	122.0 ± 35.4	117.4 ± 28.3	124.8 ± 36.5	132.7 ± 42.1	NS
Triglycerides (mg/dL)	86.3 ± 33.7	101.6 ± 46.9	108.0 ± 50.0	109.4 ± 49.8	120.3 ± 51.7	132.7 ± 36.2	6.95e-08
Glycated Haemoglobin (%)	5.7 ± 0.4	5.7 ± 0.2	5.8 ± 0.5	5.8 ± 0.7	5.8 ± 0.7	5.9 ± 0.3	NS
Glucose (mmol/L)	5.6 ± 0.8	5.8 ± 0.9	5.9 ± 0.9	6.0 ± 1.1	6.1 ± 1.0	6.3 ± 0.7	3.78e-05
Insulin (mcU/mL)	6.3 ± 3.7	6.9 ± 2.7	9.6 ± 6.4	14.1 ± 14.1	14.1 ± 8.8	22.2 ± 10.7	< 2.2e-16
HOMA IR	1.6 ± 1.1	1.8 ± 0.8	2.6 ± 2.0	3.9 ± 4.0	3.9 ± 2.6	6.4 ± 3.5	< 2.2e-16
HOMA β (%)	67.4 ± 47.0	65.8 ± 24.8	85.8 ± 53.8	112.7 ± 98.7	117.4 ± 77.3	159.7 ± 76.8	1.42e-09
Urinary Urea (g/24h)	22.6 ± 6.0	24.0 ± 4.0	22.7 ± 6.1	25.5 ± 6.8	24.6 ± 7.6	31.6 ± 12.6	1.47e-03
Albumin (g/L)	45.9 ± 4.8	47.2 ± 4.8	45.4 ±3.6	47.6 ± 4.5	44.3 ± 3.4	44.7 ± 3.5	4.83e-03
Creatinine (mmol/L)	88.6 ± 4.8	88.5 ± 17.2	90.3 ± 17.3	92.4 ± 17.2	89.2 ± 11.6	92.9 ± 27.4	NS
25(OH)D (ng/mL)	25.6 ± 8.7	24.7 ± 7.0	24.3 ± 8.5	23.4 ± 8.7	22.4 ± 8.4	25.2 ± 7.1	NS
PTH (pg/mL)	38.7 ± 27.1	40.0 ± 28.4	46.3 ± 22.5	37.1 ± 19.1	45.9 ± 22.0	46.1 ± 20.7	1.98e-03
Diastolic	77.4 ± 10.4	76.6 ± 8.2	76.2 ± 10.4	81.3± 7.6	77.7± 10.2	77.6± 8.1	NS
Systolic	134.9 ± 17.8	138.8 ±15.2	138.8± 18.2	142.3±16.7	142.7 ±17.6	141. 6 ±16.9	NS
Use of medicines/ supplements							
Statins (n=130; %)	20.5	15.0	28.3	32.3	30.0	25.0	NS
Diabetics (n=26; %)	3.2	0	10.7	2.9	11.3	8.3	NS
Hypertension (n=242; %)	30.3	35.0	51.9	46.7	63.7	100.0	4.223e-08
Vitamin D (n=26; %)	4.1	5.0	5.6	5.9	6.3	0.0	NS
Calcium (n=15; %)	1.6	15.0	3.9	2.9	0.0	0.0	2.869e-02
Physical Functioning							
Handgrip strength (kg)	39.6 ± 6.3	41.8 ± 7.5	38.2 ± 6.8	41.9±7.9	41.3 ± 7.1	44.4 ± 9.1	5.653e-04
PASE score	153.8 ± 58.1	157.4 ± 65.1	139.3 ±64.6	150.6 ± 65.2	125.2 ± 54.6	130.9 ± 61.2	2.895e-02

Population based reference ranges for: Total Cholesterol: <200mg/dL ; HDL: > 60mg/dL; LDL: <100 mg/dL; Triglycerides: <150 mg/d; Glycated Haemoglobin: < 7.5%;Glucose (serum): 4.1-5.9 mmol/L; Insulin: 2-25 mcU/ml; HOMA IR: 0.23-2.5; HOMA β (%): 0-100; Urinary Urea:10-30 g/24h; Albumin (serum): 32-49 g/L; Creatinine (serum): 50-120 mmol/L; 25(OH)D (serum): 30-100 (ng/mL); PTH (serum): 10-70 pg/mL; Diastolic pressure: <90 mmHg; Systolic pressure: < 140 mmHg; p value (chi squared test).

Figure 3.2 A | Box-plots and significant differences of metabolic parameters among clusters in women. Statistical analysis was perfomed by Kruskal - Wallis test (p-values: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)

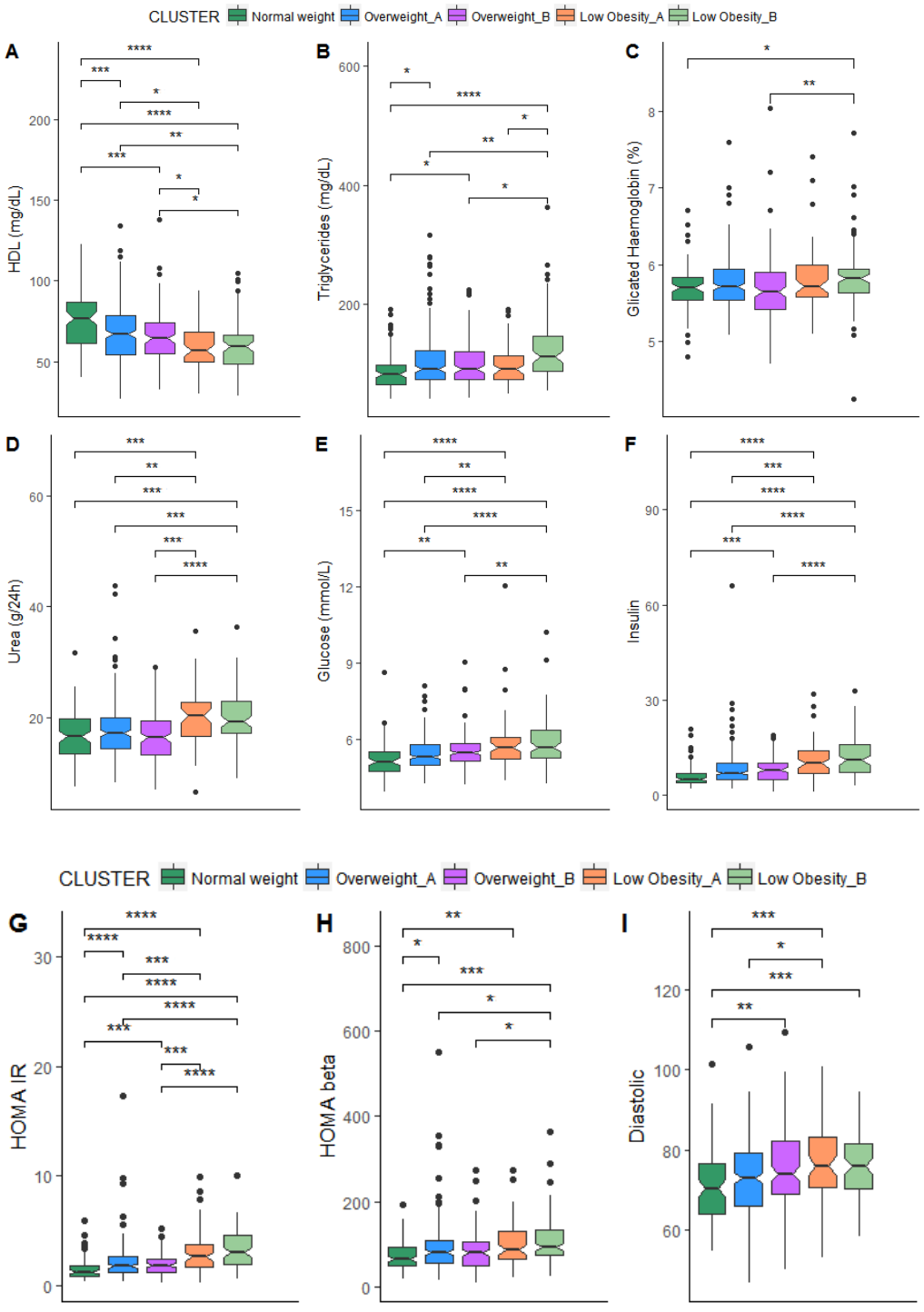


Figure 3.2 B | Box-plots and significant differences of metabolic parameters among clusters in men. Statistical analysis was perfomed by Kruskal - Wallis test (p-values: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)

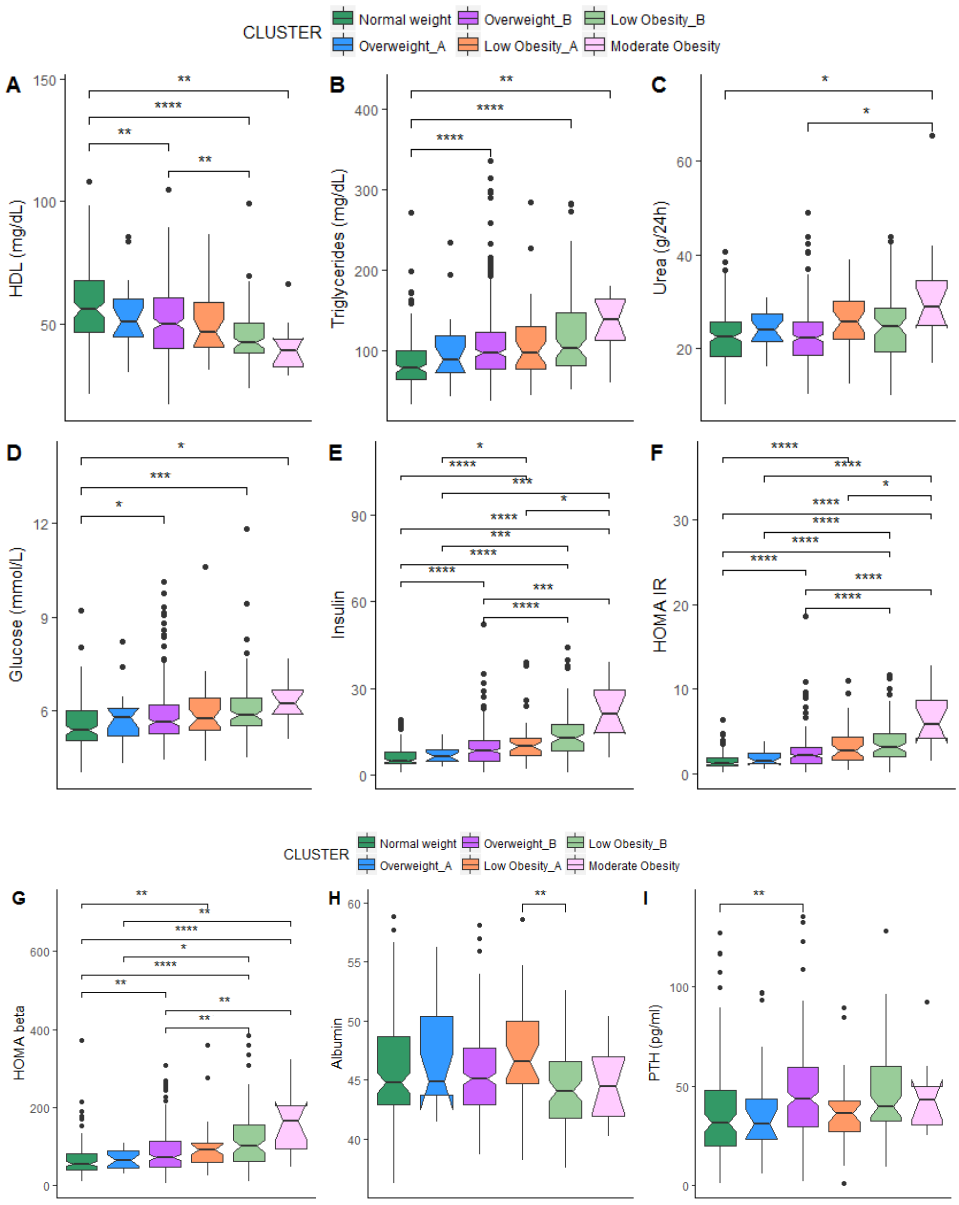


Figure 3.3 A | Percentage contribution of the countries to the clusters in female.

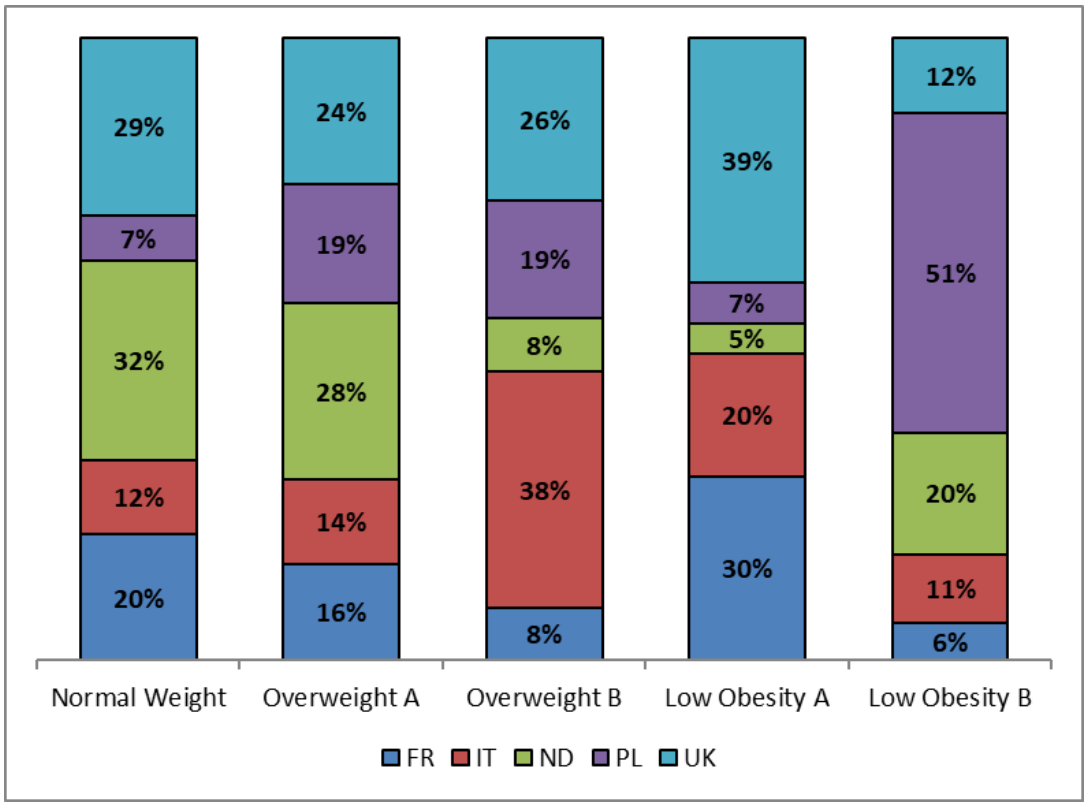
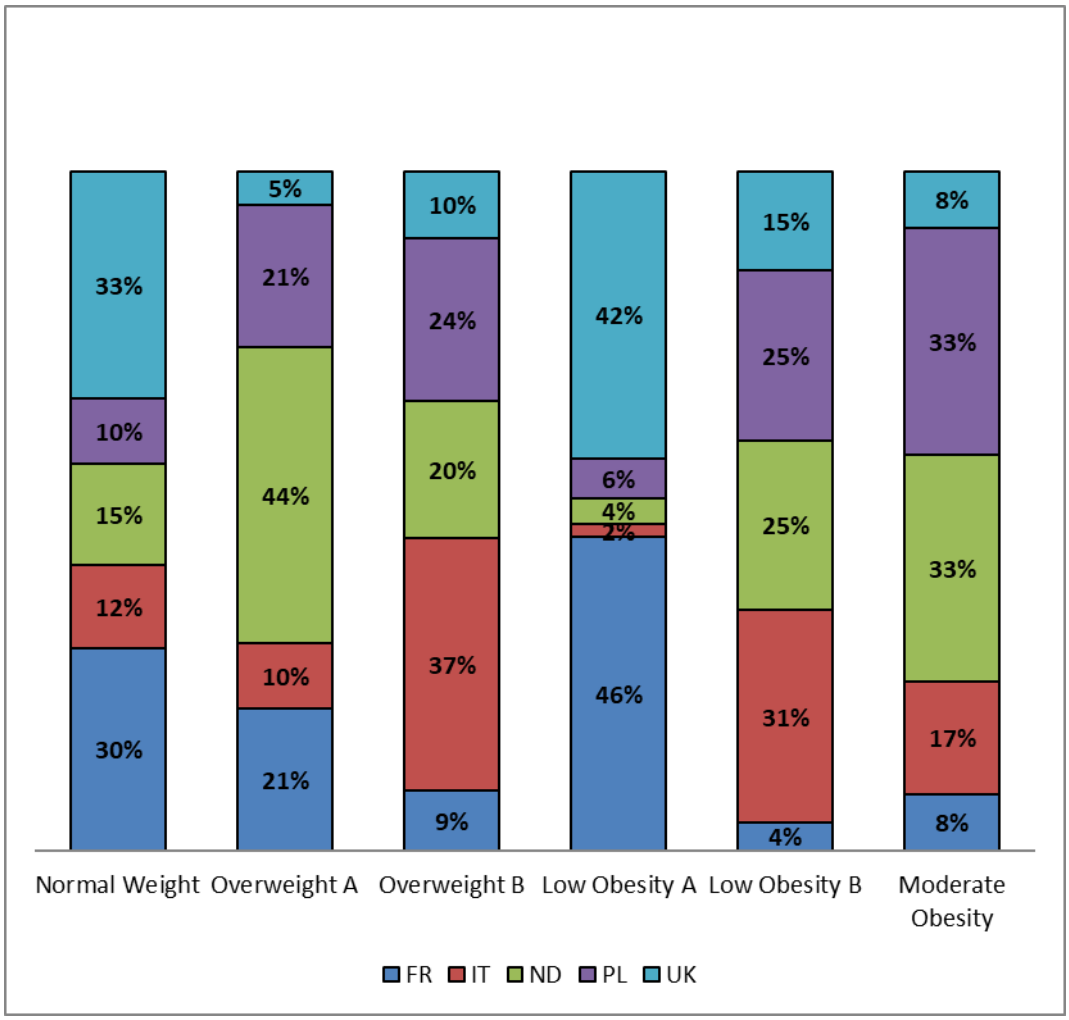


Figure 3.3 B | Percentagecontribution of the countries to the clusters in males.



