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Nutritional status and body composition by bioelectrical impedance vector analysis: a cross-sectional and longitudinal study in Mild Cognitive Impairment and Alzheimer's dementia.

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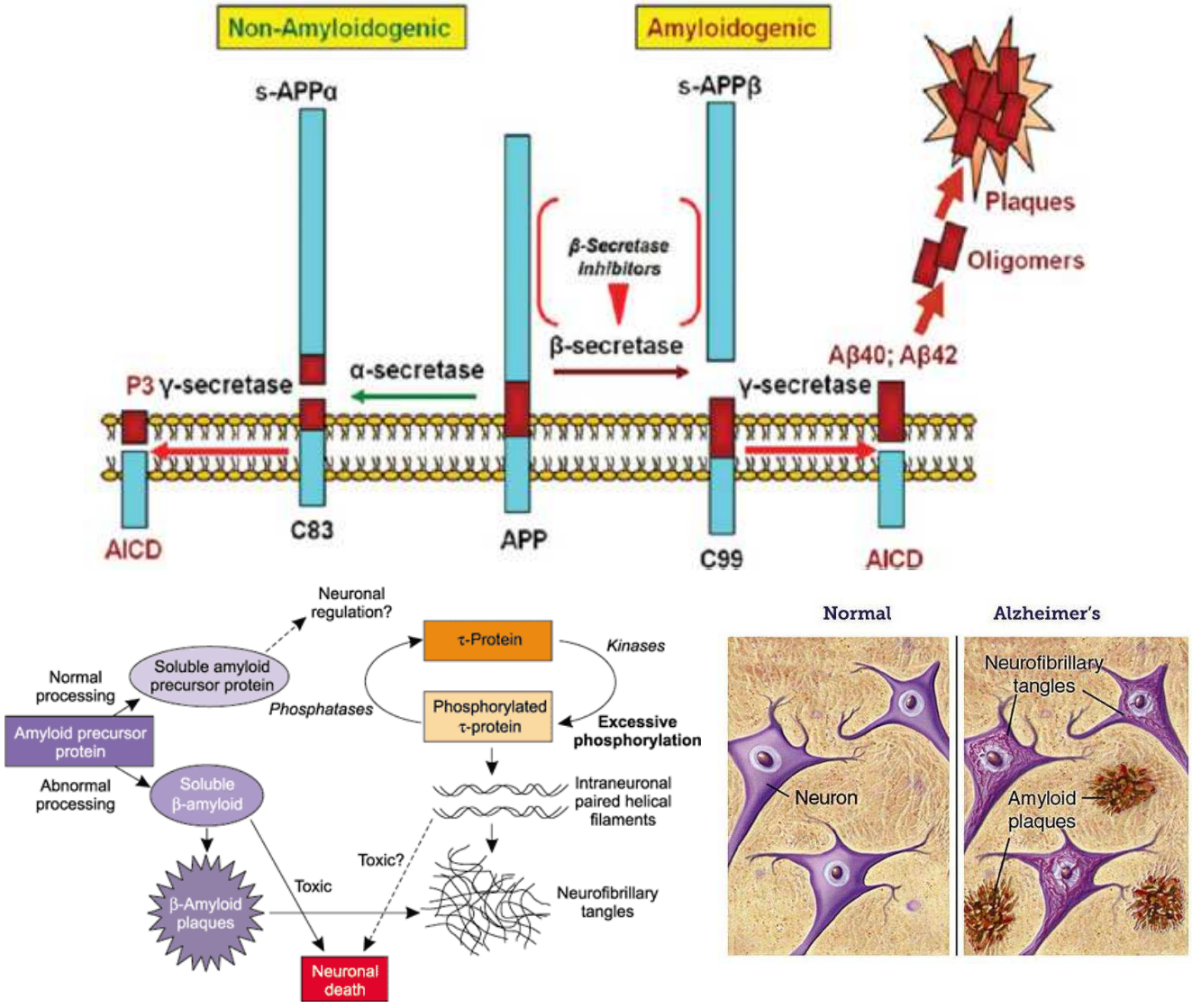
Alzheimer’s disease: from pathogenesis to biomarkers