4. ASSOCIATION OF BODY COMPOSITION WITH INFLAMMATORY AND METABOLIC MARKERS IN ELDERLY

This Chapter regard data that have already been reported in publication *Gender-specific association of body composition with inflammatory and adipose-related markers in healthy elderly Europeans from the NU-AGE study* (Santoro et al., 2018b).

4.1 INTRODUCTION

Due to its association with several diseases and decline in health status, the assessment of body composition (BC) is essential additionally, it is fundamental to characterize the metabolic status (Lemos et al., 2017). As mentioned in the general introduction, there is an association between changes in BC and aging. Those changes are mainly related to three distinct processes: i) a progressive decrease in lean mass (LM) and an increase in fat mass (FM) known as sarcopenia and sarcopenic obesity (Müller et al., 2014); ii) a redistribution of FM, central and visceral (Fox et al., 2007); iii) a reduction in height and bone mineral density (BMD) (Bazzocchi et al., 2013; Jafarinasabian et al., 2017). Moreover, the incidence of the major causes of deaths in U.S. and European population (Pischon et al.,2008; Freisling et al., 2017), i.e. type 2 diabetes, cardiovascular disease (CVD) and insulin resistance, is related to an excessive body fat accumulation.

A chronic inflammation driven by nutrient excess/overnutrition, called metaflammation, characterize the main metabolic diseases (Hotamisligil GS, 2017). It has been hypothesized that this inflammatory status may precede/contribute to inflammaging, i.e. the chronic, low-grade, systemic, inflammatory state that characterises ageing (Franceschi et al.,

2000; Franceschi et al., 2016) and that metabolic age-related dysfunctions and diseases can be considered manifestations of age acceleration (Franceschi C, 2017). Both conditions are characterized by an increased production of the pro-inflammatory cytokines, i.e. interleukin 6 (IL-6) or interleukin 8 (IL-8) (Prattichizzo et al., 2018). Interestingly inflammageing does not simply reflect an increase of pro-inflammatory markers but an overall activation of inflammatory systems that probably also promotes a concomitant rise in the levels of anti-inflammatory mediators (Franceschi et al., 2007; Morrisette-Thomas et al., 2014); Morisette et al., 2014).

Due to an increase of adipose tissue and a decrease of bone and muscle tissue during aging, there is rise in proinflammatory adipokines, chemokines and citokines and a reduction in anti-inflammatory ones which contributes to local and systemic inflammation and disturbances in glucose homeostasis (Mancuso P, 2016).

A systemic study on the age-related changes in BC was missing and, to the best of our knowledge, no study to date has evaluated the relationship between composition and regional distribution of fat, lean and bone masses and the relative inflammatory profile in healthy elderly subjects. Due to its relatively low cost, fast acquisition time and low radiation exposure, as compared to other available techniques, DXA is considered the gold standard for the assessment of human BC (Alberto Bazzocchi et al., 2013; Guglielmi et al., 2016). As mentioned, DXA can assess three body-composition components at a molecular level: BMC, LM, and FM in addition of BMD. Moreover, DXA allows to measure total-body and standard regional body composition, arms, legs, android and gynoid regions. Due to all of these advantages, DXA is the ideal method for clinical use and longitudinal studies, in both adults and children. The aim of the current study was to evaluate correlations between regional distribution of fat, lean and bone masses and several inflammatory and

adipose parameters. To fulfill this objective, the BC of 1121 gender-balanced-free-living subjects from the European NU-AGE project, “New dietary strategies addressing the specific needs of elderly population for a healthy ageing in Europe”, have been scanned by DXA.

4.2 METHODS

Study design and participants

NU-AGE (http://www.nu-age.eu/) is a one-year, multicenter, randomized, single-blind, controlled trial (registered with clinicaltrials.gov, NCT01754012) with two parallel groups (i.e., dietary intervention and control). The recruitment was carried out during April 2012 and January 2014 in five European centers located in France (Clermont-Ferrand), Italy (Bologna), the Netherlands (Wageningen), Poland (Warsaw), and the United Kingdom (UK, Norwich). The recruitment of participants has been described in detail previously (Berendsen et al., 2014; Santoro et al., 2014). Originally, 2668 man and women volunteers from the community aged 65–79 years, free of major overt chronic diseases compromising 2-year survival (i.e., cancer, dementia), free and independent living, and competent to make own decisions, were recruited in the baseline assessment. After testing the exclusion criteria, i.e. included severe heart diseases, type 1 and insulin-treated type 2 diabetes, chronic use of corticosteroids, recent use of antibiotics or vaccinations, change in habitual medication use, presence of frailty (Fried et al., 2001), malnutrition (body mass index <18.5 kg/m2 or 10% weight loss within 6 months), or food allergy/intolerance requiring special diets, 1296 were eligible to participate in the NU-AGE trial. Complete DXA scan was performed in 1121 participants, at baseline, and were included from the NU-AGE study cohort (France (N= 184; 16.4%), Italy (N=236; 21%), the Netherlands (N= 233; 20.7%), Poland (N=222; 19.8%), and UK (N=246; 21.9%)).

Assessment of Body Composition

A whole-body DXA scan has been carried out to measure total and regional BC using the following fan-beam densitometers in each of the

five recruiting centre: Lunar iDXA, GE Healthcare, Madison, WI, USA – enCORETM 2011 software version 13.6 (Bologna, Italy); Discovery QDR, Hologic Inc., Bedford, MA, USA – software version 3 (Clermont-Ferrand, France); Lunar Prodigy, GE Healthcare, Madison, WI, USA – enCORETM 2011 software version 13.6 (Wageningen, the Netherlands and Warsaw, Poland); and Discovery Wi, Hologic Inc., Bedford, MA, USA (Norwich, UK). The scanners were calibrated daily using a standard calibration block supplied by the manufacturers following standard Quality Control procedures. DXA scans were performed by trained technicians according to state-of-the-art technique and manufacturers recommendation.

No metal items were present during densitometry. Participants were positioned in the center of the scanning field in a supine position with the arms at sides and separated from the trunk. As mentioned, measurements of total-body and standard regional body composition, such as trunk, upper limbs, android and gynoid region were defined by DXA. Android and gynoid regions were not defined by the densitometer used in UK. For each region, DXA scanned the weight (in g) of total mass, FM, non-bone LM, and BMC. The weight (in g) of total mass, whole body fat mass (FM), non-bone whole body lean mass (LM), and bone mineral content (BMC). In order to reduce the possible error generated by the use of different DXA machines, specific indices have been used as reported in **Table 3.1** of third chapter. The indexes of total body FM/LM, FMI, and LMI are considered markers of general mass, android/gynoid FM is related of central/peripheral distribution of FM, while the FM/LM android, and ALMI and SMI indices are markers of central abdominal distribution, low muscle mass respectively. Moreover, bone mineral density (BMD) and T-score were also considered as markers of bone health.

Markers of inflammation and Adiposity related hormones

Fresh Blood was collected after fasting for each participant of all five recruiting centers. Then was immediately centrifuged at 2000 x g for 10 min at 4°C and separated into plasma and serum according to a standardized operating procedure. All the specimens were stored at -80 °C until the time of analysis and sent to the project partners responsible for the analyses of the markers of inflammation and adiposity related hormones. Inflammatory and adiposity related markers were analyzed by a magnetic bead-based multiplex immunoassays (Bio-Plex) (BIO-RAD laboratories, Milan, Italy) according to the manufacturer's instructions. In particular Interleukin 6 (IL-6), Interleukin 10 (IL-10), and Tumor Necrosis Factor alpha (TNFα) were measured in multiplex with Bio-Plex Pro Cytokine, Chemokine, and Growth Factor Assays (IL6 intra-assay coefficient of variation (CV), 4.01%; IL10 intra-assay CV, 3.99%; TNFα intra-assay CV, 4.55%); Transforming Growth Factor beta1 (TGF-β1 intra-assay CV, 3.83%) with Bioplex Pro TGF- beta assay; Ghrelin (inter-assay CV, 2%) and Resistin (inter-assay CV, 4%) in multiplex with Bio-Plex Pro human diabetes assay. Plates were read and analyzed by Bio-Plex Manager Software. The level of Interleukin 6 receptor alpha (IL6rα, inter-assay CV, 3.1%)), Glycoprotein 130 (gp130, inter-assay CV, 5.9%), Pentraxin-3 (inter-assay CV, 6.8%) and soluble TNFalpha receptors R1 (TNF-R1, inter-assay CV, 6.1%) and R2 (TNF-R2, inter-assay CV, 7.7%) were assessed in multiplex in a subgroup of 569 samples with Bioplex Pro human inflammation assay (gp-130, inter-assay %CV 5.9).

The quantitative determination of hsCRP, leptin, adiponectin has been performed by ProcartaPlexTM Immunoassay (eBioscience, Hatfield, UK) according to the manufacturer's instructions. Analysis was performed using Luminex 200 instrumentation (Luminex Corportation, The Netherlands). Assay sensitivities were 19.31 pg/mL for Leptin, 4.39 pg/mL for hsCRP, and 47.46 pg/mL for adiponectin.

α1 Acid glycoprotein (AGP) has been measured by an immunoturbidimetric assay (AAGP2, Tina-quant α1-Acid Glycoprotein Gen.2 COBAS, Roche Diagnostic) with a measuring range of 0.1-4.0 g/L. Plasma albumin level was analyzed using the VITROS ALB slides (Ortho-Clinical Diagnostics, UK) on an Vitros 5.1/FS analyzer. Method CV: 0.9 - 1.7%.

Statistical Methods

After testing the data distribution, according to Shapiro-Wilk test for Normality ($p < 0.01$) we decided to use non-parametric statistical tests. R studio (Version 3.3.3 for Windows) was used for the analysis and results are reported as mean and standard deviation (± SD Data were analyzed by non-parametric statistical tests, i.e. Mann - Whitney and Kruskal - Wallis tests, to determinate differences between males and females and between clusters (Santoro et al., 2018). To test differences between all pairs of clusters a pairwise comparison was used. A type I error of 0.05 (p-value) in two-tailed tests was considered significant. To assess a possible linear association between the body composition variables and markers of inflammation we used the Pearson Product-Moment Correlation, after a natural log-transformation (ln) for BC variables and a log-odds transformation for markers of inflammation. Due to multiple testing of the variables, the Benjamini–Hochberg correction was applied and both, p-value and q-value, are reported in results.

4.3 RESULTS

Participant characteristics

Table 4.1 reported all the anthropometric and metabolic measures and body composition markers analyzed in 1121 subjects from the NU-AGE cohort. Almost all of those measures are significantly different between female (55%) and males (45%), for this reason all the analyses were conducted separately. Female have lower height, weight, waist circumference and waist to hip ratio, calorie intake, glucose and haemoglobin levels than males. Moreover, the fat mass markers of females are significantly higher, such as FM, FMI, FM/LM and android FM/LM but lower android/gynoid FM, while males have significantly higher lean mass markers than females in terms of LM, ALMI, LMI, SMI and also higher bone content markers in terms of BMC, BMD and T-score than females. In terms of inflammatory parameters elderly female have also significantly higher levels of ghrelin, leptin, adiponectin, resistin and AGP, but any difference emerged for IL6, Pentraxin 3, IL10, TGFb, TNFa, IL6ra, gp130, TNFaR1 and TNFaR2 circulatin levels (Supplementary material) than elderly males.

Association of Body Composition with markers of inflammation and adiposity related hormones

The pairwise scatter plot matrices in **figure 4.1** and **figure 4.2** reports all the correlations between BC parameter and inflammatory markers in female and males, respectively. In elderly female leptin ghrelin is significantly negatively correlated with BMI, FM, FMI, and FM/LM while is significantly positively correlated with SMI. Leptin show a strong ($\rho > 0.60$, p-value < 0.05) significant positive correlation with BMI, FM, FMI, FM/LM, and a positive correlation with lean mass in terms of LMI and ALMI and bone mass, such as BMC, BMD and T score, while show a

significant negative correlation with SMI. Adiponectin is significantly negatively correlated with BMI, FM, LM, LMI. While CRP and AGP are significantly positively correlated with BMI, FM, FMI, FM/LM, LMI and ALMI, while significantly negatively correlated with SMI. No significant correlation is reported for Resistin and Albumin with the body composition markers.

Unlike female, in elderly men, ghrelin is only slightly correlated with SMI. While also in this case, leptin is significantly positively correlated with BMI, FM, FMI FM/LM, LM, LMI, BMC, BMD and T score, and significantly negatively correlated with SMI. Adiponectin is significantly negatively correlated with BMI, FM, FMI, LM, LMI, ALMI. CRP is significantly positively correlated with BMI, FM, FMI, FM/LM, while significantly negatively correlated with SMI. Albumin is significantly positively correlated with LMI, ALMI and SMI No significant correlation is reported for Resistin and AGP with the body composition markers. All the results are reported in **figure 4.2**.

The upper left part of the pairwise scatter matrix plot in **figure 4.1** and **4.2** reported all the correlation of the BC parameters in female and males, respectively. As expected, all the fat mass, lean mass and bone markers are significantly correlated among themselves and SMI is negatively correlated with the fat mass markers BMI, FM, FMI, FM/LM and positively correlated with ALMI in both female and males.

The lower right part of the pairwise scatter matrix plot in **figure 4.1** and **4.2** reported all the correlation of the inflammatory markers in female and males, respectively. In females, as expected, ghrelin is negatively associated with leptin while in males no significant correlation is reported. Moreover, leptin shows a negative correlation with adiponectin and a positive correlation with CRP in both females and males, while a positive correlation with resistin and AGP is reported only in females. AGP

is negatively related with adiponectin and positively with resistin only in females and with CRP in both genders.

No correlation was found between the BC markers and the following pro- and anti-inflammatory markers in both female and male elderly: IL6, Pentraxin 3, IL10, TGFβ1, TNFa, IL6ra, gp130, TNFaR1 and TNFaR2.

In addiction **table 4.2** and **table 4.3** reported a correlation matrix of android FM/LM and android/gynoid FM with markers of inflammation and adiposity for female and males, respectively.

In elderly female ghrelin is significantly negatively correlated with android FM/LM and android/gynoid FM, otherwise leptin is significantly positively correlated with the same BC parameters in both males and females. Another negative correlation is between adiponectin and android FM/LM and android/gynoid FM in both females and males, while CRP and AGP are significantly positively correlated in both females and male and only in female, respectively. Albumin is significantly negatively correlated with android FM/LM and android/gynoid FM. No correlation was found between android FM/LM and android/gynoid FM and resistin, IL6, Pentraxin 3, IL10, TGFβ1, TNFa, IL6ra, gp130, TNFaR1 and TNFaR2 (**Table 4.2**; **Table 4.3**).

Association of markers of inflammation and adiposity related hormones with Body Composition Clusters

As reported in chapter 3, inflammatory markers and adiposity related hormones have been evaluated among clusters of body composition markers (Santoro et al., 2018). Briefly, female and males were separately investigated and based on the BMI, FM, FMI, FM/LM, LM, LMI, ALMI, SMI, BMC, BMD and T-score five clusters have been identified for females (normal weight (NW), BMI=21.39; Overweight A (OWA), BMI=25.09; Overweight B (OWB), BMI=26.62; Low Obesity A (LOA), BMI=31.48 and

Low Obesity B (LOB), BMI=31.92) and six for men (normal weight (NW), BMI=23.98; Overweight A (OWA), BMI=25.69; Overweight B (OWB), BMI=26.27; Low Obesity A (LOA), BMI=30.06; Low Obesity B (LOB), BMI=30.42 and Moderate Obesity (MO), BMI=36.6). Those clusters can discriminate group of subjects with similar BMI but significantly different BC markers (Santoro et al., Frontiers in physiology). **Figure 4.3** shows a significant difference among the five female clusters for ghrelin (p=5.297 e-06), adiponectin (2.829 e-06), CRP (1.154e-12), leptin (p<2.2e-16), AGP (1.651e-12) and TGFβ1 (p= 0.005). Indeed, NW cluster shows higher levels of ghrelin compared with OWB and LOB clusters, and also OWA cluster has significant higher levels than LOB cluster (**Figure 4.3**). Leptin levels are different among all the five clusters, in particular are lower in NW cluster. Interestingly the two cluster with similar BMI (LOA and LOB) have different leptin levels, higher in LOB cluster than LOA (**Figure 4.3B**). Also, adiponectin levels are higher in NW females cluster compared with OWA, LOA and LOB, females in the OWA cluster have lower levels of adiponectin than OWB but higher than LOA and females in the OWB cluster have significantly higher levels than females in LOA and LOB (**Figure 4.3C**). NW female cluster shows lower levels of CRP compared with all the other four clusters, and OWA cluster have lower levels of CRP compared with LOA and LOB and those in cluster OWB have lower levels than LOA and LOB (**Figure 4.3D**). NW cluster has the lower levels of AGP compared with all the other four clusters, moreover, cluster OWA has lower AGP levels compared with OWB, LOA and LOB, and the levels of AGP are lower in OWB cluster compared with LOA and LOB (**Figure 4.3E**). While the only significant difference of TGFβ1 levels among the five female clusters is between NW and LOB (**Figure 4.3F**).

Figure 4.4 shows a significant difference among the six males clusters for ghrelin (p=0.0006417), adiponectin (p= 0.0005453), CRP (p=1.174e-06), leptin (p<2.2e-16), Albumin (p=0.004843) and AGP (0.001147). In

particular, ghrelin levels are significantly higher in NW cluster compared with LOB (**Figure 4.4A**). NW cluster shows lower levels of leptin compared with OWB, LOA, LOB, MO. Leptin levels are significantly lower in OWA cluster compared with OWB, LOA, LOB and MO, cluster OWB has significantly lower leptin level than LOA, LOB and MO, while males in LOA cluster have lower leptin levels than LOB and MO and males in cluster LOB have lower leptin levels than MO (**Figure 4.4B**). NW males cluster shows higher levels of adiponectin compared with the LOB cluster (**Figure 4.4C**). CRP and AGP levels are significantly lower in elderly men comparing NW and LOB clusters, OWA and LOB clusters, OWB and LOB clusters (**Figure 4.4D and 4.4E**). While the only significant difference of albumin levels among the six male clusters is between LOA and LOB (**Figure 4.4F**).

4.4 CONCLUSIONS

The present study reports evidence for the association between BC markers and the levels of different pro- and anti-inflammatory parameters and adiposity related hormones.

1121 healthy European elderly men and women who participated in the European project NU-AGE have been analyzed. As expected, major differences exist between BC characteristics in elderly women and men. In particular, elderly females have higher values in fat mass indices than males while males have higher values of lean mass indices and bone content than elderly females. Sex dimorphism in total BC is present since birth and continues in adulthood. Males maintain their level of lean mass into the age of 50, but then begin to lose muscle mass due to both hormonal changes and lower physical activity. Also females show a similar decrease in lean mass, but they often show greater increase in fat mass (Wells JCK, 2007), even when weight is stable (Zamboni et al., 2003). Such changes continue into old age (Bazzocchi et al., 2013; Diano et al., 2017). Among the adiposity related markers, a significant negative association between ghrelin and fat mass has been found in females but not in males, while a positive association with SMI has been highlighted in both genders. Studies have demonstrated that ghrelin levels can decreased in obesity, its levels are mainly influenced by changes in energy balance, in fact insulin may play an important role in the decrease of ghrelin levels after meals (Murdolo et al., 2003). Even if no significant differences emerged for BMI and insulin between genders, as seen females have greater fat mass than men, and this could explain the different association found in ghrelin levels. In addition, comparing ghrelin levels among the female and males' clusters previously identified in chapter 3 (Santoro et al., 2018 a) which differs for BMI and fat, lean and bone masses a gender difference emerged. Indeed, a significant negative association between BMI and ghrelin levels in female clusters has been

found, ghrelin levels decreased from NW cluster (lower BMI) to LOB cluster (higher BMI), while in males this trend is not significant.

A significant positive association between fat mass, lean mass and bone mass markers and leptin levels has been found, while SMI is negatively associated in both males and females. Leptin is an adipokine secreted by adipocytes, generally increases with weight gain, and decreases with weight loss (Spiegelman BM and Flier JS, 2001), but has been demonstrated that leptin is also produced by skeletal muscle (Fernández-Real et al., 2000; Wolks et al., 2012) as well as bone cells (Thomas T, 2004). Leptin treatment increases muscle mass and decreases the expression of atrophy-related factors such as myostatin, muscle RING-finger protein-1 (MuRF1), and muscle atrophy F-box (MAFbx) in muscle (Hamrick MX, 2017). Different studies highlight that the effects of leptin on the skeleton are quite complex, and that lower levels of leptin are associated with low bone mass primarily due to reduced cortical bone (Hamrick et al., 2008 a; Hamrick et al., 2008 b). In fact, central infusions of leptin in leptin-deficient ob/ob mice increase cortical bone formation and total bone mass (Bartell SM et al., 2011). Moreover, it has been demonstrated than osteoporotic subjects have lower levels of leptin in the bone marrow microenvironment (Pino et al., 2010).

Adiponectin, together with leptin, is able to regulate the energy homeostasis. Studies have demonstrated that adiponectin levels can decreased in obesity and insulin resistance (Mancuso P, 2016). In the 1121 subjects analyzed no correlation was found between adiponectin and bone markers, while an inverse relationship emerged with fat and lean markers in both males and females. However, our results concord with a recent paper by Baker and colleagues demonstrating that in elderly high levels of serum adiponectin are significantly correlated with low BMI, fat and lean mass BC markers (Baker et al., 2018)(Baker et al., 2018)(Baker et al., 2018)(Baker et al., 2018). Moreover, in males and females' clusters

adiponectin levels decrease as increase BMI. However, it is interesting to note that among the five clusters of females, the two clusters with similar BMI (25.09 and 26.62 respectively) have different adiponectin levels, higher ones are in the overweight group characterized by higher levels of fat and bone mass and lower levels of lean mass, indeed, a similar trend can be found among the two low obesity clusters (BMI 31.48 and 31.92 respectively) even if it is not significant. While the only significant difference of adiponectin levels among the six male clusters is between the low obesity B cluster and the normal weight which have higher levels and an increasing trend emerged when comparing the two clusters (overweight A vs B and low obesity A vs B) with similar BMI but different amount of fat, lean and bone mass.

Regarding inflammatory markers, a significant positive correlation between CRP and fat mass markers emerged while there is a negative association with SMI in both females and males. CRP and AGP are positively correlated with lean mass markers in female. In addition, AGP is positively correlated LMI and negatively associated with SMI in female, while in male the only significant association between AGP levels and BC markers is a positive correlation with android/gynoid FM ratio. A negative association between albumin and central adiposity markers (android FM/LM and android/gynoid FM ratio) has been found in females while in males, albumin is positively associated with lean mass markers (LMI, ALMI and SMI). Different studies had demonstrated that an increase in fat mass is correlated with markers of inflammation in elderly (Brinkley et al., 2012; Schrager et al., 2007). The obesity-related inflammation and its mechanisms are not entirely understood, expansion of adipose tissue is mainly influenced by changes in energy balance which may play a major role. The expansion of adipose tissue leads the activation of macrophage to secrete inflammatory cytokines such as TNFα and IL-6 (Kern et al., 2001). In addition, leptin may play a role as a pro-inflammatory molecule

in the setting of obesity (Matarese et al., 2005) along with resistin, whereas adiponectin and ghrelin have anti-inflammatory properties. Indeed, adiponectin inhibit inflammation by blocking NF-kB activation and reducing cytokines like TNFα, IL-6, and IL-18 (Yamaguchi et al., 2005; Chandrasekar et al., 2008). In addition, adiponectin plays a pro-inflammatory role in arthritic joints by promoting COX2 expression and PGE2 synthesis, which are related to an increase in inflammation and pain (Bas et al., 2014). Through the efflux of anti-inflammatory and pro-inflammatory adipokines into the systemic circulation, adipose tissue plays an important role in regulating the inflammatory response in the setting of caloric restriction, obesity, and aging. However, it is possible that the association with inflammatory markers differs depending on gender and adipose tissue location. The two most commonly measured inflammatory proteins in nutritional investigations are CRP and AGP, which are measures of acute and chronic inflammation, respectively (Suchdev et al., 2017). Our results show that there is a positive correlation between CRP and fat mass in both genders, but LMI and ALMI correlate only in female. Moreover, in females' clusters CRP and AGP increased with BMI and a similar trend can be seen also in males' clusters. It was demonstrated that significant differences in the effect of aging on the human immune system emerged between female and males, with a stronger pro-inflammatory response in female (Marttila et al., 2013). No differences emerged in CRP levels between males and females (median 0.84 mgL-1 and 0.87 mgL-1, respectively), however, females have a significative higher concentration of AGP compared to males (median 0.67 gL-1 and 0.61 gL-1, respectively). Moreover, AGP shows a positive correlation with fat mass and LMI in female. Differences in haemoglobin levels, which is lower in females than in males (median 13.7 gdL-1 and 14.9 gdL-1, respectively), could contribute to the different inflammatory status (Suchdev et al., 2017). CRP and albumin can act, respectively, as

positive and negative acute phase reactants. This seems to provide a relation to the increased inflammatory state in elderly females. Different studies had demonstrated an association between the specific pattern of increased CRP and decreased albumin concentrations with sarcopenia, frailty and vascular and non-vascular mortality in elderly subjects (Clarke et al., 2008; Hubbard et al., 2009). Even if no correlation has been found between BC markers and indexes and circulating levels of a series of pro- and anti-inflammatory molecules such as IL6, Pentraxin 3, IL10, TGFβ1, TNFα, IL6rα, gp130, TNFαR1 and TNFαR2, there are evidence that there are association between fat mass, BMI and waist circumference and inflammatory markers (Brinkley et al., 2012; Schrager et al., 2007; Cesari et al., 2005). TGFβ1 in females increase with BMI and this can be explained by the size of the cohort used, by the technique used to identify BC and many other factors. The strength of this study is the size of the sample used for the analysis, consisting of healthy elderly subjects aged between 65 and 79, who are representative of the European population.

Moreover, DXA, which is used for the assessment of BC, is a powerful and comprehensive tool and a gold-standard technique at this level. A limitation can be associated with the voluntariness of subjects to participate to the NU-AGE study, in fact, in all the five countries considered the participants represent a population that is particular interested in health and nutritional aspects and as this it has higher knowledge on these issues than the general population at the same age.

On the whole, all the BC markers studied in this paper are positively or negatively associated with adipose related and inflammatory markers, excepted SMI which represents a marker of sarcopenia, together with ALMI (Kim et al., 2016; Guglielmi et al., 2016). Our results show that SMI association with adiposity related and inflammatory markers are always discordant in both females and males except for the positive correlation of albumin levels in males. In particular, in both females and males, SMI

is positively correlated with an anti-inflammatory molecule, i.e. ghrelin, while negatively associated with leptin, CRP and AGP which are considered pro-inflammatory markers. In elderly sarcopenia is related with a higher increase of inflammatory status, therefore the results obtained with SMI are more reliable respect of results highlight with ALMI when both are considered as markers of sarcopenia. These results fit with the open debate on the use of optimal quantitative markers of sarcopenia (Kim et al., 2016). Moreover, a negative association between SMI and BMI and fat mass markers and positive association with ALMI but not with LM and LMI have been identified, while ALMI is positively correlated with BMI and fat mass markers an also with LM and LMI. These results showed that it is likely that ALMI still represents the general lean mass instead of being a marker of sarcopenia, however further studies are needed to verify this hypothesis.

Table 4.1 | Characteristics of participants

	Female *n = 620*	**Male** *n = 501*	***p-value***	***q-value***
Age (*years*)	70.7 ± 3.9	71.0 ± 4.1	NS	NS
Weight *(kg)*	67.7 ± 11.2	80.6 ± 12.6	<2.2e-16	<2.2e-16
Height *(cm)*	160.0 ± 6.7	173.0 ± 6.4	<2.2e-16	<2.2e-16
BMI (kg/m^2)	26.5 ± 4.1	26.9 ± 3.7	1.16e-02	NS
Hip circumference (*cm*)	103.3 ± 9.1	101.5 ± 7.6	1.32e-03	NS
Waist circumference (*cm*)	86.9 ± 10.8	96.7 ± 11.1	<2.2e-16	<2.2e-16
Waist/Hip ratio	0.85 ± 0.31	0.95 ± 0.06	<2.2e-16	<2.2e-16
Calorie intake (*kcal*)	1680.9 ± 328	2123.3 ± 445	<2.2e-16	<2.2e-16
Physical activity (Pase Score)	127.8 ± 48.9	140.9 ± 59.5	3.53e-04	NS
Metabolic parameters				
Glucose	5.52 ± 0.77	5.85 ± 0.95	7.92e-11	1.54e-07
Insulin	8.75 ± 5.57	10.03 ± 7.85	NS	NS
HOMA IR	2.21 ± 1.58	2.70 ± 2.36	5.47e-03	NS
HOMA beta	90.43 ± 52.88	89.06 ± 63.57	2.08e-02	NS
Haemoglobin (g/dL)	13.7 ± 0.9	14.9 ± 1.0	<2.2e-16	5.66e-14
Body composition markers				
FM (*kg*)	26.2 ± 8.06	22.0 ± 8.37	<2.2e-16	<2.2e-16
FMI (kg/m^2)	10.3 ± 3.16	7.35 ± 2.74	<2.2e-16	<2.2e-16
LM (*kg*)	40.3 ± 4.97	57.1 ± 6.71	<2.2e-16	<2.2e-16
ALMI (kg/m^2)	6.56 ± 0.77	8.47 ± 0.87	<2.2e-16	<2.2e-16
LMI (kg/m^2)	15.7 ± 1.53	19.1 ± 1.80	<2.2e-16	<2.2e-16
FM/LM	0.65 ± 0.19	0.39 ± 0.14	<2.2e-16	<2.2e-16
SMI	0.25 ± 0.03	0.32 ± 0.04	<2.2e-16	<2.2e-16
BMC (*g*)	2092 ± 357	2948 ± 483	<2.2e-16	<2.2e-16
BMD (g/cm^2)	1.03 ± 0.11	1.19 ± 0.11	<2.2e-16	<2.2e-16
T-score	-0.82 ± 1.20	-0.19 ± 1.20	< 2.2e-16	4.92e-14
Android/Gynoid FM*	0.50 ± 0.15	0.78 ± 0.21	<2.2e-16	<2.2e-16
Android FM/LM*	0.79 ± 0.30	0.61 ± 0.25	2.70e-16	4.92e-13
Inflammatory parameters				
Ghrelin (pg/ml)	1631 [842 - 4427]	1256 [582 - 3538]	9.86e-05	
Leptin (ng/ml)	4.39 [2.86 - 6.21]	1.86 [0.94 - 3.16]	<2.2e-16	<2.2e-16
Adiponectin (µg/ml)	14.09 [9.76 - 19.96]	7.33 [5.03 - 10.51]	<2.2e-16	<2.2e-16
Resistin (pg/ml)	5850 [4287 - 752]	6222 [4756 - 8310]	5.67e-03	
CRP (mg/L)	0.87 [0.44 - 1.72]	0.84 [0.41 - 1.78]	NS	NS
AGP (mg/ml)	0.67 [0.57 - 0.79]	0.61 [0.51 - 0.73]	1.24e-08	2.32e-05
Albumin (g/L)	44.90 [42.50 - 47.50]	44.95 [42.78 - 48.00]	NS	NS

Table 4.2 | Correlation Matrix for android Fat Mass/lean Mass and android/Gynoid Fat mass with inflammatory and adiposity related markers in Females. *p < .05. **p < .01. ***p < .001

	ANDR FM/LM	ANDR/GYN FM	Ghrelin	Leptin	Adiponectin	Resistin	CRP	AGP	Albumin	IL-6	IL-6 ra	GP-130	Pentraxin-3	TNF α	TNF-R1	TNF-R2	IL-10	TGF β
ANDR FM/LM	1																	
ANDR/ GYN FM	.76***	1																
Ghrelin	-.30***	-.20*	1															
Leptin	.68***	.42***	-.22***	1														
Adiponectin	-.27***	-.48***	.08	-.23***	1													
Resistin	.08	.04	-.09	.17*	-.10	1												
CRP	.28***	.22**	-.17	.24***	-.16	.12	1											
AGP	.32***	.31***	-.12	.27***	-.28***	.18*	.47***	1										
Albumin	-.27***	-.21**	.09	-.06	.00	-.08	-.08	.00	1									
IL-6	-.05	-.02	.30***	-.13	-.03	-.07	.03	.07	.01	1								
IL-6 ra	.00	.01	.10	.09	.02	.19	-.02	-.02	-.10	-.12	1							
GP-130	.02	-.02	-.01	.08	.10	.11	-.05	-.11	-.13	-.14	.70***	1						
Pentraxin-3	.09	.00	-.20	.11	.14	-.02	.01	.00	-.11	-.09	.42***	.63***	1					
TNF α	.04	.04	.28***	-.05	.00	-.12	.03	.02	.01	.63***	-.12	-.11	-.12	1				
TNF R1	.19	.10	-.05	.24*	-.04	.25**	.08	.08	-.14	-.11	.65***	.78***	.52***	-.07	1			
TNF R2	.20	.12	.01	.22	.01	.23*	.13	.07	-.19	-.05	.68***	.77***	.57***	-.04	.83***	1		
IL-10	-.03	-.01	.30***	-.11	.03	-.08	-.01	-.01	.02	.63***	-.09	-.07	-.13	.64***	-.04	.00	1	
TGF β	.13	.09	-.08	-.04	-.04	.03	.05	.05	.07	.18**	-.40***	-.37***	-.23*	.19**	-.28***	-.31***	.15	1

Table 4.3 | Correlation Matrix for android Fat Mass/lean Mass and android/Gynoid Fat mass with inflammatory and adiposity related markers in Males. *p < .05. **p < .01. ***p < .001

	ANDR FM/LM	ANDR/GYN FM	Ghrelin	Leptin	Adiponectin	Resistin	CRP	AGP	Albumin	IL-6	IL-6 ra	GP-130	Pentraxin-3	TNFα	TNF-R1	TNF-R2	IL-10	TGFβ
ANDR FM/LM	1																	
ANDR/GYN FM	.79***	1																
Ghrelin	-.15	-.10	1															
Leptin	.76***	.56***	-.13	1														
Adiponectin	-.25***	-.43***	.01	-.27***	1													
Resistin	-.01	.05	-.03	.03	-.04	1												
CRP	.30***	.30***	-.06	.25***	-.16	.10	1											
AGP	.20	.22*	.04	.13	-.15	.12	.45***	1										
Albumin	-.19	-.12	.07	-.05	-.01	-.05	-.14	-.04	1									
IL-6	.08	.08	.35***	-.02	.02	.04	.22***	.22**	-.05	1								
IL-6 ra	-.01	-.06	.05	.03	.05	-.02	-.10	-.07	-.08	-.12	1							
GP-130	-.11	-.15	-.03	-.07	.14	-.03	-.15	-.05	-.04	-.08	.72***	1						
Pentraxin-3	-.11	-.12	-.21	-.11	.19	-.07	-.08	.01	-.06	-.08	.57***	.70***	1					
TNF α	-.02	.00	.41***	-.11	-.02	.01	.03	.08	.04	.64***	-.09	-.01	-.16	1				
TNF R1	-.02	-.04	.03	.01	.00	.16	.02	.06	-.12	.00	.64***	.77***	.61***	.03	1			
TNF R2	.03	-.01	.06	.07	.06	.19	.02	.08	-.16	.03	.66***	.76***	.63***	.01	.85***	1		
IL-10	.02	.03	.32***	-.09	.00	.03	.01	.10	-.08	.51***	.07	.09	-.04	.66***	.09	.12	1	
TGF β	.09	.08	-.11	-.01	.02	.12	.02	.07	-.02	.03	-.37***	-.32**	-.28*	-.03	-.28*	-.29*	.03	1

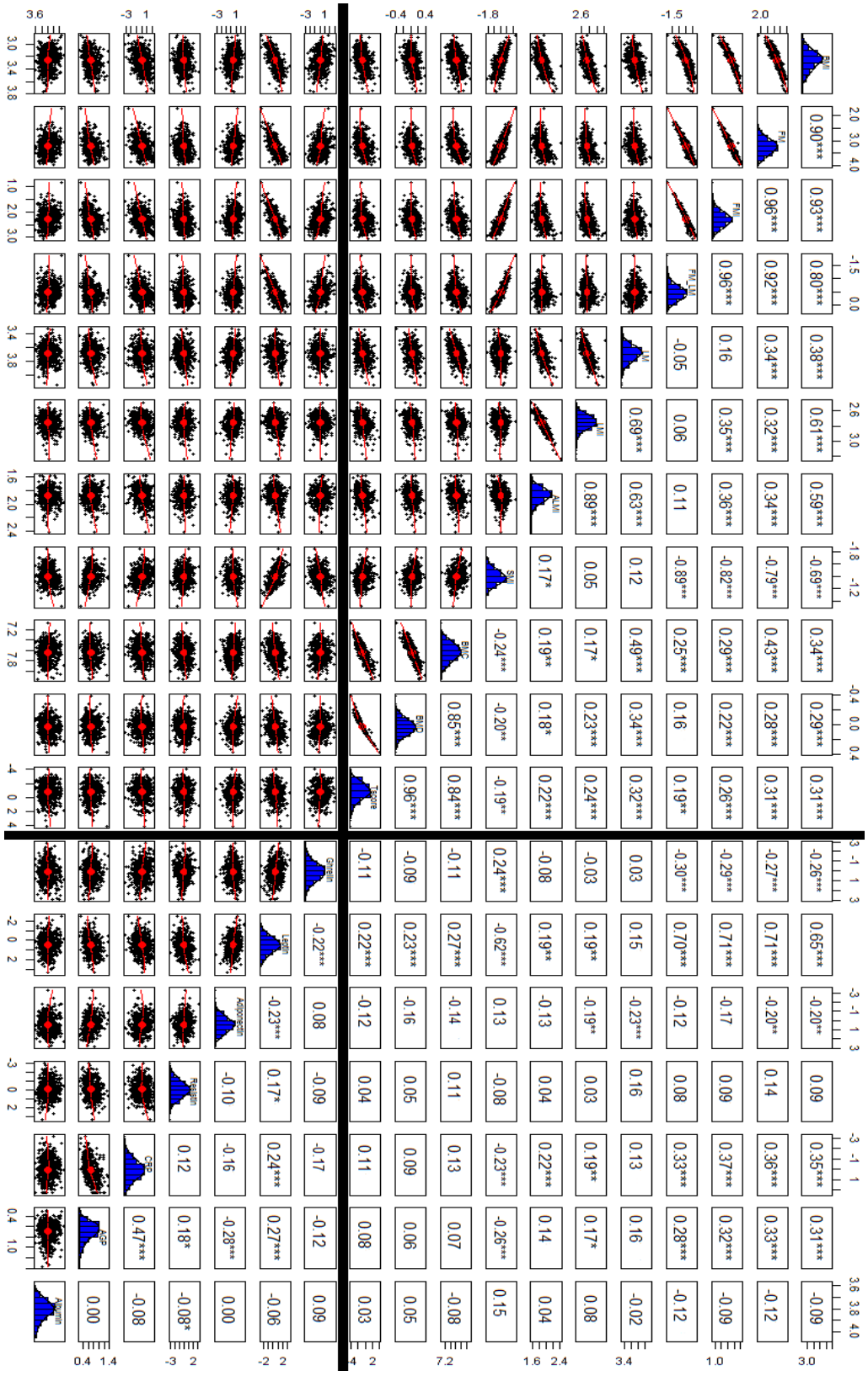


Figure 4.1 | Pairwise scatter plot matrix. histogram and correlation coefficients of all Body Composition parameters and Inflammatory parameters in female. Pairwise scatter plots are in lower triangle boxes, histograms are in the diagonal boxes and correlation coefficients between variables are in the upper triangle boxes.