The term "dementia" currently indicates a syndrome which involves a progressive alteration of previously acquired cognitive functions of such severity that interfere with activities of daily living and quality of life. The skills implicated include attention, language, learning and memory, executive functions (i.e. programming skills, strategy development, problem solving, abstract thinking,), perception and social interaction; concomitant mood and behavior disorders contribute to complicate the clinical picture. This definition underlies a wide range of diseases that can be etiologically divided in primary (or degenerative) and secondary to defined causal factors, only a few of them curable. Degenerative dementia is classified in different irreversible forms, distinguishable from each other mainly through clinical features and supportive instrumental exams: in most cases a definitive diagnosis cannot be achieved in life. The improvement of scientific knowledge and the resulting development of increasingly precise criteria over the years has increased the specificity of the diagnosis of different form of dementia. Alzheimer's disease (AD) has been recognized as a clinical entity more than 100 years ago and represents the most frequent form of dementia (accounting for 60-80% of dementia cases) and the most common neurodegenerative disorder worldwide, affecting nearly 40 million people (Prince et al., 2013) of all races and ethnic groups. The high global prevalence and the economic impact on families, caregivers and society make AD a public health priority. Currently, two main forms of AD are recognized: a genetically determined familial form (FAD) that occurs in about 1-5% of AD cases and a sporadic late onset AD (LOAD) which is determined multifactorially and accounts for all the other cases. These two forms share underlying neuropathology, so the symptoms of both forms are similar. FAD is characterized by an early onset and associated with mutations in the amyloid precursor protein (APP) gene and the genes for presenilin 1 (PS1) or 2 (PS2), which are the components of γ-secretase complex responsible for cleavage and release of amyloid β (Aβ) from APP; this results in an imbalance between production and clearance of Aβ peptides and, as a

consequence, their accumulation in toxic aggregates in the brain, called amyloid plaques (Siegel GJ et al, 1999). Also neurofibrillary degeneration characterized by abnormal hyperphosphorylation and aggregation of protein tau (neurofibrillary tangles) plays a pivotal role in the pathogenesis of AD. (Figure 1).

Figure 1. Pathogenesis of Alzheimer's disease.



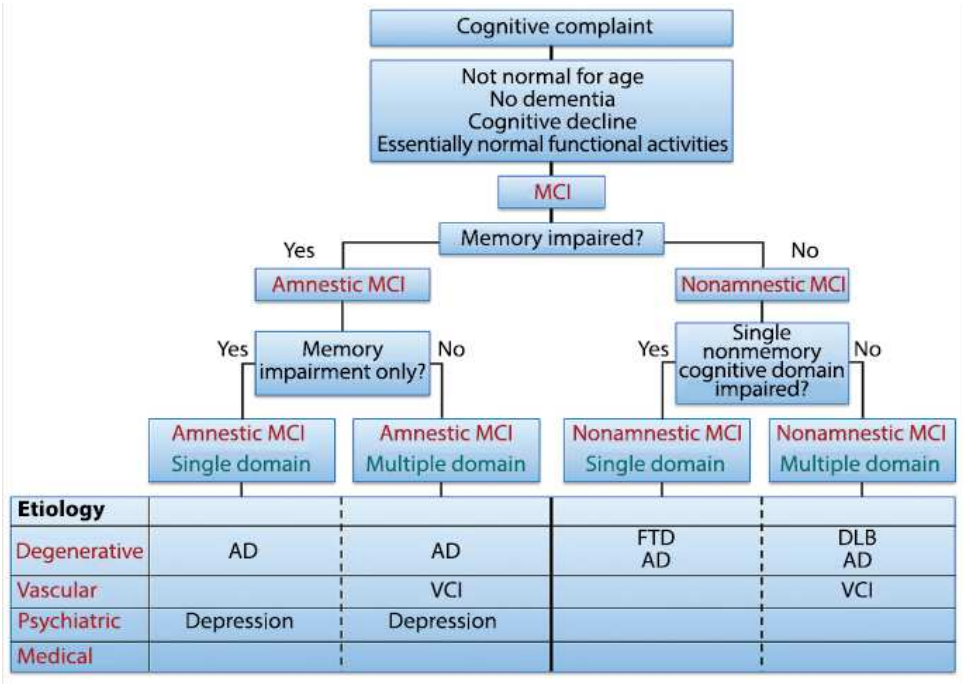
The formation of amyloid plaques and neurofibrillary tangles is thought to contribute to synaptic degeneration and neuronal loss resulting in atrophy of specific areas of the brain (entorinal cortex, hippocampus, amygdala, and basal telencephalus) (Auld et al., 2002) and the subsequent symptoms

of AD. Aß and tau are directly measurable in cerebrospinal fluid and reflect the amount of cerebral pathological aggregates of AD.

LOAD is not clearly associated with any distinct mutation profile, although there is an increased likelihood of disease in carriers of a particular allelic form (ε4) of the apolipoprotein E that is a regulator of lipid metabolism with affinity for the Aβ protein aggregated in extracellular deposits, namely senile plaques (Strittmatter et al., 1993).