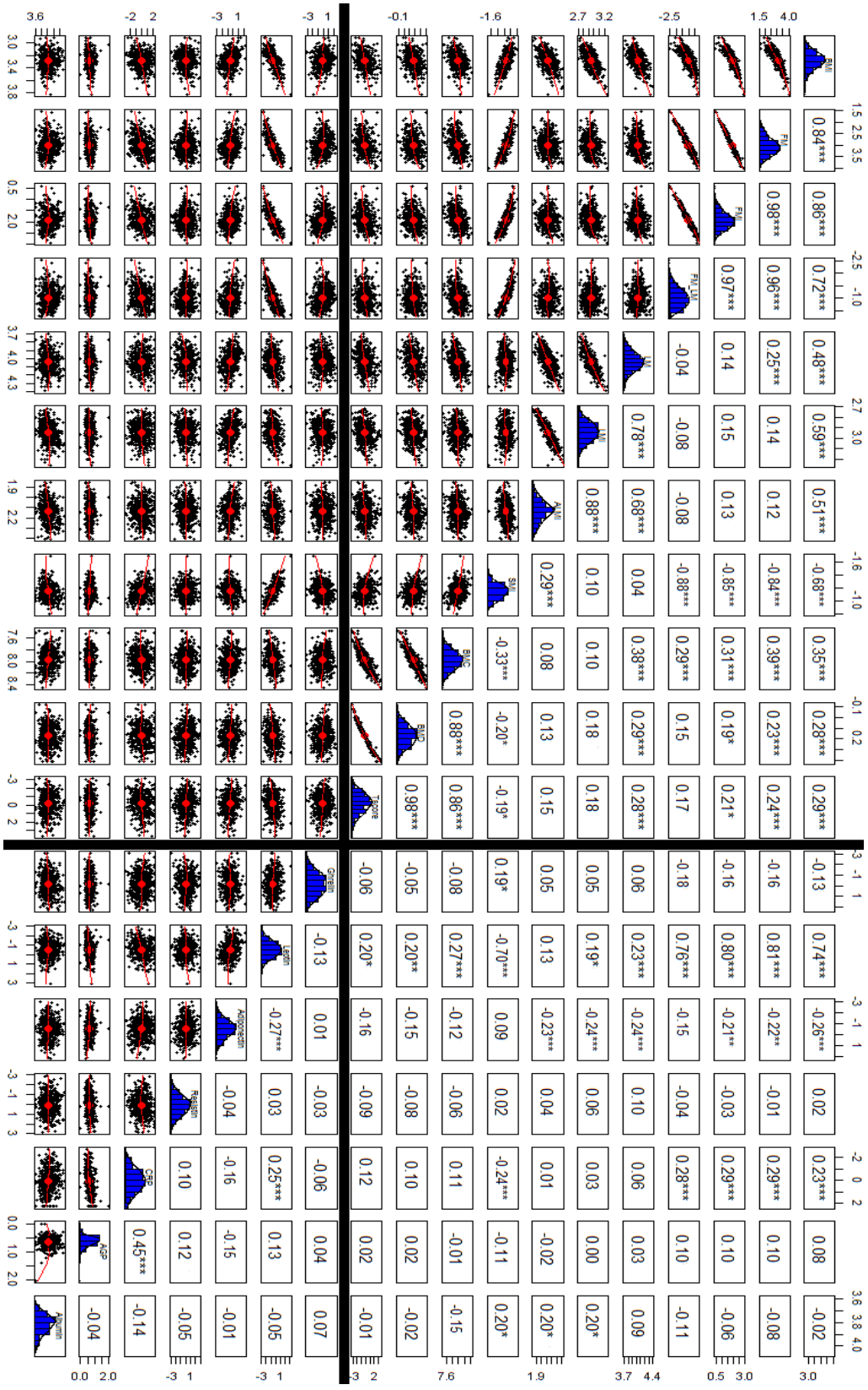


Figure 4.2 | Pairwise scatter plot matrix. histogram and correlation coefficients of all Body Composition parameters and Inflammatory parameters in Male. Pairwise scatter plots are in lower triangle boxes, histograms are in the diagonal boxes and correlation coefficients between variables are in the upper triangle boxes.

Figure 4.3 | Female clusters' boxplot

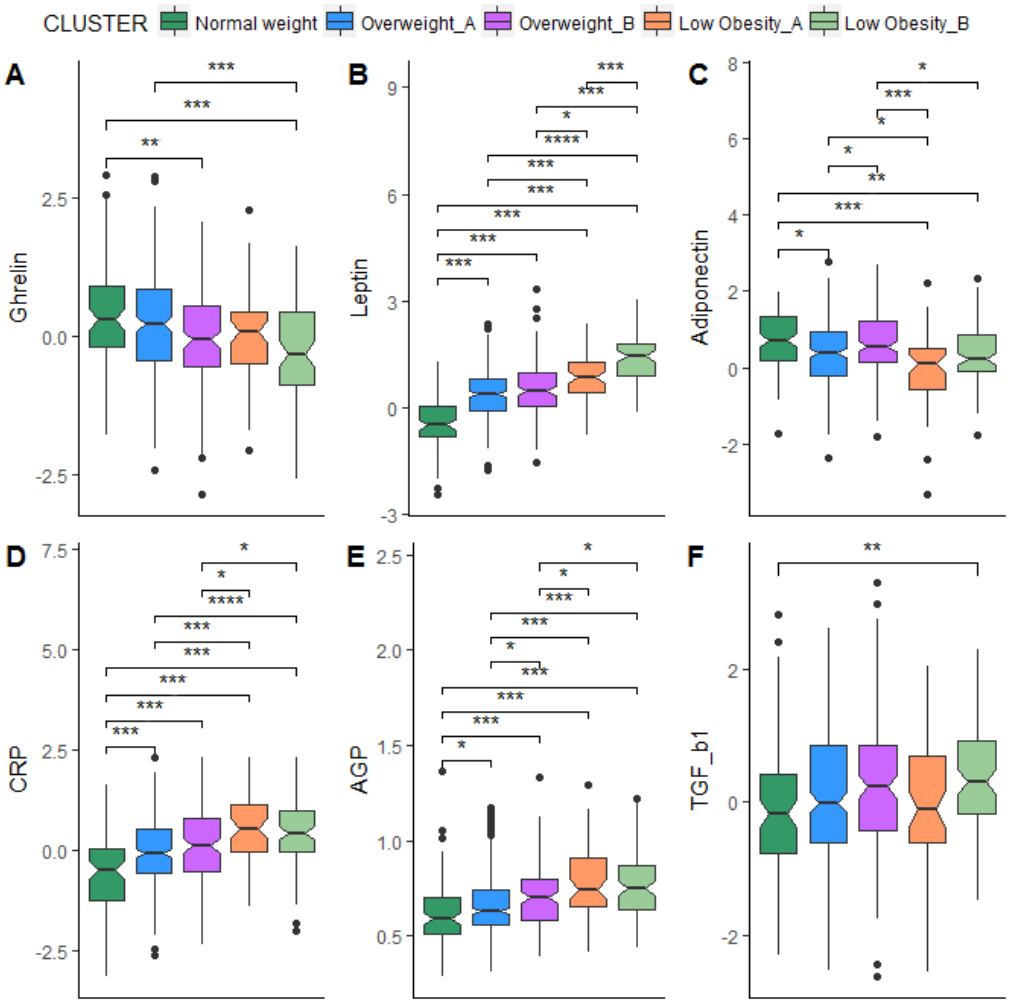
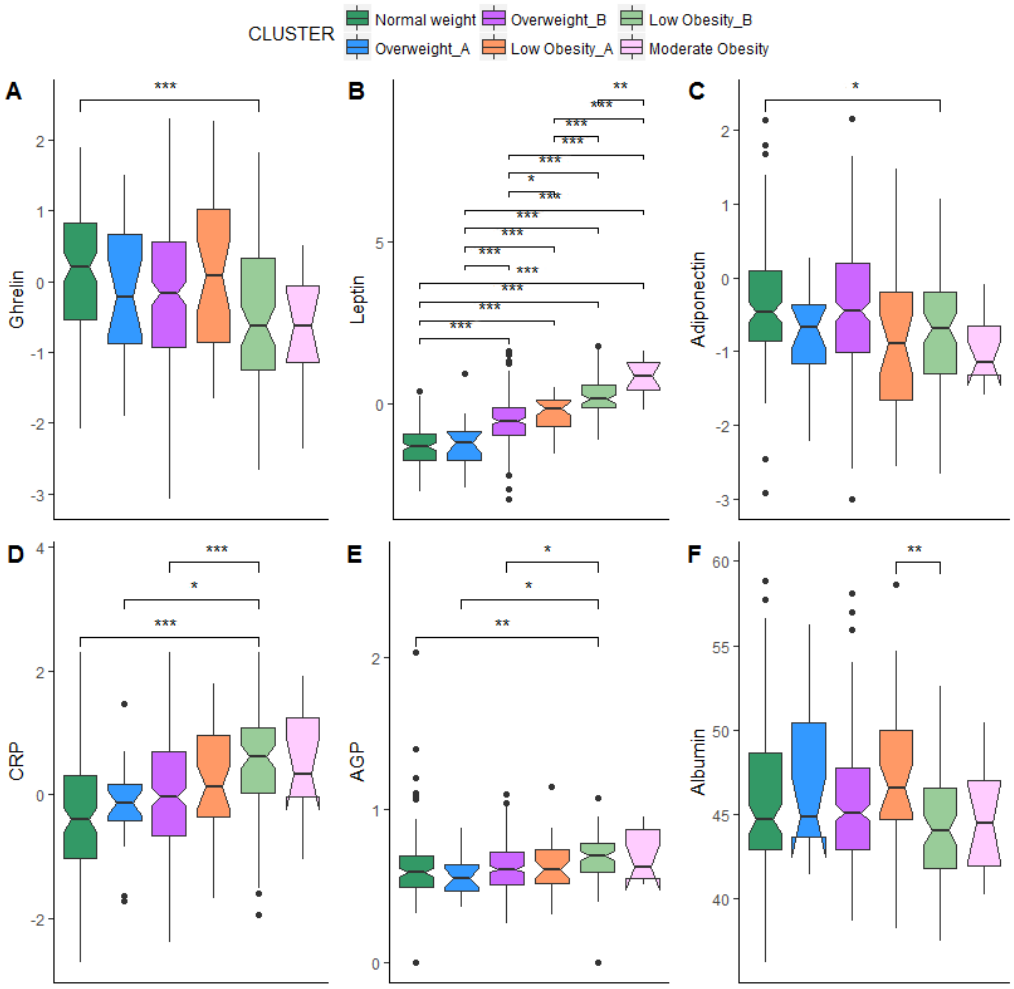


Figure 4.4 | Male clusters' boxplot



5. BODY COMPOSITION AND FRAILTY

This Chapter reports on data not yet published.

5.1 INTRODUCTION

Human aging is an inevitable and irreversible biological process, although with the improvement of lifestyle and health care, there has been an increase in average life span. A recent study from Eurostat (Eurostat, 2020 b) shows that the European union's population will decrease by 7% between 2019 and 2100. However, people aged 65 years and over will increase from 20% in 2018 to 31% in 2100 (**Figure 5.1**).

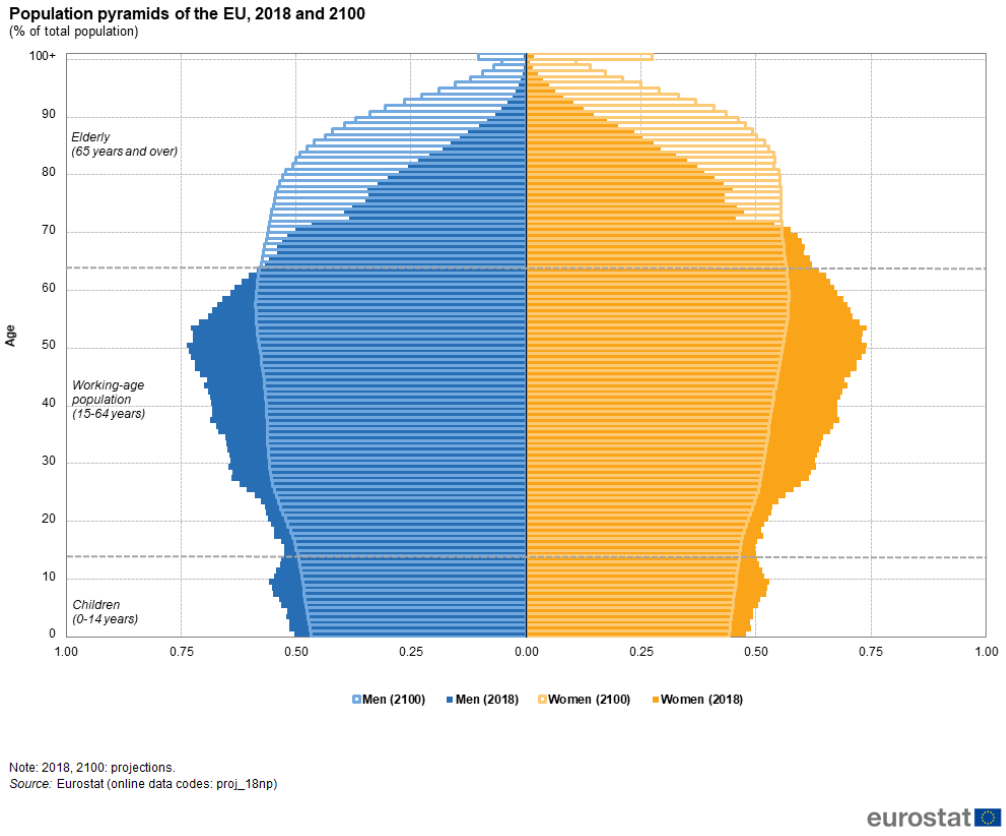


Figure 5.1 | Population pyramids of EU, 2018 and 2100 (Eurostat, 2019).

However, the effects of the Covid-19 outbreak on mortality could change this trend. Indeed, if we analyzed mortality data of 2020 the number of deaths across the 31 European countries starts to rise abruptly at the beginning of March, in week 10, compared to previous years (average over 2016 to 2019) (Eurostat, 2020 c). In Italy, as in other European countries, the epidemic has hit vulnerable people hardest, and the increase in mortality is greatest in the 65-79 age group, for both men and women (Istat, 2020). The reasons for such an increased susceptibility are still matter of debate, however, it is clear that comorbidities (including diabetes and cardiovascular diseases) are strong risk factors for severe forms of Covid19. As a whole, it appears that the elderly population is at risk because two possible main reasons: comorbidities and increased inflammatory reactions (inflammaging), two phenomena that usually accompany the aging process. In fact, one of the most universal features of the ageing process appears to be a chronic, low-grade inflammatory state indicated inflammaging (Franceschi et al., 2007; Cevenini et al., 2013) that is associated with increased risk of age-related diseases (Franceschi and Campisi, 2014; Ferrucci and Fabbri, 2018).

In particular, inflammaging is marked by a complex reshape in the production of pro- and anti-inflammatory mediators, which, as a whole, tilts the balance toward an increase of the level of basal inflammation. As an example, aging is characterized by a decreased production of the anti-inflammatory interleukin 10 (IL-10) and an increase of the pro-inflammatory interleukin 6 (IL-6), (Marcos-Pérez et al., 2020), this one in particular is considered a risk factor for many of the major age-associated diseases, including obesity, cardiovascular diseases, sarcopenia and frailty (Santoro et al., 2020). Even if many studies have shown a positive correlation between frailty and inflammatory parameters, such as IL-6 or C reactive Protein (CRP), no single predictive molecular markers have been identified so far (Kane AE, Sinclair DA, 2019).