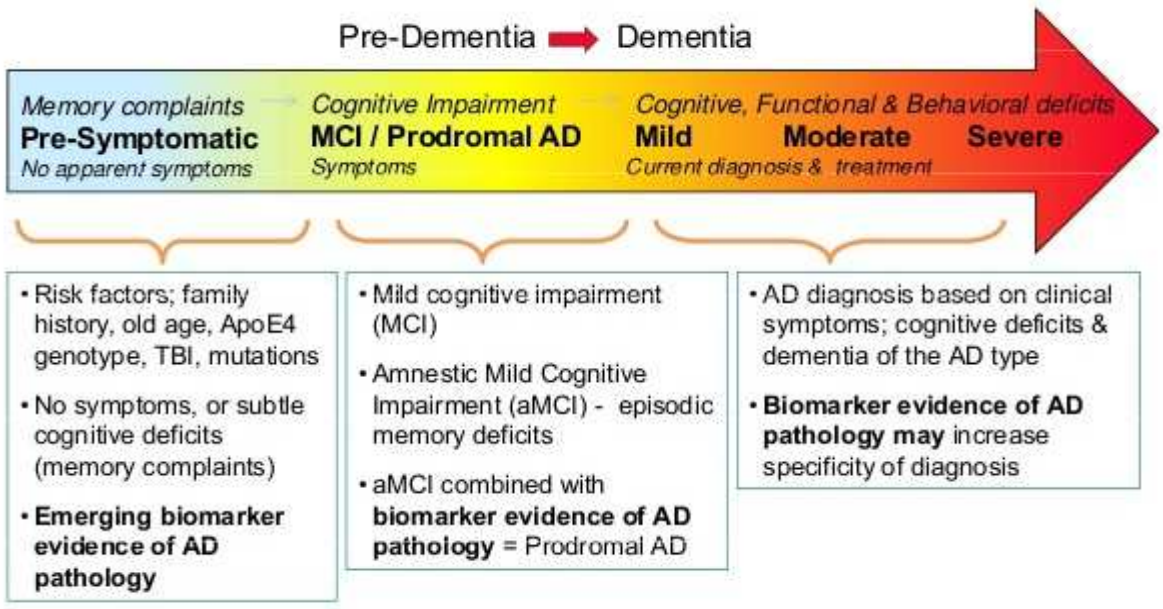
The main known risk factor for the development of the sporadic form of AD is aging; indeed, the age-specific prevalence of AD almost doubles every 5 years after age 65 (Qiu et al., 2009). Another risk factor for developing dementia and AD is a clinical condition called Mild Cognitive Impairment (MCI) (Petersen et al., 2014). MCI, first described in 1999 (Petersen et al., 1999) but previously depicted with other terms (such as cognitive impairment not dementia, etc.), represents an intermediate stage between the expected cognitive decline of normal aging (and level of education) and the more pronounced decline of dementia; it is characterized by a slight but noticeable and measurable decline in cognitive abilities, not severe enough to interfere with day-to-day life and ordinary activities. MCI prevalence is around 15-20% in people aged 60 years and older with an annual rate of progression to dementia that varies between 8% and 15% per year (Petersen, 2016); a high frequency of subjects with MCI remains at that stage for years, others may even revert to normal cognition (Canevelli et al., 2016). Clinic-based studies have showed that up to 80% of subjects with MCI develop dementia after six years (Petersen, 2004). MCI that primarily affects memory function is known as "amnestic MCI", to distinguish it from "non-amnestic MCI" in which cognitive skills other than memory are affected; an additional distinction can be made between “single domain” and “multiple domain”. This classification by subtype relates not only to clinical presentation, but also to outcomes (and therefore to underlying aetiology and pathology), even if subtyping may depend on how extensive is the battery of neuropsychological tests applied. As shown in the figure 2, all different subtypes of MCI can be a prelude of AD. So MCI could be considered as a risk factor for AD, but at the same time, a prodromic phase of AD (figure 3).

Figure 2. Classification of Mild Cognitive Impairment in suptypes and implication for aetiology.



from Petersen et al., 2016

Figure 3. Stages of Alzheimer's disease.



In 2007, the International Working Group (IWG) for New Research Criteria for the Diagnosis of AD (Dubois et al., 2007) provided a new conceptual framework that proposes to integrate the clinical diagnosis of AD with the presence of biomarkers (Table 1).

Table 1. **Putative Biomarkers for Alzheimer's Disease Currently Being Used**

1. Markers of amyloid-beta (Aβ) protein deposition in the brain
    a. Low cerebrospinal fluid Aβ42
    b. Positive positron emission tomography amyloid imaging
2. Markers of downstream neurodegeneration
    a. Elevated cerebrospinal fluid tau (total and phosphorylated)
    b. Decreased metabolism in temporal and parietal cortex on 18flurodeoxyglucose positron emission tomography
    c. Atrophy on magnetic resonance imaging in temporal (medial, basal, and lateral) and medial parietal cortex

From Dubois et al., 2007

The aim of these diagnostic criteria and of the subsequent National Institute on Aging–Alzheimer's Association (NIA–AA) criteria of 2011 (McKhann et al., 2011), have been to expand coverage of the full range of dementia stages, from the asymptomatic through the most severe stages. Potentially, their most important practical application is to allow earlier intervention in the prodromal stage of the disease (such as MCI condition) and to facilitate research studies for secondary prevention of AD in a preclinical phase. NIA–AA criteria specified the criteria for making a diagnosis of "MCI due to AD" based on clinical criteria in combination with additional information from structural magnetic resonance imaging, FDG-PET, PIB-PET, and cerebrospinal fluid biomarkers.

The term 'biomarker' is often used indiscriminately to describe any gene or protein expression change, but it has been better defined by the NIH Biomarkers Definitions Working Group as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention" (Marras et al., 2002). Several reports have examined the use and qualification of clinical biomarkers

(Freeman et al., 2010). More specifically, biomarkers for AD could be used for several objectives: diagnostic markers can be useful to recognize AD in a prodromal phase (MCI due to AD) and to differentiate this condition from different underlying etiologies, which will be important for choosing a correct treatment, when effective therapy is available; prognostic markers may define the likelihood of cognitive and functional progression (potentially a defined period of time) for MCI to a more severe stage of MCI (from single to multiple domain) or to dementia; finally, staging markers are helpful to describe disease severity and theragnostic markers to support treatment choice. A biomarker which might be useful for defining an aetiology could not be beneficial for characterizing a prognosis and vice versa. So, different properties of biomarkers may have differential utility over the short- and long-term and this knowledge should drive their use in the research and clinical context (Albert et al., 2011).

In 2014 IWG-2 criteria reconsidered the biomarker support necessary for AD diagnosis by anchoring all diagnostic criteria to the need of in-vivo evidence of AD pathophysiology; an important change in IWG-2 criteria is that topographical markers of AD were recommended as staging rather than as diagnostic markers (Table 2).

Table 2. Comparison of the IWG-2 and NIA-AAA criteria

IWG-2	*NIA-AA*
Pathophysiological markers • ↓ $A\beta_{42}$ together with ↑T-tau or P-tau in CSF • ↑ tracer retention on amyloid PET	Aβ biomarkers • ↓ Aβ42 in CSF • ↑ tracer retention on amyloid PET
Topographical markers • AD-like pattern of atrophy on brain MRI • AD-like pattern of hypometabolism on FDG-PET	Markers of neuronal injury • ↑ T-tau or P-tau in CSF • AD-like pattern of atrophy on brain MRI • AD-like pattern of hypometabolism on FDG-PET
AD autosomal dominant mutation • PSEN1, PSEN2 or APP	

AD = Alzheimer's disease; Aβ = amyloid-beta; CSF = cerebrospinal fluid; FDG = fluorodeoxyglucose; IWG = International Working Group; MRI = magnetic resonance imaging; NIA-AA = National Institute on Aging-Alzheimer's Association; PET = positron emission tomography.

From Molin et. al, 2016

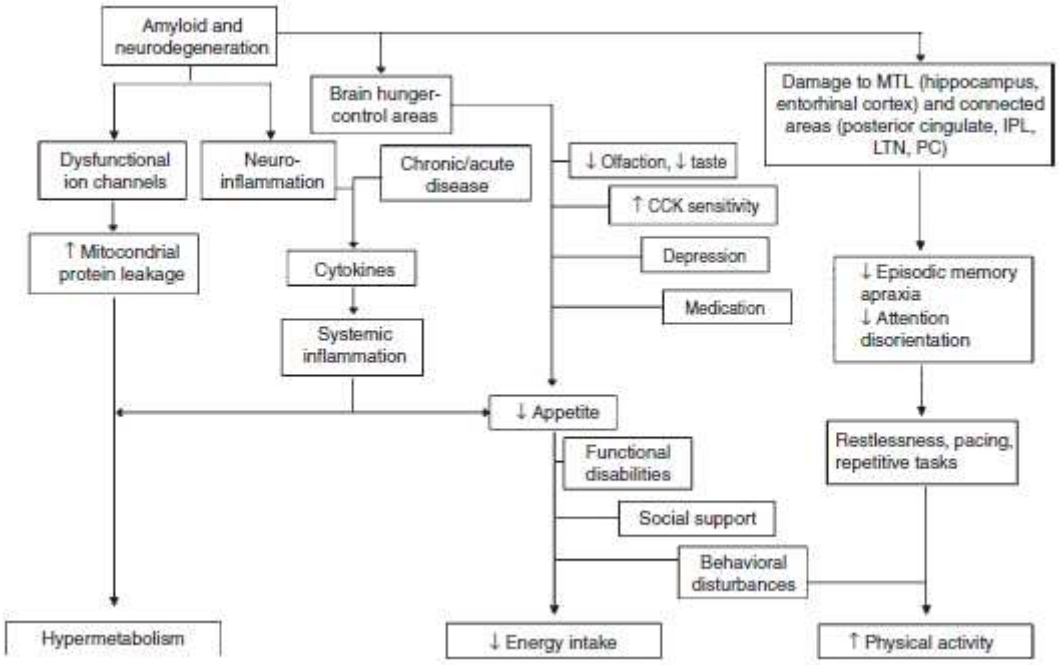
Body composition as a potential biomarker in Alzheimer's disease