Frailty is a condition of decreased capability to cope with and recover from stresses even of mild intensity. As already mentioned in Chapter 1, frailty is a strong predictor of disability, hospitalization and mortality and a criterion for non-eligibility for invasive treatments. The Frailty phenotype described by Fried and colleagues (Fried LP et al., 2001) includes involuntary weight loss, exhaustion, low physical activity, low gait speed, and low grip strength and is considered a “physical” frailty. At variance, the Frailty Index includes anomalous laboratory results or presence of diseases and it is considered as a measure of decline in health (Rockwood, Mitnitski, 2007). According to the standardized phenotype proposed by Fried and colleagues, frailty is defined by verifying if three or more out of the five criteria are met. People can be divided in three different groups: non-frail (none of these criteria are met), pre-frail (one or two features are met) and frail (three or more criteria are met). The prevalence of frailty in EU’s population aged 65–74 years old is about 6.0% while pre-frail subjects are 41.7%, moreover the prevalence increases with age with 16.0% of frail and 50.5% of pre-frail for people between 75 and 84 years old (Manfredi et al., 2019). Furthermore, it has been demonstrated that excess visceral adipose tissue (VAT) rather than accumulation of subcutaneous adipose tissue (SAT) represents the cause of atherosclerotic cardiovascular events (Sato et al., 2018) and an elevated waist circumference and body fat mass are risk factors for frailty in the elderly (Xu Et al., 2020). The connections between BC and frail status are largely unexplored, with particular regards for parameters related to inflammaging. The aim of this third study was to detect differences of body composition and health markers that characterize pre-frail or frail individuals. In addition, through regression analysis we define a “frailty signature”. To fulfill this objective, Italian Non-frail and Pre-frail subjects from the NU-AGE Project were selected while Italian Frail subjects belong to the PRO-AGE project.

5.2 METHODS

Study design and population

Within the framework of the European NU-AGE project – New dietary strategies addressing the specific needs of elderly population for a healthy ageing in Europe (Grant Agreement no. 266486, Coordinator Prof. Claudio Franceschi, registered at clinicaltrials.gov as NCT01754012) 2668 free-living elderly people, aged between 65 and 79 y.o., free of major diseases and competent to make own decision, were selected within five European countries (Italy, UK, France, Poland and The Netherlands). After testing the exclusion criteria, i.e. included severe heart diseases, type 1 and insulin-treated type 2 diabetes, chronic use of corticosteroids, recent use of antibiotics or vaccinations, change in habitual medication use, malnutrition (body mass index <18.5 kg/m2 or 10% weight loss within 6 months), or food allergy/intolerance requiring special diets, 1296 were eligible to participate.

Moreover, the presence of frailty (Fried et al., 2001) was one of the exclusion criteria in the NU-AGE project, as the aim was to include healthy elderly (Berendsen et al., 2014).

So, in collaboration with the Nestlé Institute of Health Sciences (NIHS) from Lausanne (Switzerland), one of the partners of the NU-AGE study, a new project has been defined and funded to recruit frail subjects, named PRO-AGE: “Omics for Aging-ProAGE” (n. 14.02. NIHS Code NPDI n. DUND-100373). PRO-AGE uses the same protocol as NU-AGE for the recruitment of subjects, the age is between 65 and 79 years and it has been run in Italy (Bologna). Frailty has been assessed using the standard phenotype proposed by Fried et colleagues and verifying the presence of at least 3, or more, criteria. Given that the 23 PRO-AGE subjects were recruited in Italy, we decided to only include in our study the 271 NU-AGE Italian subjects. Complete DXA scan was performed in 292 participants, at

baseline, and were included from the NU-AGE and PRO-AGE studies cohorts, N= 271 and N = 21 (after removing two drop-out), respectively. NU-AGE was approved by the Ethics Committee of the coordinator center: the Independent Ethics Committee of the S. Orsola-Malpighi Hospital Bologna (Italy), and by the local/national Ethics Committees of all the other four recruiting centers: the South-East 6 Person Protection Committee (France), the Wageningen University Medical Ethics Committee (Netherlands), the National Research Ethics Committee–East of England (UK), and the Bioethics Committee of the Polish National Food and Nutrition Institute (Poland). PRO-AGE was also approved by the Independent Ethic Committee of the S. Orsola-Malpighi Hospital Bologna (Italy).

Assessment of Body Composition

A whole-body DXA scan has been carried out to measure total and regional BC using the fan-beam densitometer Lunar iDXA, GE Healthcare, Madison, WI, USA – enCORETM 2011 software version 13.6 (Bologna, Italy); The scanner was calibrated daily using a standard calibration block supplied by the manufacturers following standard Quality Control procedures. DXA scans were performed by trained technicians according to state-of-the-art technique and manufacturers recommendation.

No metal items were present during densitometry. Participants were positioned in the center of the scanning field in a supine position with the arms at sides and separated from the trunk. As mentioned, measurements of total-body and standard regional body composition, such as trunk, upper limbs, android and gynoid region were defined by DXA. For each region, DXA scanned the weight (in g) of total mass, FM, non-bone LM, and BMC. The weight (in g) of total mass, whole body fat mass (FM), non-bone whole body lean mass (LM), and bone mineral

content (BMC). Specific indices have been used as reported in Table 1 of third chapter. The indexes of total body FM/LM, FMI, and LMI are considered markers of general mass, android/gynoid FM is related of central/peripheral distribution of FM, while the FM/LM android, and ALMI and SMI indices are markers of central abdominal distribution, low muscle mass respectively. Bone mineral density (BMD) and T-score were also considered as markers of bone health. Moreover, DXA has embedded algorithms to specifically estimate the amount of VAT and SAT in the android region (Ponti et al., 2020; Bilsborough et al., 2014).

Data Collection

Height was measured with a stadiometer to the nearest 0.1 cm. Weight was measured to the nearest 0.1 kg with a calibrated scale while wearing light clothes. Body Mass Index (BMI) was calculated as weight [kg]/height[m]2. All measures were taken by trained research assistants.

Plasma total, HDL and LDL cholesterol (mg/dL) and triglycerides (mg/dL) were measured with standard methods.

Concentrations of parathyroid hormone (PTH) in all serum samples was measured with an ELISA kit (intact PTH; MD Biosciences Inc.). Intra-assay and inter- assay CVs were 3.0% and 5.1%, respectively (at a concentration of 47.7 and 52.6 pg/ml, respectively). A 24-h urine collection was obtained for estimation of sodium, potassium, urea and creatinine excretion. The first urine of the day was discarded and all urine over the following 24h were collected. Urinary sodium and potassium were measured by direct potentiometry assay, Olympus AU400 chemistry analyzer by Beckman and urinary creatinine was measured by colorimetric method based on the Jaffe reaction. Glycated haemoglobin was measured on fresh blood by standard methods. Glucose (mg/dL) and

insulin (mcU/mL), were measured on frozen blood and frozen urine (urea) in a centralized centre with standard methodologies.

Dietary intake was assessed by means of validate version of the 7-day food records completed by the participants (Ortega et al., 2015). Consumed foods were converted using a software exploiting local food composition tables: INRAN and IEO. The variability of the food composition was assessed analyzing calories and nutrients of 16 basic foods, i.e. semi-skimmed meal, egg, apple, orange, chicken, breast, beef filet, salmon, tomatoes, peas, nuts, potatoes, lager beer, red brown whole meal, spinach, extra-virgin olive oil).

Statistical analyses

After testing the data distribution, according to Shapiro-Wilk test for Normality ($p < 0.01$) we decided to use non-parametric statistical tests. Characteristics of the studied population and sub-groups were analyzed using Kruskal - Wallis tests or Fisher's exact tests for numerical or categorical data, respectively. R studio (Version 3.3.3 for Windows) was used for the analysis and results are reported as mean and standard deviation (± SD). Because of the 85% of the frail subjects present in the PRO-AGE cohort were females we decided to focus our analyses only on women, in order to avoid sex biases. The significant BC markers plus age and Neutrophils, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC) and Urinary Nitrogen were used to build a multinomial logistic regression. In order to reduce multicollinearity, i.e. the presence of correlation between predictors that can cause less precise estimates, the Spearman's rank correlation coefficient was used to determinate correlation between variables and the most correlated were eliminated one by one to reduce redundant information. In addition, Variance Inflation Factor (VIF) was

used to test multicollinearity and only predictors with VIF < 2 were included in this model. Because of the relatively small sample size in sub-groups, a cross-validation technique was used to ensure that the multinomial logistic regression models were robust. The dataset was iteratively randomly split into a training set, to fit the model, and a test set, to evaluate it, with the "Leave-one-out cross-validation" (LOOCV). The cross-validation has a single hyperparameter "k" that controls the number of splits of the dataset into train and test sets, in the LOOCV k is the number of examples (**Figure 5.2**), for this reason has the maximum computational cost and it is appropriate for small datasets.

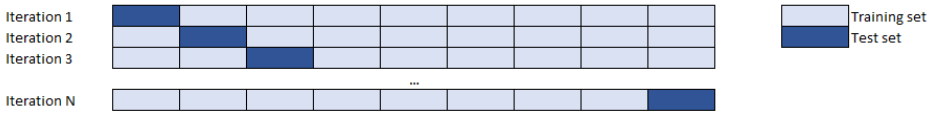


Figure 5.2 | Example of iterations of cross-validation, the sample is split into a training and test set.

The prediction model performance was evaluated using a confusion matrix and area under ROC curves (AUC) (Xia et al., 2013) [R package "pROC" (Robin et al., 2011)].

5.3 RESULTS

Table 5.1 reported all the anthropometric measures and body composition markers analyzed in 292 Italian subjects from the NU-AGE and PRO-AGE cohorts. Differences among Non-Frail, Pre-Frail and Frail subjects are significant for anthropometric measures: frail subjects have higher weight, BMI, waist and hip circumference than the other two groups, while height is lower in frail subjects and waist to hip circumference ratio is not different among the three groups. BC parameters highlight a higher presence of fat mass markers in frail subject with significant differences compared to non-frail and pre-frail in terms of FM, FMI, FM/LM, android FM/LM and gynoid FM/LM. Non-frail subjects have significantly higher lean mass markers in terms of LM and SMI and also higher BMC.

As expected, the Frail group is represented for the 85.7% of female subjects, the prevalence of frailty is indeed higher in women when compared with men (9,1 % and 6.0%, respectively) (Manfredi et al., 2019). For this reason, we decided to analyze females apart. **Table 5.2** reported the anthropometric measures and body composition markers analyzed in 158 Italian female subjects from the NU-AGE and PRO-AGE cohorts. As for the whole Italian group, differences among Non-Frail, Pre-Frail and Frail female subjects are significant for anthropometric measures and BC parameters. In particular, frail subjects have a significantly higher weight, BMI, waist and hip circumference, while no difference emerged for waist to hip circumference ratio. Moreover, frail subjects have significantly higher fat mass markers, such as FM, FMI, FM/LM, Android/Gynoid FM, Android FM/LM, Gynoid FM/LM, VAT and SAT but also higher lean mass markers in terms of LMI and ALMI. While SMI, which represents a marker of sarcopenia (Kim et al., 2016; Guglielmi et al., 2016) is lower than the other two groups. The mean cell volume (MCV) and mean cell hemoglobin (MCH) were lower in frail subject than

the other two groups while white blood cells (WBC), neutrophils and nitrogen were significantly higher. No differences emerged for Total Cholesterol, HDL, LDL, Triglycerides, Glucose and Insulin, while PTH is significantly lower in frail subjects (**Table 5.3**). The significant BC markers, with the exception of PTH, which was measured in a subgroup of subjects, plus age and Neutrophils, MCH, MCHC, WBC and Urinary Nitrogen were studied to build a multinomial logistic regression. **Figure 5.3** reports the correlation matrix based on Spearman's rank correlation. Orange circles highlights negative significant correlations, while violet ones refer to significant positive correlations among variables. As expected, all the fat mass, lean mass and bone markers are significantly correlated among themselves and SMI is negatively correlated with the fat mass markers such as BMI, Waist and Hip Circumference, FM, FMI, FM/LM, while there is no correlation with LMI and ALMI. Obviously, MCV and MCH were positively correlated as well as WBC and Neutrophils. As mentioned, the presence of correlation between predictors can cause multicollinearity and less precise estimates, so the most correlated variables ($\rho < 0.65$) were excluded one by one to reduce redundant information. Age, ALMI, SMI, SAT, MCV, WBC and Urinary Nitrogen were used to build a multinomial logistic regression. The best reduced model included age, SAT, MCV and urinary nitrogen (**Table 5.4**). To evaluate the performance of this model, the AUC, i.e. Area under the ROC curve, was computed with a prediction capacity of 0.83 and accuracy of 0.74. In particular, table shows the sensibility (%) and specificity (%) of the classification for Non-Frail, Pre-Frail and Frail subjects (**Table 5.5**).

5.4 CONLUSIONS

The present study reports evidence of the presence of differences among non-frail and pre-frail subjects and frailty ones.

271 healthy non-frail and pre-frail Italian elderly who participated in the European project NU-AGE and 21 Italian frail participants of the PRO-AGE project have been analyzed. As expected, major differences exist between BC characteristics in elderly frails. It has been shown that BMI and obesity are associated with increased risk of frailty in the elderly (Blaum et al., 2005; Sewo et al., 2016), this is confirmed by our results in both all Italians and the subgroup of females. Though BMI has always been considered a valid tool to assess overall adiposity, it fails to distinguish between the relative contribution of fat mass and lean mass (Blundell et al., 2014). Frail women have higher LMI values than the other two groups, this may be due to the fact that this group have a higher weight than non-frail or pre-frail women. In fact, despite higher values of LMI, the ratio of FM to LM turns out to be significantly higher in frail elderly women. Moreover, the frail group have significantly lower level of SMI, which represents a marker of sarcopenia (Kim et al., 2016; Guglielmi et al., 2016), the combination of high levels of FM and low levels of SMI is more associated with health risk and disability than individual conditions (Roubenoff et al., 2004). SAT and VAT were correlated with multiple metabolic risk factors (Fox et al., 2007), as expected our results reported high level of VAT and SAT for the frail group, in addition SAT seems to be a valid predictor of frailty in elderly female.

The inclusion and exclusion criteria in the NU-AGE and PRO-AGE projects allowed to select healthy subjects, as a result all urinary and blood markers are within the range, although, as shown in the results, there are differences for frail subjects. Low levels of haemoglobin are often associated with low muscle strength or fatigue in frail individuals (Roy et al., 2011), although no significant difference emerged, frail individuals

have the lowest values. In addition, it has been demonstrated that Frailty is associated with higher numbers of neutrophils and monocytes in both males and female (Samson et al., 2019), in accordance with our results. A recent review highlights that different studies have demonstrated a correlation between increased levels of parathyroid hormone (PTH) and frailty while other studies have found that there was no relationship (Saedi et al., 2019). In fact, our results reported significant lower level of PTH for frailty subjects.

As expected, the Frail group is represented for the 85.7% of female subjects, the prevalence of frailty is indeed higher in women when compared with men (9,1 % and 6.0%, respectively) (Manfredi et al., 2019).

However, body composition parameters and urinary and blood markers do not allow discrimination between pre-frail and non-frail or frail females. In fact, all the 25 non-frail subjects of the test set were classified correctly, as well as the 5 frail subjects, while all the 10 pre-frail subjects were classified as non-frail. Despite the Fried et al. scale is the most used, the presence of some components of this phenotype, i.e. low hand grip strength or low gait speed, are more relevant than others. This may affect the correct detection of the pre-frail subject, in fact a systematic review by Fernandez-Garrido et al. (2014) show that the prevalence of pre-frailty can change in different cohorts of people aged over 65, ranging between 35 and 60%. Moreover, 4 out 10 of pre-frail subjects used as test set reverted their status into 'Non-Frail' after one year. **Figure 5.4** shows the presence of criteria in Italian pre-frail subjects, only 9 elderly had 2 criteria, while 47 have only one objective or subjective criteria (25 and 22, respectively). The study has some weaknesses, such as the fact that the number of frail subjects is low, this is because finding frail individuals who fit the PRO-AGE project's inclusion and exclusion criteria is difficult. Although the weaknesses, there are also strengths. Indeed, the fact of

using standardized and accurate tools to study body composition, i.e. DXA, is certainly important.

Table 5.1 | Anthropometric measures and BC markers of Italian Subjects

Characteristics	**Non-Frail (N = 215)**	**Pre-Frail (N = 56)**	**Frail (N = 21)**	***p-value***
Age (*years*)	71.4 ± 3.9	72.7 ± 3.7	74.1 ± 4.3	1.94e-03
Female sex	99 (46.0)	41 (73.2)	18 (85.7)	1.43e-05
Weight (*kg*)	74.5 ± 12.5	71.4 ± 12.6	78.9 ± 13.1	ns
Height (*cm*)	165.0 ± 9.1	159.4 ± 8.4	154.2 ± 7.5	3.27e-05
BMI (kg/m^2)	26.9 ± 3.6	28.0 ± 4.6	32.8 ± 5.9	1.75e-02
Waist circumference (*cm*)	93.4 ± 11.6	92.8 ± 10.8	101.9 ± 11.4	6.80e-03
Hip circumference (*cm*)	101.0 ± 7.1	103.5 ± 8.9	112.3 ± 10.5	9.40e-04
Waist to Hip circumference ratio	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	ns
Body Composition parameters				
FM (*kg*)	26.3 ± 7.2	27.7 ± 8.1	36.4 ± 8.7	9.54e-03
FMI (kg/m^2)	9.7 ± 2.9	11.1 ± 3.4	15.4 ± 4.3	3.54e-06
FM/LM	0.59 ± 0.18	0.68 ± 0.2	0.90 ± 0.19	1.17e-06
LM (*kg*)	45.7 ± 8.6	41.4 ± 7.7	40.5 ± 6.5	3.05e-04
LMI (kg/m^2)	16.6 ± 2.0	16.3 ± 2.1	17.0 ± 2.3	ns
ALMI (kg/m^2)	7.5 ± 1.1	7.2 ± 1.1	7.4 ± 1.2	ns
SMI	0.27 ± 0.03	0.26 ± 0.03	0.23 ± 0.02	9.04e-07
T-score	-0.72 ± 1.1	-0.56 ± 1.27	-0.63 ± 1.2	ns
BMC (*g*)	2500 ± 585	2274 ± 587	2024 ± 491	1.02e-04
BMD (g/cm^2)	1.07 ± 0.15	1.05 ± 0.16	1.03 ± 0.13	ns
Android/Gynoid FM	0.67 ± 0.23	0.61 ± 0.2	0.65 ± 0.13	ns
Android FM/LM	0.77 ± 0.27	0.84 ± 0.31	1.14 ± 0.22	2.78e-04
Gynoid FM/LM	0.60 ± 0.23	0.71 ± 0.22	0.96 ± 0.24	8.22e-06
VAT (*g*)	1587 ± 942	1409 ± 897	1815 ± 831	4.39e-03
SAT (*g*)	1039 ± 453	1225 ± 524	1695 ± 736	1.07e-06
l1 l4 T-score	-0.83 ± 1.4	-0.85 ± 1.35	-0.40 ± 1.85	ns
l1 l4 BMD (g/cm^2)	1.10 ± 0.18	1.08 ± 0.17	1.14 ± 0.22	ns
Neck T-score	-1.41 ± 0.91	-1.37 ± 1.07	-1.68 ± 0.7	ns
Neck BMD (g/cm^2)	0.85 ± 0.13	0.84 ± 0.14	0.80 ± 0.10	ns