Among systemic manifestations of AD which still do not find a unique interpretation, progressive weight loss (WL) has been described for the first time by Alois Alzheimer himself in 1907, who recognized a "slow progressive decline in body weight" in his first patient. Since then, several studies in the Eighties have confirmed in a large subpopulation of AD patients a non-intentional WL and a malnutrition, resulting in a change of body composition (Asplund et al., 1981; Cronin-Stubbs et al., 1997); in 1984 WL was even listed in NINCDS-ADRDA criteria of AD (McKhann et al., 1984).

Since 2005, reversible acetylcholinesterase inhibitors have been introduced for the symptomatic treatment of AD; adverse events consistent with the cholinergic actions of these drugs include loss of appetite and nausea, which doubtless can impact on food intake. However, a study of 1997 highlighted that WL in naïve patients with AD (0.52 kg/m^2/year) was higher than that of elderly non-dementia patients (0.14 kg/m^2/year). Moreover, a study conducted in 2013 in over 300 AD patients with a maximum follow-up of about 3 years, showed that long-term treatment with galantamine had no effect on weight (Droogsma et al., 2013).

Several mechanisms are hypothesized at the basis of WL (Sergi et al., 2013) it could be explained by the presence of a hypermetabolism and/or a reduction in energy intake and/or an increased physical activity; etiological factors that may support each of these hypotheses are illustrated in Figure 4**.**

Figure 4. Mechanisms potentially causing a weight loss in Alzheimer's disease.



MTC, medial temporal cortex; ACC, anterior cingulated cortex; MTL, medial temporal lobe; IPL, inferior parietal lobe; LTN, lateral temporal neocortex; PC, prefrontal cortex; CCK, cholecystokinin

From Sergi et.al, 2013

Amyloidogenesis and neurofibrillary degeneration may be associated with progressive WL by three hypothetical mechanisms:

1) Increased physical activity and, consequently, energy consumption.

One of the first clinical signs of AD consists on the loss of episodic memory, a disorder that results in ineffective storage of new information: patients become restless, are committed in repetitive tasks (Lopez et al., 1999) and spend more energy in trying to carry out everyday activities. Apathy, spatial-temporal disorientation (which contributes to increased physical exercise and walking) and anxiety resulting from the perception of the AD patient’s difficulties also contribute to the increase in energy expenditure. In advanced stages of AD, the occurrence of aberrant motor activity (e.g. wandering) is frequently observed; patients also frequently exhibit psychotic behavioural symptoms

such as agitation and aggression, and these symptoms have been associated to a WL of more than 5 kg during a 6-month follow-up (White et al., 2004). The hypothesis of an increased energy consumption as the cause of the WL is most attributable to behavioural disorders of advanced stage; this assumption however is in contrast with the demonstration that weight reduction appears more pronounced in the initial stage of AD (0.59 kg/m^2/year) rather than in the severe stage (0.47 kg/m^2/year) (Cronin-Stubbs et al., 1997).

2) Reduced energy intake due to poor food intake.

AD patients may forget to eat or experience aversion to some food (Miyamoto et al., 2011). Cognitive deficits affect the ability to provide, to purchase and to prepare food (Tracy et al., 2001). Progressive lifestyle changes may eventually lead to depart from a healthy diet regime. Earlier changes in appetite-regulating mechanisms have also been described, namely a decreased ability to smell and a loss of taste possibly due to amyloid plaques in brain areas responsible for these functions (cingulate cortex, olfactory epithelium); a premature sense of satiety may be caused by a higher sensitivity to cholecystokinin (Morley, 2001). Reduction in dietary intake may also be caused by neuroinflammation through the production of pro-inflammatory cytokines (TNFα, IL-1, IL-6) which reduce hunger, by possibly concomitant mood deflection and/or chronic therapies which can lead to anorexia, constipation and further contribute to decreasing the sense of taste and smell (Plata-Salamán, 1996; Kishi and Elmquist, 2005).

With the progression of dementia and functional deficits, caregivers support becomes increasingly important to provide adequate energy intake to the patient; they can offset the patient's difficulties by purchasing food, preparing meals, modifying a food's consistency to make it simpler to swallow. Indeed during advanced stages, patients may experience difficulty in carrying food to their mouths and in chewing, thus contributing to weight loss (Berkhout et al., 1998).

A discrepancy between needs, intake and nutrient utilization entails a state of functional and structural alteration of the body, which is defined as malnutrition. This clinical condition, which may occur due to poor or inadequate dietetic regimen as well as to deficiency or excess of certain

nutrients, is most frequently observed in advanced age. The Mini Nutritional Assessment (MNA) is a validated nutrition screening test for the elderly population, consisting of 18 questions, divided into 3 sections: anthropometry (body mass index [BMI], arm and calf circumference, weight loss), eating habits (number of full meals per day, fluid introduced, vegetables, fruit and protein intake, mode of feeding), cognitive status and disability (mobility, psychological stress or acute illness, neuropsychological problems, prescription drugs per day, pressure sores or skin ulcers, self view of nutritional status and health). Scores <17 indicate malnutrition, scores between 17 and 23.5 indicate risk of malnutrition and scores ≥ 24 indicate a normal nutrition. The prevalence of malnutrition in community-dwelling elderly studied with MNA (21 studies, n = 14149 elderly) was around 2% and risk of malnutrition around 24 % (Guigoz, 2006). A Dutch study conducted in 2014 found no significant differences of MNA scores between mild AD patients and healthy elderly controls; however, mean scores of MNA in AD resulted lower than cut-off of normal nutritional status (< 24.0), suggesting an increased risk of malnutrition only in a subgroup of AD (Olde Rikkert et al., 2014). These results are in agreement with those of another Dutch study (Droogsma et al., 2013) that showed that AD patients did not suffer from protein-energy malnutrition, with the exception of a subgroup of patients (14%) at risk for malnutrition (MNA scores 17-23.5). Several studies showed a positive association between the risk of malnutrition and later stages of AD (Sandman et al., 1987; Guerin et al., 2005; Gillioz et al., 2009; Buffa et al., 2010; Droogsma et al., 2013).

3) Hypermetabolism

Among the several biological factors thought to be involved in the onset and progression of AD, an important role could be played by an imperfect functioning of mitochondria. These organelles are the energetic centers of the cell due to their main function of ATP production through the coupling of the electron-transport system with phosporylation. During simultaneous exposure to amyloid β and phosphorylated tau, conformational, alterations in electrical potential and mobility, as well as different response to oxidative stress of mitochondria in neurons of rats have been described (Quintanilla et al., 2012). Mitochondrial dysfunction could justify the reduction of cerebral