BMI, body mass index; FM, fat mass; FMI, fat mass index; LM, lean mass; LMI, non-bone lean mass index; ALMI, non-bone appendicular lean mass index; SMI, skeletal mass index; BMC, bone mineral content; BMD, bone mineral density; Values are means ± SDs, unless otherwise stated. NS, not statistically significant. p-value (Fisher's exact test and Kruskal - Wallis tests);

Table 5.2 | Anthropometric measures and BC markers of Italian Females

Characteristics	**Non-Frail (N = 99)**	**Pre-Frail (N = 41)**	**Frail (N = 18)**	***p-value***
Age (*years*)	71 ± 4	73 ± 4	74 ± 4	4.60e-03
Weight (*kg*)	66.8 ± 10.5	68.5 ± 10.8	76.9 ± 12.8	8.65e-03
Height (*cm*)	157.9 ± 6.3	156.1 ± 6.2	152.8 ± 7.0	1.78e-02
BMI (kg/m^2)	26.4 ± 3.9	28.2 ± 4.9	32.7 ± 6.3	1.29e-04
Waist circumference (*cm*)	86.7 ± 10.1	90.8 ± 10.4	99.8 ± 10.6	2.21e-05
Hip circumference (*cm*)	101.4 ± 8.3	104.3 ± 8.5	112.4 ± 11.3	1.84e-04
Waist to Hip circumference ratio	0.85 ± 0.07	0.87 ± 0.06	0.89 ± 0.08	ns
Body Composition parameters				
FM (*kg*)	27.1 ± 7.6	28.9 ± 7.7	36.6 ± 9.2	3.46e-04
FMI (kg/m^2)	10.9 ± 3.1	11.97 ± 3.2	15.0 ± 4.51	2.34e-05
FM/LM	0.71 ± 0.18	0.76 ± 0.17	0.94 ± 0.18	3.51e-05
LM (*kg*)	37.8 ± 4.2	37.7 ± 3.8	38.5 ± 4.3	ns
LMI (kg/m^2)	15.2 ± 1.4	15.71 ± 2.01	16.6 ± 2.1	4.29e-03
ALMI (kg/m^2)	6.59 ± 0.80	6.83 ± 1.05	7.25 ± 1.09	1.28e-02
SMI	0.25 ± 0.02	0.24 ± 0.02	0.22 ± 0.02	1.71e-04
T-score	-1.21 ± 0.97	-0.93 ± 1.11	-0.80 ± 1.09	ns
BMC (*g*)	1982 ± 269	1987 ± 298	1862 ± 291	ns
BMD (g/cm^2)	0.96 ± 0.10	0.98 ± 0.10	0.99 ± 0.11	ns
Android/Gynoid FM	0.51 ± 0.14	0.54 ± 0.15	0.62 ± 0.10	3.71e-03
Android FM/LM	0.81 ± 0.29	0.90 ± 0.30	1.16 ± 0.23	3.31e-05
Gynoid FM/LM	0.80 ± 0.19	0.81 ± 0.15	1.01 ± 0.23	6.86e-04
VAT (g)	1073 ± 570	1206 ± 699	1470 ± 583	1.54e-02
SAT (g)	1249 ± 451	1376 ± 508	2036 ± 774	1.35e-04
l1 l4 T-score	-1.49 ± 1.22	-1.19 ± 1.21	-0.58 ± 1.94	ns
l1 l4 BMD (g/cm^2)	1.00 ± 0.15	1.03 ± 0.14	1.11 ± 0.23	ns
Neck T-score	-1.57 ± 0.91	-1.53 ± 1.12	-1.69 ± 0.71	ns
Neck BMD (g/cm^2)	0.79 ± 0.11	0.80 ± 0.13	0.79 ± 0.10	ns

BMI, body mass index; FM, fat mass; FMI, fat mass index; LM, lean mass; LMI, non-bone lean mass index; ALMI, non-bone appendicular lean mass index; SMI, skeletal mass index; BMC, bone mineral content; BMD, bone mineral density; Values are means ± SDs, unless otherwise stated. NS, not statistically significant. p-value (Fisher's exact test and Kruskal - Wallis tests);

Table 5.3 | Urinary and Blood Markers of Italian Females

Urinary and Blood Markers	**Non-Frail (N = 99)**	**Pre-Frail (N = 41)**	**Frail (N = 18)**	**p-value**
RBC (*x10^3/ul*)	4.62 ± 0.33	4.64 ± 0.32	4.69 ± 0.32	ns
HGB (*g/dl*)	13.54 ± 0.93	13.42 ± 0.94	13.06 ± 1.12	ns
HCT (*%*)	42.06 ± 2.59	41.85 ± 2.56	40.71 ± 3.24	ns
MCV (*fl*)	91.16 ± 3.60	90.36 ± 3.98	86.91 ± 6.80	2.62e-02
MCH (*pg*)	29.34 ± 1.25	28.97 ± 1.32	27.88 ± 2.38	3.42e-02
MCHC (*g/dl*)	32.18 ± 0.65	32.07 ± 0.70	32.16 ± 0.84	ns
WBC (*x10^3/ul*)	5.44 ± 1.23	5.35 ± 1.25	6.83 ± 1.54	8.24e-04
Neutrophils *(x10^9/L)*	3.02 ± 0.88	3.03 ± 1.00	4.11 ± 1.31	3.04e-03
Lymphocytes *(x10^9/L)*	1.77 ± 0.49	1.67 ± 0.44	1.95 ± 0.48	ns
Monocytes *(x10^9/L)*	0.48 ± 0.13	0.46 ± 0.13	0.53 ± 0.16	ns
Eosinophils *(x10^9/L)*	0.15 ± 0.10	0.16 ± 0.08	0.17 ± 0.10	ns
Basophils *(x10^9/L)*	0.03 ± 0.01	0.03 ± 0.02	0.04 ± 0.03	ns
Platelets (*x10^3/ul*)	233.6 ± 49.4	230.4 ± 57.3	235.3 ± 53.3	ns
Glycated Hemoglobin (*%*)	5.62 ± 0.34	5.76 ± 0.64	5.82 ± 0.87	ns
Urinary Sodium (*mmol/L*)	72.1 ± 41.6	67.8 ± 32.4	74.6 ± 27.8	ns
Urinary Potassium (*mmol/L*)	39.0 ± 26.8	37.0 ± 35.4	34.2 ± 8.8	ns
Urinary Creatinine (*g/24h*)	0.95 ± 0.22	0.95 ± 0.23	0.90 ± 0.20	ns
Urinary Urea (*g/24h*)	16.4 ± 4.3	15.9 ± 5.3	15.3 ± 4.2	ns
Urinary Nitrogen (*g/24h*)	0.44 ± 0.20	0.42 ± 0.18	2.24 ± 0.98	4.84e-07
Total Cholesterol (*mg/dL*)	208.5 ± 34.2	201.1 ± 28.1	194.7 ± 42.6	ns
HDL (*mg/dL*)	61.8 ± 15.9	62.0 ± 11.9	58.2 ± 17.7	ns
LDL (*mg/dL*)	125.6 ± 29.3	119.9 ± 29.4	121.4 ± 30.1	ns
Triglycerides (*mg/dL*)	105.7 ± 46.2	95.8 ± 29.1	125.4 ± 54.3	ns
Glucose (*mg/dL*)	99.7 ± 10.3	101.9 ± 15.6	109.2 ± 31.1	ns
Insulin (*mcU/ml*)	8.9 ± 5.6	8.7 ± 5.2	10.8 ± 5.4	ns
PTH (*pg/mL*)	51.9 ± 25.6	56.1 ± 26.6	34.6 ± 11.2	1.83e-03

Population based reference ranges for: RBC: 4.2-5.5 x10^3/ul; HGB: 13.0-16.5 g/dl; HCT: 39.0-54.0 %; MCV: 82.0-99.0 fl; MCH: 27.0-32.0 pg; MCHC: 33.0-38.0 g/dl; WBC: 4.8-8.5 x10^3/ul; Neutrophils: 2.0-7.5 *x10^9/L*; Lymphocytes: 1.0-4.0 *x10^9/L*; Monocytes: 0.2-1.0 *x10^9/L*; Eosinophils: 0.0-0.5 *x10^9/L*; Basophils: 0.0-0.15 *x10^9/L*; Platelets: 130-400 x10^3/ul; Glycated Haemoglobin: < 7.5%; Urinary Sodium: 50-250 (mmol/L); Urinary Potassium: 30-120 (mmol/L); Urinary Creatinine: D: 0.7-1.6 (g/24h); Urinary Urea:10-30 g/24h; Urinary Nitrogen (g/24h): 10-35; Total Cholesterol: <200mg/dL; HDL: > 60mg/dL; LDL: <100 mg/dL; Triglycerides: <150 mg/dL; Glucose (serum): 60-110 mg/dL; Insulin: 2-25 mcU/ml; PTH (serum): 10-70 pg/mL; p- value (Kruskal – Wallis test).

Figure 5.3 | Correlation Matrix based on Spearman's rank correlation. Orange circles highlights inverse significant correlations, while violet ones refer to significant positive correlations among variables.

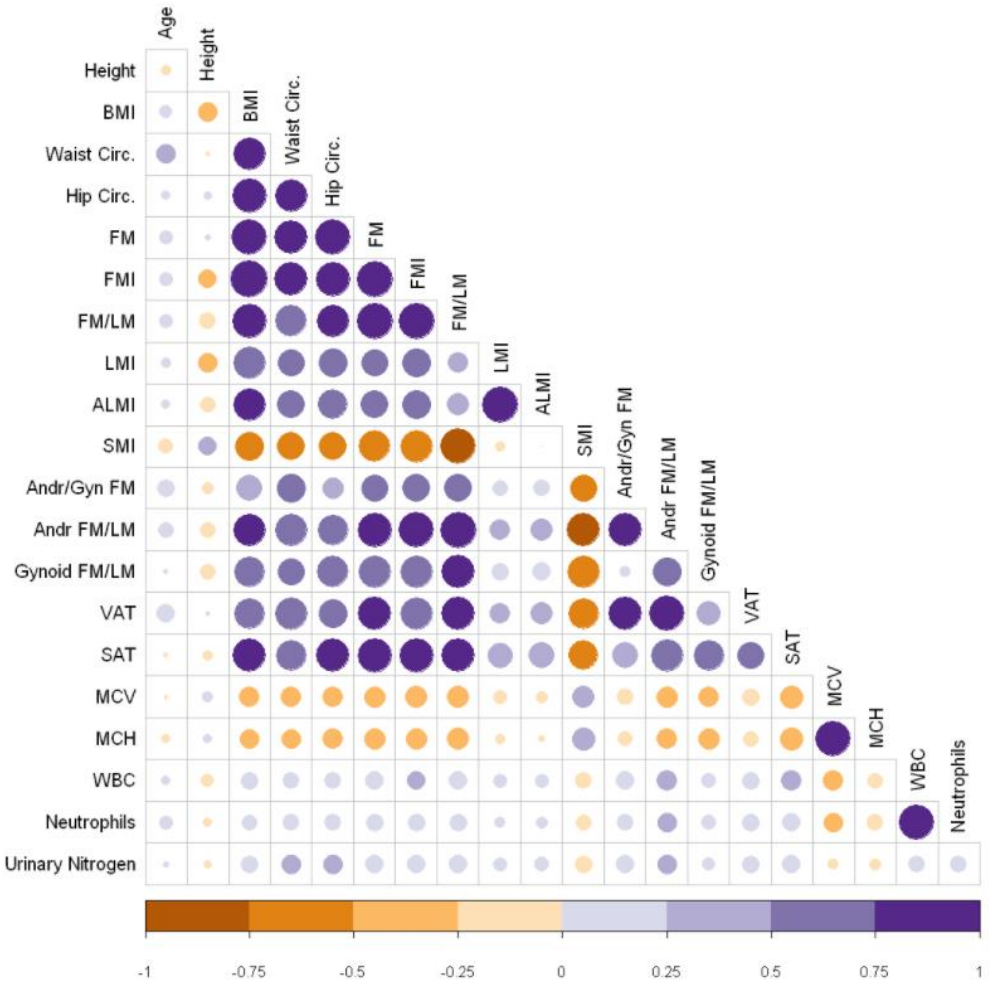


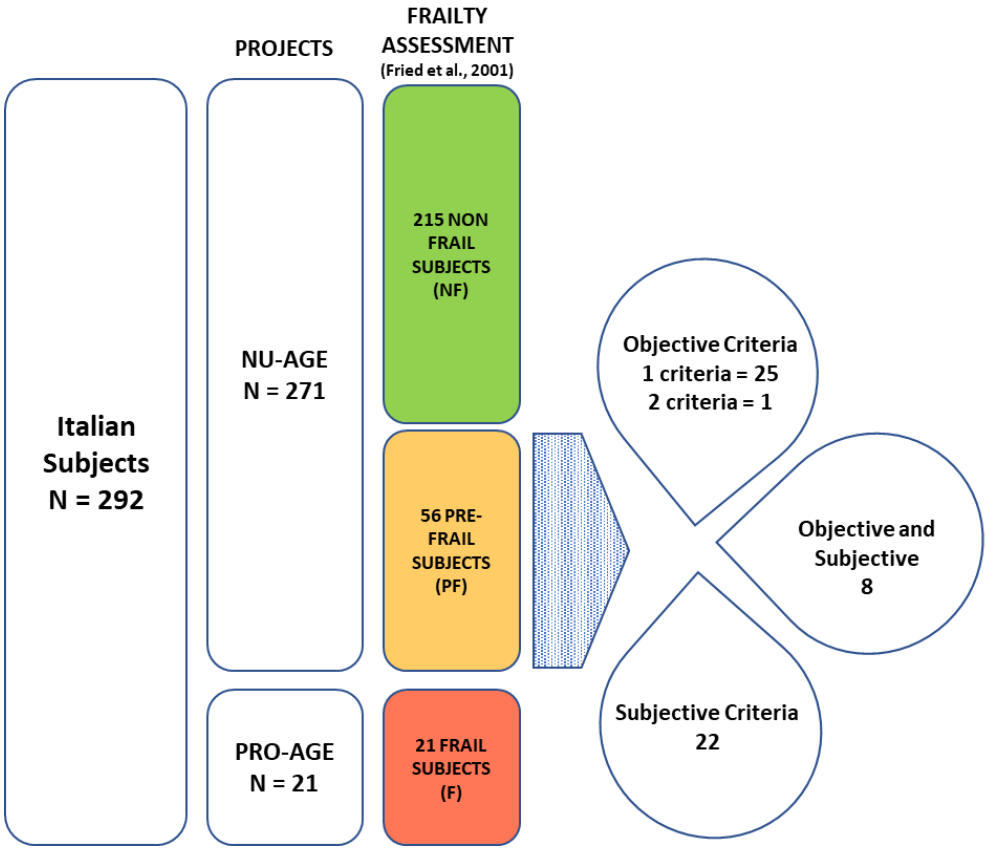
Table 5.4 | Multinomial logistic regression for the prediction of Pre-Frail and Frail status at baseline from BC and Urinary and Blood Markers.

		Coefficent β	Standard error	Pr(>|z|)		Odd ratios	Confidence Interval
Pre-Frail	(Intercept)	-4.5e-01	6.9e-04	< 2.2e-16	***		
	Age	8.4e-02	4.1e-02	0.04	*	1.09	(1.003; 1.180)
	SAT	4.3e-04	4.0e-04	ns		1.00	(0.999; 1.001)
	MCV	-7.8e-02	3.2e-02	0.02	*	0.92	(0.867; 0.986)
	Urinary Nitrogen	5.1e-02	1.5e-01	ns		1.05	(0.771; 1.436)
Frail	(Intercept)	6.1e-01	8.2e-04	< 2.2e-16	***		
	Age	1.8e-01	6.9e-02	0.009	**	1.20	(1.045; 1.374)
	SAT	1.9e-03	6.0e-04	< 0.001	***	1.00	(1.000; 1.003)
	MCV	-1.9e-01	5.6e-02	< 0.001	***	0.82	(0.735; 0.918)
	Urinary Nitrogen	2.8e-01	1.8e-01	ns		1.32	(0.919; 1.910)

Table 5.5 | Sensibility and specificity of the multinomial logistic regression

	Sensibility (%)	Specificity (%)
Non-Frail	71 %	100 %
Pre-Frail	0 %	74%
Frail	100 %	100%

Figure 5.4 | Frailty status distribution of Italian subjects and a focus on criteria in pre-frail subjects. Subjective criteria include: unintentional weight loss (4.5 kg in the year before the evaluation), self-reported exhaustion and reduce energy consumption while objective criteria include hand grip strength and low gait speed.



6. FINAL REMARKS

In this final chapter I wrap up the main conclusions of the three studies reported in this PhD work. As mentioned in Chapter 2, "Aims of the studies", the main scientific questions I had try to answer were:

- Is body composition in elderly across Europe different? Are there Body composition differences by sex?
- Which are the inflammatory and metabolic markers associated with body composition in the elderly?
- Which are the main differences of body composition and health markers that characterize frail individuals? Is there a BC "frailty signature"?

The 1121 elderly participants to the European project NU-AGE have been thoroughly studied for their dietary intake (Berendsen et al., 2014) and their anthropometric, metabolic, physical and cognitive status (Santoro et al., 2014), in particular DXA scan was performed to evaluate their BC in terms of fat, lean and bone mass.

Due to significant aging-related depletion of sex hormones such as rapid loss of estradiol and progesterone in women after menopause, it has been thought in the past that, as far as BC, women would become more similar to men as they get older. On the contrary, many results (Lauretta et al., 2017), including ours, demonstrate that there is a great difference among BC in elderly women and men. In fact, female aged 65 or more years old tend to have higher fat mass, in particular in the gynoid region, but lower lean and bone mass than males aged same. In addition, women and man are different in terms of bone content, in fact the prevalence of osteoporosis is higher in women. Our results showed also geographic differences in BC across the 5 countries of NU-AGE project: Italy, France,

the Netherlands, Poland, and the United Kingdom. The highest values of lean mass markers were reported for French subjects as respect to other countries. On the contrary, Polish participants have the higher values of fat mass markers while the highest value of bone mass markers is reported for Dutch elderly. These differences among the 5 countries could be attributed to genetic predisposition, dietary habits, lifestyle or physical activity, stress or education, because the criteria for exclusion and inclusion in the study were the same for all countries (Santoro et al., 2014). After identifying through a cluster analysis six clusters for elderly males and five clusters for elderly females using the values of FM, FMI, LM, LMI, ALMI, FM/LM, SMI, t-score, BMC and BMD in addition to BMI, different metabolic parameters were compared across BC clusters. It is well known that an increase in fat mass, together with aging, can cause medical complications, such as hypertension, diabetes and cardiovascular disease. Even if the metabolic parameters are in the normal range, due to the exclusion and inclusion criteria of the project, differences between clusters emerged. In fact, subjects within the cluster with lowest BMI have the highest HDL cholesterol levels and the lowest triglycerides, glucose, insulin, HOMA, urea compared with the other clusters and females have the lowest level of diastolic pressure. At variance, the clusters with higher BMI have the lowest HDL cholesterol levels and the highest triglycerides, glucose, insulin, HOMA (IR and β) and urea and diastolic pressure (only females) levels compared with the other clusters. The similar levels of total cholesterol and LDL among the clusters may be explained by the fact that the number of subjects taking statins does not change among clusters. Additionally, subjects in cluster with highest values of LM markers have also highest values of handgrip strength.