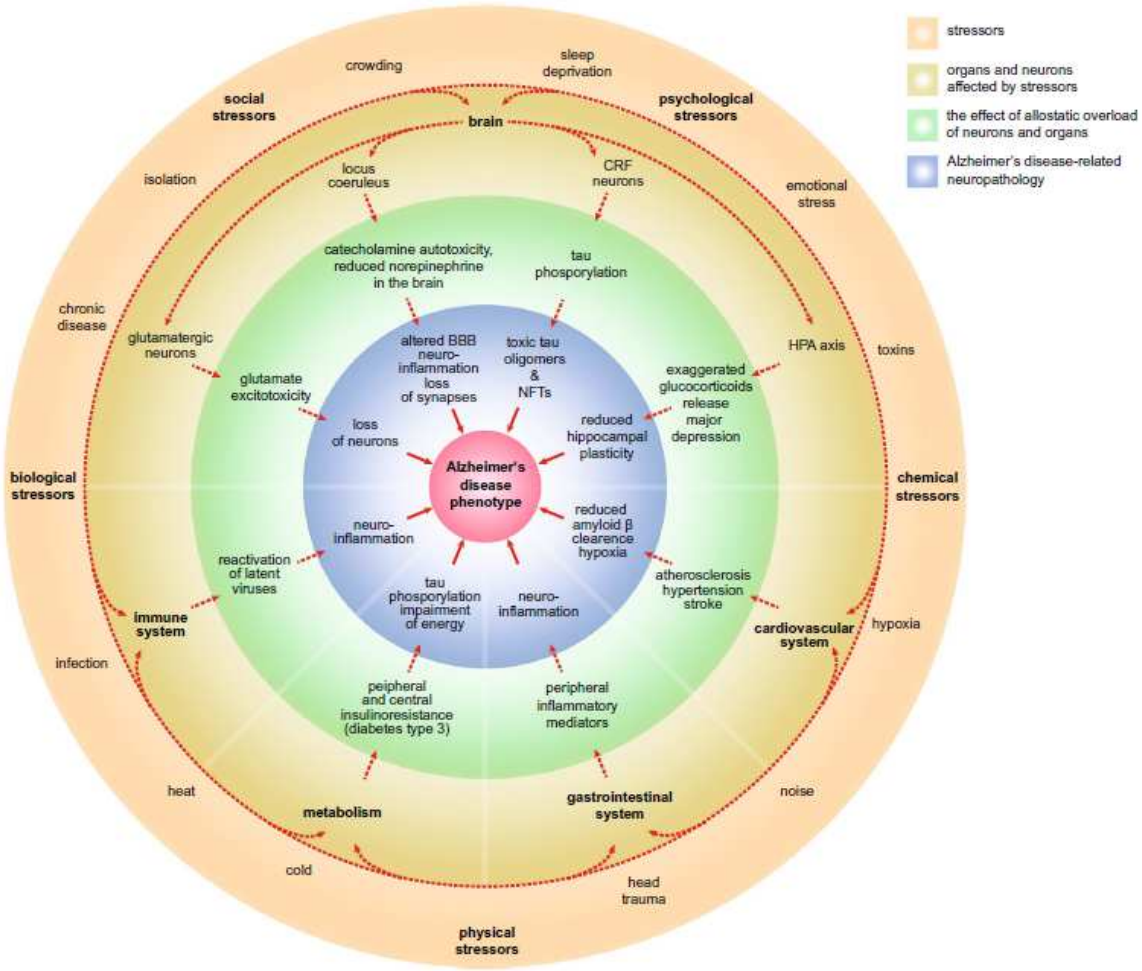
metabolism, especially in the temporal-parietal cortex typically involved in AD (Sullivan and Brown, 2005). Mitochondria actually supply brain cells with about 90% of the total required energy (Wallace, 1997). A decoupling of the electron-transport system, which is a distinctive feature of brown adipose tissue, leads to a lower ATP production whilst heat production increases; this mechanism represents a cytoprotective strategy occurring during aging process in order to reduce the production of free radicals (Speakman et al., 2004). However, decoupling in altered mitochondria may increase the production of free radicals, inducing cellular damage and increasing the permeability of the mitochondrial membrane to H+ ions, thus supporting the decoupling process in a vicious circle (Brookes, 2005). An increased mitochondrial oxidative activity in muscle tissue of AD patients with respect to controls have been found in a study of 1991 (Mariani et al., 1991). In 2004, AD mitochondrial cascade hypothesis has been proposed as a key for interpretation of amyloid cascade in sporadic late-onset AD: mitochondrial dysfunction could trigger APP expression and processing or Aβ accumulation. This hypothesis suggested that gene inheritance defines an individual's baseline mitochondrial function; inherited and environmental factors determinate rates at which mitochondrial function changes over time: so baseline mitochondrial function and mitochondrial change rates could influence brain aging as well as AD chronology (Swerdlow and Khan, 2004).

Hypermetabolism would also seem to be increased by neuroinflammation: amyloid deposits are able to activate astrocytes and microglia in the production of cytokines, leading to a systemic inflammatory response (Visser et al., 2002; Rebeck et al., 2010; Patra and Arora, 2012).

Pro-inflammatory molecules are released also from microbiota, due to changes in permeability of the gut due to chronic stress (Rieder et al., 2017), thus affecting functions of microglia; microbiota seems also implicated in the regulation of the formation of amyloid plaques (Fung et al., 2017).

Many stressors have been suggested as initiators of AD neuropathology (Figure 5)

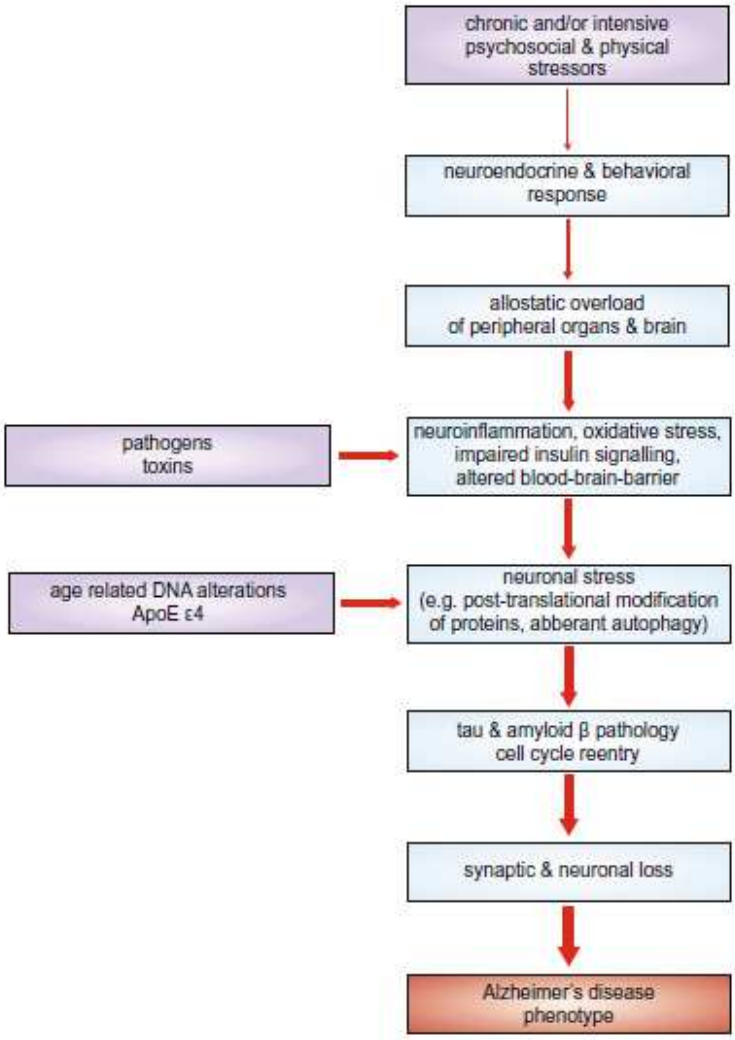
Figure 5. From stressors to Alzheimer's disease-related neuropathology.