Moreover, it has been reported that an increase in fat mass is correlated with markers of inflammation among elderly (Brinkley et al., 2018; Schrager et al., 2007), our results highlights a significant negative

association between ghrelin and fat mass in females but not in males, while a positive association with SMI has been found in both genders. Leptin may play a role as a pro-inflammatory molecule in the setting of obesity (Matarese et al., 2005) along with resistin, whereas adiponectin and ghrelin have anti-inflammatory properties. Leptin is an adipokine secreted by adipocytes, generally increases with weight gain, and decreases with weight loss (Spiegelman BM and Flier JS, 2001), but it has been demonstrated that leptin is also produced by skeletal muscle (Fernández-Real et al., 2000; Wolks et al., 2012) as well as bone cells (Thomas T, 2004) in fact a significant positive association between fat mass, lean mass and bone mass markers and leptin levels has been found in our study. However, our results concord with a recent paper by Baker and colleagues demonstrating that in the elderly high serum levels of adiponectin are significantly correlated with low BMI, fat and lean mass BC markers (Baker et al., 2018)(Baker et al., 2018)(Baker et al., 2018)(Baker et al., 2018). Moreover, in males and females' clusters adiponectin levels decrease as BMI increases. However, it is interesting to note that among the five clusters of females, the two clusters with similar BMI (25.09 and 26.62 respectively) have different adiponectin levels, those with higher levels are in the overweight group also characterized by higher levels of fat and bone mass and lower levels of lean mass. A similar result is found among the two low obesity clusters (BMI 31.48 and 31.92 respectively) even if it is not significant.

At variance, the only significant difference of adiponectin levels among the six male clusters is found between the low obesity B and the normal weight clusters which have higher levels. A similar trend also emerged when comparing the two clusters (overweight A vs B and low obesity A vs B) with similar BMI but different amount of fat, lean and bone mass.

Regarding inflammatory markers, a significant positive correlation between CRP and fat mass markers emerged while there is a negative

association with SMI in both females and males. CRP and AGP are positively correlated with lean mass markers in female. In addition, AGP is positively correlated LMI and negatively associated with SMI in female, while in male the only significant association between AGP levels and BC markers is a positive correlation with android/gynoid FM ratio. Different studies had demonstrated an association between the specific pattern of increased CRP and decreased albumin concentrations with sarcopenia, frailty and vascular and non-vascular mortality in elderly subjects (Clarke et al., 2008; Hubbard et al., 2009). Even if no correlation has been found between BC markers and indexes and circulating levels of a series of pro- and anti-inflammatory molecules such as IL6, Pentraxin 3, IL10, TGFβ1, TNFα, IL6rα, gp130, TNFαR1 and TNFαR2, there are evidence that there are associations between fat mass, BMI and waist circumference and inflammatory markers (Brinkley et al., 2012; Schrager et al., 2007; Cesari et al., 2005).

Finally, a focus on the Italian population was carried out in 271 healthy non-frail and pre-frail elderly who participated in the European project NU-AGE and 21 frail participants of the PRO-AGE project. The prevalence of frailty is higher in women when compared with men (9,1 % and 6.0%, respectively) (Manfredi et al., 2019), considering that the results of our study confirm these findings, i.e. Frail group is represented for the 85.7% of female subjects, we decided to analyze females apart.

As expected, major differences exist between BC characteristics in elderly frails. Frail women have higher LMI values than the other two groups, this may be due to the fact that this group have a higher weight than non-frail or pre-frail women. In fact, despite higher values of LMI, the ratio of FM to LM turns out to be significantly higher in frail elderly women. Moreover, the frail group have significantly lower level of SMI, which represents a marker of sarcopenia (Kim et al., 2016; Guglielmi et al., 2016), the combination of high levels of FM and low levels of SMI is more

associated with health risk and disability than individual conditions (Roubenoff et al., 2004). SAT and VAT were correlated with multiple metabolic risk factors (Fox et al., 2007), as expected our results reported high level of VAT and SAT for the frail group, in addition SAT seems to be a valid predictor of frailty in elderly female. Low levels of haemoglobin are often associated with low muscle strength or fatigue in frail individuals (Roy et al., 2011), although no significant difference emerged, frail individuals have the lowest values. In addition, it has been demonstrated that Frailty is associated with higher numbers of neutrophils and monocytes in both males and female (Samson et al., 2019), in accordance with our results.

In conclusion, the results presented in this study provide a synthesis of the health status of elderly subjects in Europe that can be used as a reference for studies related to gender differences in body composition, disease conditions, and differences between European countries. The study has some weaknesses, such as the fact that the subjects are healthy volunteers, highly educated and interested in nutrition and health issues and therefore may not be representative of the population of the same age. In addition, the last part of the study (on frailty) has a low sample size, due to the fact that only Italian women were analyzed. The main strength is constituted by the use of a standardized and accurate method as DXA was performed at European level to assessed BC composition, as well as the availability of a high number of parameters per each subject yielding data of a high quality.

As a whole, this work indicated the importance of BC in the aging process, however, as we focused mainly on healthy volunteers and only in the last part of the study we included a certain number of people with signs of frailty, the conclusions could underestimate the importance of BC parameters in frankly pathological situations (characterized for instance

by elevated inflammaging), therefore this warrants future studies that will include not only healthy elderly volunteers but also patients.

7. REFERENCES

Arsenault, B. J., Beaumont, E. P., Després, J.-P., and Larose, E. (2012). Mapping body fat distribution: A key step towards the identification of the vulnerable patient? Annals of Medicine 44, 758–772. doi:10.3109/07853890.2011.605387.

Baker JF, Newman AB, Kanaya A, et al (2018) The Adiponectin Paradox in the Elderly: Associations With Body Composition, Physical Functioning, and Mortality. The Journals of Gerontology: Series A 0:1–7. doi: 10.1093/gerona/gly017

Bartell SM, Rayalam S, Ambati S, et al (2011) Central (ICV) leptin injection increases bone formation, bone mineral density, muscle mass, serum IGF-1, and the expression of osteogenic genes in leptin-deficient ob/ob mice. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 26:1710–20. doi: 10.1002/jbmr.406

Bas S, Finckh A, Puskas GJ, et al (2014) Adipokines correlate with pain in lower limb osteoarthritis: different associations in hip and knee. International Orthopaedics 38:2577–2583. doi: 10.1007/s00264-014-2416-9

Bazzocchi, A., Diano, D., Ponti, F., Andreone, A., Sassi, C., Albisinni, U., et al. (2013). Health and ageing: a cross-sectional study of body composition. Clin. Nutr. 32, 569–578. doi: 10.1016/j.clnu.2012.10.004.

Bazzocchi A, Diano D, Ponti F, et al (2014) A 360-degree overview of body composition in healthy people: Relationships among anthropometry, ultrasonography, and dual-energy x-ray absorptiometry. Nutrition 30:696–701. doi: 10.1016/j.nut.2013.11.013

Bazzocchi, A., Ponti, F., Albisinni, U., Battista, G., and Guglielmi, G. (2016). DXA: Technical aspects and application. European Journal of Radiology 85, 1481–1492. doi:10.1016/j.ejrad.2016.04.004.

Beavers, K. M., Beavers, D. P., Houston, D. K., Harris, T. B., Hue, T. F., Koster, A., et al. (2013). Associations between body composition and gait-speed decline: results from the Health, Aging, and Body

Composition study 1–4. Am J Clin Nutr 97, 552–60. doi:10.3945/ajcn.112.047860.

Berendsen, A., Santoro, A., Pini, E., Cevenini, E., Ostan, R., Pietruszka, B., et al. (2014). Reprint of: a parallel randomized trial on the effect of a healthful diet on inflammageing and its consequences in European elderly people: design of the NU-AGE dietary intervention study. Mech. Ageing Dev. 136–137, 14–21. doi: 10.1016/j.mad.2014. 03.001

Berendsen AAM, van de Rest O, Feskens EJM, Santoro A, Ostan R, Pietruszka B, Brzozowska A, Stelmaszczyk-Kusz A, Jennings A, Gillings R, Cassidy A, Caille A, Caumon E, Malpuech-Brugere C, Franceschi C, de Groot LCPGM. Changes in Dietary Intake and Adherence to the NU-AGE Diet Following a One-Year Dietary Intervention among European Older Adults-Results of the NU-AGE Randomized Trial. Nutrients. 2018 Dec 4;10(12):1905. doi: 10.3390/nu10121905. PMID: 30518044; PMCID: PMC6315357.

Bilsborough JC, Greenway K, Opar D, Livingstone S, Cordy J, Coutts AJ. The accuracy and precision of DXA for assessing body composition in team sport athletes. J Sports Sci. 2014;32(19):1821-8. doi: 10.1080/02640414.2014.926380. Epub 2014 Jun 10. PMID: 24914773.

Bjorntorp, P. (1991). Metabolic implications of body fat distribution. Diabetes Care 14, 1132–1143.

Blaum CS, Xue QL, Michelon E, Semba RD, Fried LP. The association between obesity and the frailty syndrome in older women: the women's health and aging studies. J Am Geriatr Soc. 2005;53 (6):927–934. doi:10.1111/jgs.2005.53.issue-6

Blundell, J., Dulloo, A., Salvador, J., and Fruhbeck, G. (2014). Beyond BMI – phenotyping the obesities. Obes Facts 7, 322–328.

Blundell, J. E., Baker, J. L., Boyland, E., Blaak, E., Charzewska, J., De Henauw, S., et al. (2017). Variations in the Prevalence of Obesity Among European Countries, and a Consideration of Possible Causes. Obes Facts 10, 25–37. doi:10.1159/000455952.

Bosy-Westphal, A., Kahlhöfer, J., Lagerpusch, M., Skurk, T., and Müller, M. J. (2015). Deep body composition phenotyping during weight cycling: Relevance to metabolic efficiency and metabolic risk. Obesity Reviews 16, 36–44. doi:10.1111/obr.12254.

Brinkley TE, Hsu F-C, Beavers KM, et al (2012) Total and Abdominal Adiposity Are Associated With Inflammation in Older Adults Using a Factor Analysis Approach. The Journals of Gerontology: Series A 67:1099–1106. doi: 10.1093/gerona/gls077

Britton, KA, Massaro, JM, Murabito, JM, Kreger, BE, Hoffmann, U, Fox, C. (2013). Body fat distribution, incident cardiovascular disease, cancer, and all-cause

Carmienke, S., Freitag, M. H., Pischon, T., Schlattmann, P., Fankhaenel, T., Goebel, H., et al. (2013). General and abdominal obesity parameters and their combination in relation to mortality: a systematic review and meta-regression analysis. Eur. J. Clin. Nutr. 67, 573–585. doi: 10.1038/ejcn. 2013.61

Cesari M, Kritchevsky SB, Baumgartner RN, et al (2005) Sarcopenia, obesity, and inflammation--results from the Trial of Angiotensin Converting Enzyme Inhibition and Novel Cardiovascular Risk Factors study. The American journal of clinical nutrition 82:428–34. doi: 10.1093/ajcn.82.2.428

Cevenini, E., Monti, D., and Franceschi, C. (2013). Inflamm-ageing. Curr. Opin.Clin. Nutr. Metab. Care 16, 14–20. doi: 10.1097/MCO.0b013e32835ada13

Chalise, P., Koestler, D. C., Bimali, M., Yu, Q., and Fridley, B. L. (2014). Integrative clustering methods for high-dimensional molecular data. Transl Cancer Res 3, 202–216.

Chandrasekar B, Boylston WH, Venkatachalam K, et al (2008) Adiponectin blocks interleukin-18-mediated endothelial cell death via APPL1-dependent AMP-activated protein kinase (AMPK) activation and IKK/NF-kappaB/PTEN suppression. The Journal of biological chemistry 283:24889–98. doi: 10.1074/jbc.M804236200

Cheng FW, Gao X, J. G. (2015). Weight change and all-cause mortality in older adults: a meta-analysis. J Nutr Gerontol Geriatr 34, 343–368.

Clarke R, Emberson JR, Breeze E, et al (2008) Biomarkers of inflammation predict both vascular and non-vascular mortality in older men. European Heart Journal 29:800–809. doi: 10.1093/eurheartj/ehn049

Diano D, Ponti F, Guerri S, et al (2017) Upper and lower limbs composition: a comparison between anthropometry and dual-

energy X-ray absorptiometry in healthy people. Archives of Osteoporosis. doi: 10.1007/s11657-017-0374-8

Elmer, R., and Alswat, K. A. (2017). Gender Disparities in Osteoporosis. J Clin Med Res 9, 382–387. doi:10.14740/jocmr2970w.

Eurostat (2016). European Health Interview Survey. Almost 1 adult in 6 in the EU is considered obese. EUROSTAT: news release 2014, 1–5.

Eurostat (2019). The EU's population projected up to 2100.

Eurostat (2020). Elderly population across EU regions. (a)

Eurostat (2020). World Population Day: Population trends up to 2100. (b)

Eurostat (2020). Weekly death statistics. (c)

Fernandez-Garrido, J., Ruiz-Ros, V., Buigues, C., Navarro-Martinez, R., and Cauli, O. (2014). Clinical features of prefrail older individuals and emerging peripheral biomarkers: a systematic review. Arch. Gerontol. Geriatr. 59, 7–17. doi: 10.1016/j.archger.2014.02.008

Fernández-Real J-M, Vayreda M, Casamitjana R, et al (2000) The fat-free mass compartment influences serum leptin in men. European Journal of Endocrinology 142:25–29.

Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. Nat Rev Cardiol. 2018 Sep;15(9):505-522. doi: 10.1038/s41569-018-0064-2. PMID: 30065258; PMCID: PMC6146930.

Fox CS, Massaro JM, Hoffmann U, et al (2007) Abdominal visceral and subcutaneous adipose tissue compartments: Association with metabolic risk factors in the framingham heart study. Circulation 116:39–48. doi: 10.1161/CIRCULATIONAHA.106.675355

Franceschi C, Bonafè M, Valensin S, et al (2000) Inflamm-aging. An evolutionary perspective on immunosenescence. Annals of the New York Academy of Sciences 908:244–254. doi: 10.1111/j.1749-6632.2000.tb06651.x

Franceschi, C., Capri, M., Monti, D., Giunta, S., Olivieri, F., Sevini, F., et al. (2007). Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech. Ageing Dev. 128, 92–105.doi: 10.1016/j.mad.2006.11.016

Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci. 2014 Jun;69 Suppl 1:S4-9. doi: 10.1093/gerona/glu057. PMID: 24833586.

Franceschi C, Garagnani P, Vitale G, et al (2016) Inflammaging and "Garb-aging." Trends in Endocrinology & Metabolism 0:2609–2617. doi: 10.1016/j.tem.2016.09.005

Franceschi C (2017) Healthy ageing in 2016: Obesity in geroscience — is cellular senescence the culprit? Nature Reviews Endocrinology 13:76–78. doi: 10.1038/nrendo.2016.213

Franceschi C, Garagnani P, Morsiani C, Conte M, Santoro A, Grignolio A, Monti D, Capri M, Salvioli S. The Continuum of Aging and Age-Related Diseases: Common Mechanisms but Different Rates. Front Med (Lausanne). 2018 Mar 12;5:61. doi: 10.3389/fmed.2018.00061. PMID: 29662881; PMCID: PMC5890129.

Freisling H, Arnold M, Soerjomataram I, et al (2017) Comparison of general obesity and measures of body fat distribution in older adults in relation to cancer risk: meta-analysis of individual participant data of seven prospective cohorts in Europe. doi: 10.1038/bjc.2017.106

Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci. 2001;56(3):146–56.

Guglielmi, G., Ponti, F., Agostini, M., Amadori, M., Battista, G., and Bazzocchi, A. (2016). The role of DXA in sarcopenia. Aging Clin. Exp. Res. 28, 1047–1060. doi: 10.1007/s40520-016-0589-3

Hamrick MW, Ding K-H, Ponnala S, et al (2008) Caloric Restriction Decreases Cortical Bone Mass but Spares Trabecular Bone in the Mouse Skeleton: Implications for the Regulation of Bone Mass by Body Weight. Journal of Bone and Mineral Research 23:870–878. doi: 10.1359/jbmr.080213 (a)

Hamrick MW, Ferrari SL (2008) Leptin and the sympathetic connection of fat to bone. Osteoporosis International 19:905–912. doi: 10.1007/s00198-007-0487-9 (b)

Hamrick MW (2017) Role of the Cytokine-like Hormone Leptin in Muscle-bone Crosstalk with Aging. Journal of Bone Metabolism 24:1. doi: 10.11005/jbm.2017.24.1.1

Hinton, B. J., Fan, B., Ng, B. K., and Shepherd, J. A. (2017). Dual energy X-ray absorptiometry body composition reference values of limbs and trunk from NHANES 1999–2004 with additional visualization methods. PLOS ONE | 12, e0174180. doi:10.1371/journal.pone.0174180.

Hotamisligil GS (2017) Inflammation, metaflammation and immunometabolic disorders. Nature. doi: 10.1038/nature21363

Hubbard RE, O'Mahony MS, Savva GM, et al (2009) Inflammation and frailty measures in older people. Journal of cellular and molecular medicine 13:3103–9. doi: 10.1111/j.1582-4934.2009.00733.x

Istat 2020. Rapporto annual 2020 in pillole.

Jafarinasabian P, Inglis JE, Reilly W, Kelly OJ (2017) Aging human body: changes in bone, muscle and body fat with consequent changes in nutrient intake. doi: 10.1530/JOE-16-0603

Kane AE, Sinclair DA. Frailty biomarkers in humans and rodents: Current approaches and future advances. Mech Ageing Dev. 2019 Jun;180:117-128. doi: 10.1016/j.mad.2019.03.007. Epub 2019 Apr 17. PMID: 31002925; PMCID: PMC6581034.

Kelly, T. L., Wilson, K. E., and Heymsfield, S. B. (2009). Dual Energy X-Ray Absorptiometry Body Composition Reference Values from NHANES. PLoS ONE 4. doi:10.1371/journal.pone.0007038.

Kennedy BK, Berger SL, Brunet A, Campisi J, Cuervo AM, Epel ES, Franceschi C, Lithgow GJ, Morimoto RI, Pessin JE, Rando TA, Richardson A, Schadt EE, Wyss-Coray T, Sierra F. Geroscience: linking aging to chronic disease. Cell. 2014 Nov 6;159(4):709-13. doi: 10.1016/j.cell.2014.10.039. PMID: 25417146; PMCID: PMC4852871.

Kern PA , Ranganathan S , Li C , Wood L RG. (2001) Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am J Physiol Endocrinol Metab 1:E745 – E751.

Kim KM, Jang HC, Lim S (2016) Differences among skeletal muscle mass indices derived from height-, weight-, and body mass index-

adjusted models in assessing sarcopenia. Korean Journal of Internal Medicine 31:643–650. doi: 10.3904/kjim.2016.015

Lauretta, R., Sansone, M., Romanelli, F., and Appetecchia, M. (2017). Gender in endocrinological diseases: biological and clinical differences. Ital J Gender-Specific Med 3, 109–116. Available at: http://www.gendermedjournal.it/r.php?v=2882&a=29060&l=333657&f=allegati/02882_2017_03/fulltext/109-116_Review_Appetecchia.pdf [Accessed May 3, 2018].

Lemos T, Gallagher D (2017) Current body composition measurement techniques. Current Opinion in Endocrinology & Diabetes and Obesity 24:310–314. doi: 10.1097/MED.0000000000000360

Mancuso P (2016) The role of adipokines in chronic inflammation. ImmunoTargets and therapy 5:47–56. doi: 10.2147/ITT.S73223

Manfredi G, Midão L, Paúl C, Cena C, Duarte M, Costa E. Prevalence of frailty status among the European elderly population: Findings from the Survey of Health, Aging and Retirement in Europe. Geriatr Gerontol Int. 2019 Aug;19(8):723-729. doi: 10.1111/ggi.13689. Epub 2019 May 30. PMID: 31146300.

Marcos-Pérez D, Sánchez-Flores M, Proietti S, Bonassi S, Costa S, Teixeira JP, Fernández-Tajes J, Pásaro E, Laffon B, Valdiglesias V. Association of inflammatory mediators with frailty status in older adults: results from a systematic review and meta-analysis. Geroscience. 2020 Aug 15. doi: 10.1007/s11357-020-00247-4. Epub ahead of print. PMID: 32803650.

Marttila S, Jylhävä J, Nevalainen T, et al (2013) Transcriptional analysis reveals gender-specific changes in the aging of the human immune system. PloS one 8:e66229. doi: 10.1371/journal.pone.0066229

Matarese G, Moschos S, Mantzoros CS (2005) Leptin in immunology. Journal of immunology (Baltimore, Md : 1950) 174:3137–42. doi: 10.4049/JIMMUNOL.174.6.3137

Miljkovic, I., Kuipers, A. L., Cauley, J. A., Prasad, T., Lee, C. G., Ensrud, K. E., et al. (2015). Greater Skeletal Muscle Fat Infiltration Is Associated With Higher All-Cause and Cardiovascular Mortality in Older Men ; for the Osteoporotic Fractures in Men Study Group. J Gerontol A Biol Sci Med Sci 70, 1133–1140. doi:10.1093/gerona/glv027.

Morrisette-Thomas V, Cohen AA, Fü lö T, et al (2014) Inflamm-aging does not simply reflect increases in pro-inflammatory markers. Mechanisms of Ageing and Development 139:49–57. doi: 10.1016/j.mad.2014.06.005

Morley, J. E., Vellas, B., van Kan, G. A., Anker, S. D., Bauer, J. M., Bernabei, R., et al. (2013). Frailty consensus: a call to action. J. Am. Med. Dir. Assoc. 14, 392–397. doi: 10.1016/j.jamda.2013.03.022.

Müller MJ, Geisler C, Pourhassan M, et al (2014) Assessment and definition of lean body mass deficiency in the elderly. European Journal of Clinical Nutrition 68:1220–1227. doi: 10.1038/ejcn.2014.169

Murdolo G, Lucidi P, Di Loreto C, et al (2003) Insulin is Required for Prandial Ghrelin Suppression in Humans. Diabetes. doi: 10.2337/diabetes.52.12.2923

Ortega RM, Pérez-Rodrigo C, López-Sobaler AM. Dietary assessment methods: dietary records. Nutr Hosp. 2015 Feb 26;31 Suppl 3:38-45. doi: 10.3305/nh.2015.31.sup3.8749. PMID: 25719769.

Petak, S., Barbu, C. G., Yu, E. W., Fielding, R., Mulligan, K., Sabowitz, B., et al. (2013). The Official Positions of the International Society for Clinical Densitometry: Body Composition Analysis Reporting. Journal of Clinical Densitometry 16, 508–519. doi:10.1016/j.jocd.2013.08.018.

Pino AM, Ríos S, Astudillo P, et al (2010) Concentration of adipogenic and proinflammatory cytokines in the bone marrow supernatant fluid of osteoporotic women. Journal of Bone and Mineral Research 25:492–498. doi: 10.1359/jbmr.090802

Pischon T, Boeing H, Hoffmann K, et al (2008) General and Abdominal Adiposity and Risk of Death in Europe. N Engl J Med 20359:2105–20. doi: 10.1056/NEJMoa0801891

Ponti F, Santoro A, Mercatelli D, Gasperini C, Conte M, Martucci M, Sangiorgi L, Franceschi C, Bazzocchi A. Aging and Imaging Assessment of Body Composition: From Fat to Facts. Front Endocrinol (Lausanne). 2020 Jan 14;10:861. doi: 10.3389/fendo.2019.00861. PMID: 31993018; PMCID: PMC6970947.

Prattichizzo F, De Nigris V, Spiga R, et al (2018) Inflammageing and metaflammation: The yin and yang of type 2 diabetes. Ageing Research Reviews 41:1–17. doi: 10.1016/j.arr.2017.10.003

Prentice, A. M., and Jebb, S. A. (2001). Beyond body mass index. Obes. Rev. 2, 141–147. doi: 10.1046/j.1467-789x.2001.00031.x

Robin, X., Turck, N., Hainard, A., Tiberti, N., Lisacek, F., Sanchez, J. C., et al. (2011). pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics 12:77. doi: 10.1186/1471-2105-12-77

Roubenoff R. Sarcopenic obesity: the confluence of two epidemics. Obes Res. 2004;12(6):887–888. doi:10.1038/oby.2004.107

Roy CN. Anemia in frailty. Clin Geriatr Med. 2011 Feb;27(1):67-78. doi: 10.1016/j.cger.2010.08.005. PMID: 21093723; PMCID: PMC2998908.

Reinders, I., Murphy, R. A., Brouwer, I. A., Visser, M., Launer, L., Siggeirsdottir, K., et al. (2015a). Original Contribution Muscle Quality and Myosteatosis: Novel Associations With Mortality Risk The Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study. Am J Epidemiology 183. doi:10.1093/aje/kwv153.

Reinders, I., Murphy, R. A., Koster, A., Brouwer, I. A., Visser, M., Garcia, M. E., et al. (2015b). Muscle Quality and Muscle Fat Infiltration in Relation to Incident Mobility Disability and Gait Speed Decline: the Age, Gene/Environment Susceptibility-Reykjavik Study. J Gerontolog A Biol Sci Med Sci, 1030–1036. doi:10.1093/gerona/glv016.

Rockwood K, Mitnitski A. 2007 Frailty in relation to the accumulation of deficits. Journals Gerontol Biol Sci Med Sci. 62:722–727

Saedi AA, Feehan J, Phu S, Duque G. Current and emerging biomarkers of frailty in the elderly. Clin Interv Aging. 2019 Feb 19;14:389-398. doi: 10.2147/CIA.S168687. PMID: 30863033; PMCID: PMC6388773.

Samson LD, Boots AMH, Verschuren WMM, Picavet HSJ, Engelfriet P, Buisman AM. Frailty is associated with elevated CRP trajectories and higher numbers of neutrophils and monocytes. Exp Gerontol. 2019 Oct 1;125:110674. doi: 10.1016/j.exger.2019.110674. Epub 2019 Jul 20. PMID: 31336145.

Santoro A, Bazzocchi A, Guidarelli G, Ostan R, Giampieri E, Mercatelli D, Scurti M, Berendsen A, Surala O, Jennings A, Meunier N, Caumon E, Gillings R, Kadi F, Capel F, Cashman KD, Pietruszka B, Feskens EJM, De Groot LCPGM, Battista G, Salvioli S, Franceschi C. A Cross-Sectional Analysis of Body Composition Among Healthy Elderly From the European NU-AGE Study: Sex and Country Specific Features. Front Physiol. 2018 Nov 30;9:1693. doi: 10.3389/fphys.2018.01693. PMID: 30555339; PMCID: PMC6283977. (a)

Santoro A, Guidarelli G, Ostan R, Giampieri E, Fabbri C, Bertarelli C, Nicoletti C, Kadi F, de Groot LCPGM, Feskens E, Berendsen A, Brzozowska A, Januszko O, Kozlowska K, Fairweather-Tait S, Jennings A, Meunier N, Caumon E, Napoli A, Mercatelli D, Battista G, Capri M, Franceschi C, Bazzocchi A. Gender-specific association of body composition with inflammatory and adipose-related markers in healthy elderly Europeans from the NU-AGE study. Eur Radiol. 2019 Sep;29(9):4968-4979. doi: 10.1007/s00330-018-5973-2. Epub 2019 Feb 4. PMID: 30715588; PMCID: PMC6682581. (b)

Santoro A, Martucci M, Conte M, Capri M, Franceschi C, Salvioli S. Inflammaging, hormesis and the rationale for anti-aging strategies. Ageing Res Rev. 2020 Aug 16:101142. doi: 10.1016/j.arr.2020.101142. Epub ahead of print. PMID: 32814129.

Sato F, Maeda N, Yamada T, Namazui H, Fukuda S, Natsukawa T, Nagao H, Murai J, Masuda S, Tanaka Y, Obata Y, Fujishima Y, Nishizawa H, Funahashi T, Shimomura I. Association of Epicardial, Visceral, and Subcutaneous Fat With Cardiometabolic Diseases. Circ J. 2018 Jan 25;82(2):502-508. doi: 10.1253/circj.CJ-17-0820. Epub 2017 Sep 27. PMID: 28954947.

Schrager MA, Metter EJ, Simonsick E, et al (2007) Sarcopenic obesity and inflammation in the InCHIANTI study. J Appl Physiol 102:919–925. doi: 10.1152/japplphysiol.00627.2006

Sewo Sampaio PY, Sampaio RA, Coelho Júnior HJ, et al. Differences in lifestyle, physical performance and quality of life between frail and robust Brazilian community-dwelling elderly women. Geriatr Gerontol Int. 2016;16(7):829–835. doi:10.1093/gerona/60.10.1278

Spiegelman BM, Flier JS (2001) Obesity and the Regulation Review of Energy Balance total fast of approximately 150 days! This impressive energy reserve is due both to the high energy content of triglycerides versus polysaccharides, and the fact. Cell 104:531–543.

Suchdev PS, Williams AM, Mei Z, et al (2017) Assessment of iron status in settings of inflammation: challenges and potential approaches. The American journal of clinical nutrition 106:1626S–1633S. doi: 10.3945/ajcn.117.155937

Thomas T (2004) The complex effects of leptin on bone metabolism through multiple pathways. Current Opinion in Pharmacology 4:295–300. doi: 10.1016/J.COPH.2004.01.009

Torriani, M., Gill, C. M., Daley, S., Oliveira, A. L., Azevedo, D. C., and Bredella, M. A. (2014). Compartmental neck fat accumulation and its relation to cardiovascular risk and metabolic syndrome 1–3. Am J Clin Nutr 100, 1244–51. doi:10.3945/ajcn.114.088450.

Wells JCK (2007) Sexual dimorphism of body composition. Best Practice and Research: Clinical Endocrinology and Metabolism 21:415–430. doi: 10.1016/j.beem.2007.04.007

Wolsk E, Mygind H, Grøndahl TS, et al (2012) Human skeletal muscle releases leptin in vivo. Cytokine 60:667–673. doi: 10.1016/J.CYTO.2012.08.021

Xia, J., Broadhurst, D. I., Wilson, M., and Wishart, D. S. (2013). Translational biomarker discovery in clinical metabolomics: an introductory tutorial. Metabolomics 9, 280–299. doi: 10.1007/s11306-012-0482-9

Xu L, Zhang J, Shen S, Hong X, Zeng X, Yang Y, Liu Z, Chen L, Chen X. Association Between Body Composition and Frailty in Elder Inpatients. Clin Interv Aging. 2020 Mar 4;15:313-320. doi: 10.2147/CIA.S243211. PMID: 32184580; PMCID: PMC7061425.

Yamaguchi N, Argueta JGM, Masuhiro Y, et al (2005) Adiponectin inhibits Toll-like receptor family-induced signaling. FEBS Letters 579:6821–6826. doi: 10.1016/j.febslet.2005.11.019

Zamboni M, Zoico E, Scartezzini T, et al (2003) Body composition changes in stable-weight elderly subjects: the effect of sex. Aging clinical and experimental research 15:321–7.

Zong, G., Zhang, Z., Yang, Q., Wu, H., Hu, F. B., and Sun, Q. (2017). Total and regional adiposity measured by dual-energy X-ray absorptiometry and mortality in NHANES 1999-2006. Obesity 24, 2414–2421. doi: 10.1002/oby. 21659

8. APPENDIX

Scientific papers published by the author Giulia Guidarelli during the period of the PhD Course.

Cazzaniga S, Naldi L, Damiani G, Atzori L, Patta F, **Guidarelli G**, Bettoli V. Validation of a visual-aided questionnaire for the self-assessment of hidradenitits suppurativa. J Eur Acad Dermatol Venereol. 2018 Nov;32(11):1993-1998. doi: 10.1111/jdv.15050. Epub 2018 May 28. PMID: 29729101.

Conte M, Ostan R, Fabbri C, Santoro A, **Guidarelli G**, Vitale G, Mari D, Sevini F, Capri M, Sandri M, Monti D, Franceschi C, Salvioli S. Human Aging and Longevity Are Characterized by High Levels of Mitokines. J Gerontol A Biol Sci Med Sci. 2019 Apr 23;74(5):600-607. doi: 10.1093/gerona/gly153. PMID: 29955888.

Giampieri E, Ostan R, **Guidarelli G**, Salvioli S, Berendsen AAM, Brzozowska A, Pietruszka B, Jennings A, Meunier N, Caumon E, Fairweather-Tait S, Sicinska E, Feskens EJM, de Groot LCPGM, Franceschi C, Santoro A. A Novel Approach to Improve the Estimation of a Diet Adherence Considering Seasonality and Short Term Variability - The NU-AGE Mediterranean Diet Experience. Front Physiol. 2019 Mar 5;10:149. doi: 10.3389/fphys.2019.00149. PMID: 30890946; PMCID: PMC6413567.

Montesanto A, De Rango F, Pirazzini C, **Guidarelli G**, Domma F, Franceschi C, Passarino G. Demographic, genetic and phenotypic characteristics of centenarians in Italy: Focus on gender differences. Mech Ageing Dev. 2017 Jul;165(Pt B):68-74. doi: 10.1016/j.mad.2017.04.008. Epub 2017 Apr 29. PMID: 28461103.

Montiel Rojas D, Nilsson A, Ponsot E, Brummer RJ, Fairweather-Tait S, Jennings A, de Groot LCPGM, Berendsen A, Pietruszka B, Madej D, Caumon E, Meunier N, Malpuech-Brugère C, **Guidarelli G**, Santoro A, Franceschi C, Kadi F. Short Telomere Length Is Related to Limitations in Physical Function in Elderly European Adults. Front Physiol. 2018 Aug 10;9:1110. doi: 10.3389/fphys.2018.01110. PMID: 30147659; PMCID: PMC6096049.

Ostan R, Guidarelli G, Giampieri E, Lanzarini C, Berendsen AAM, Januszko O, Jennings A, Lyon N, Caumon E, Gillings R, Sicinska E, Meunier N, Feskens EJM, Pietruszka B, de Groot LCPGM, Fairweather-Tait S, Capri M, Franceschi C, Santoro A. Cross-Sectional Analysis of the Correlation Between Daily Nutrient Intake Assessed by 7-Day Food Records and Biomarkers of Dietary Intake Among Participants of the NU-AGE Study. Front Physiol. 2018 Oct 1;9:1359. doi: 10.3389/fphys.2018.01359. PMID: 30327612; PMCID: PMC6174234.

Santoro A, Bazzocchi A, **Guidarelli G**, Ostan R, Giampieri E, Mercatelli D, Scurti M, Berendsen A, Surala O, Jennings A, Meunier N, Caumon E, Gillings R, Kadi F, Capel F, Cashman KD, Pietruszka B, Feskens EJM, De Groot LCPGM, Battista G, Salvioli S, Franceschi C. A Cross-Sectional Analysis of Body Composition Among Healthy Elderly From the European NU-AGE Study: Sex and Country Specific Features. Front Physiol. 2018 Nov 30;9:1693. doi: 10.3389/fphys.2018.01693. PMID: 30555339; PMCID: PMC6283977.

Santoro A, **Guidarelli G**, Ostan R, Giampieri E, Fabbri C, Bertarelli C, Nicoletti C, Kadi F, de Groot LCPGM, Feskens E, Berendsen A, Brzozowska A, Januszko O, Kozlowska K, Fairweather-Tait S, Jennings A, Meunier N, Caumon E, Napoli A, Mercatelli D, Battista G, Capri M, Franceschi C, Bazzocchi A. Gender-specific association of body composition with inflammatory and adipose-related markers in healthy elderly Europeans from the NU-AGE study. Eur Radiol. 2019 Sep;29(9):4968-4979. doi: 10.1007/s00330-018-5973-2. Epub 2019 Feb 4. PMID: 30715588; PMCID: PMC6682581.

Tavella T, Rampelli S, **Guidarelli G**, Bazzocchi A, Gasperini C, Pujos-Guillot E, Comte B, Barone M, Biagi E, Candela M, Nicoletti C, Kadi F, Battista G, Salvioli S, O'Toole PW, Franceschi C, Brigidi P, Turroni S, Santoro A. Elevated gut microbiome abundance of Christensenellaceae, Porphyromonadaceae and Rikenellaceae is associated with reduced visceral adipose tissue and healthier metabolic profile in Italian elderly. Gut Microbes. 2021 Jan-Dec;13(1):1-19. doi: 10.1080/19490976.2021.1880221. PMID: 33557667; PMCID: PMC7889099.