From Mravec et al, 2017

Chronic and/or psychosocial and physical stressors can contribute to neuroinflammation through a mechanism of activation of signals processed by neurons synthesizing glutamate, norepinephrine from locus coeruleus, corticotropin-releasing factor (CRF), other neurotransmitters and neuromodulators. Repeatedly or chronically elevated mediators of the neuroendocrine stress response have a direct damaging role on the brain by impairing neuronal metabolism, plasticity and survival (Mravec et al., 2017). (Figure 6)

Figure 6. Development of Alzheimer's disease phenotype as a consequence of a multistep-pathological cascade activated by primary etiological factors.

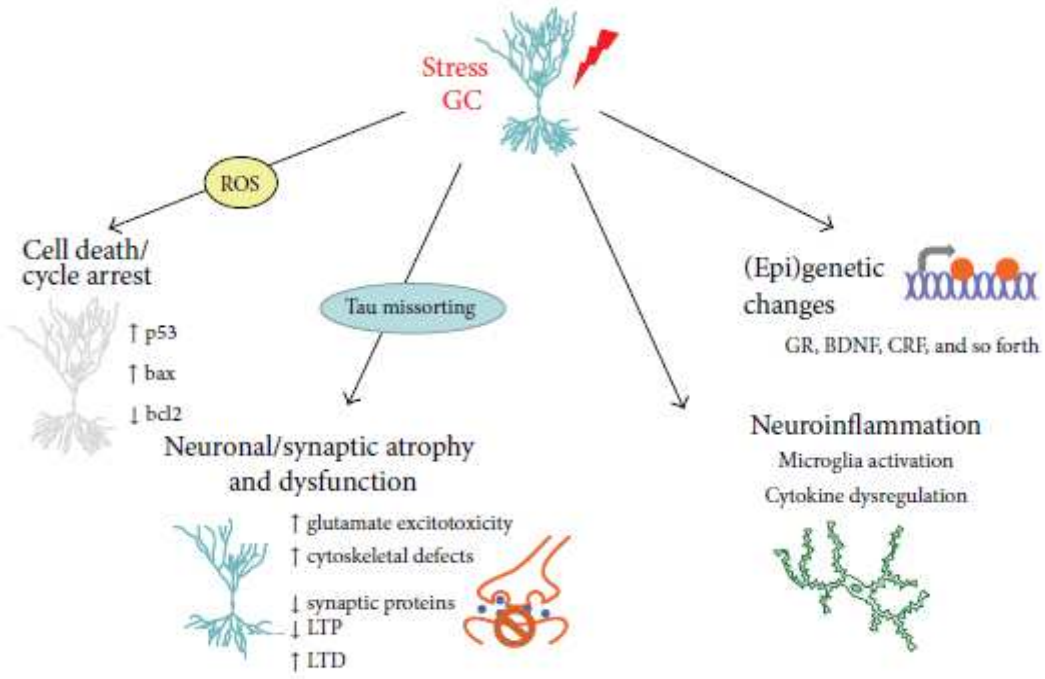


From Mravec et al, 2017

The effector molecules of the hypothalamic–pituitary–adrenal axis are glucocorticoids (GC) which exert pivotal effects such as regulation of glucose utilization by brain tissue, appetite, feeding and memory formation (Sapolsky et al., 2000). High density of GC binding receptors is known in hippocampus; whereas central role of GC is to maintain homeostasis, a persistent elevated level of GC due to chronic stress could instead reduce synaptic plasticity and the number of neurons in hippocampus, by damping of brain-derived neurotrophic factor (Lucassen et al., 2015). Moreover, through the activation of glycogen synthase kinase-3, high GC increases phosphorylation of tau (Yi

et al., 2017). Some recent evidences also suggest a potential neuroinflammatory role of GC (in contrast to their classical view) (Vyas et al., 2016) (Figure 7).

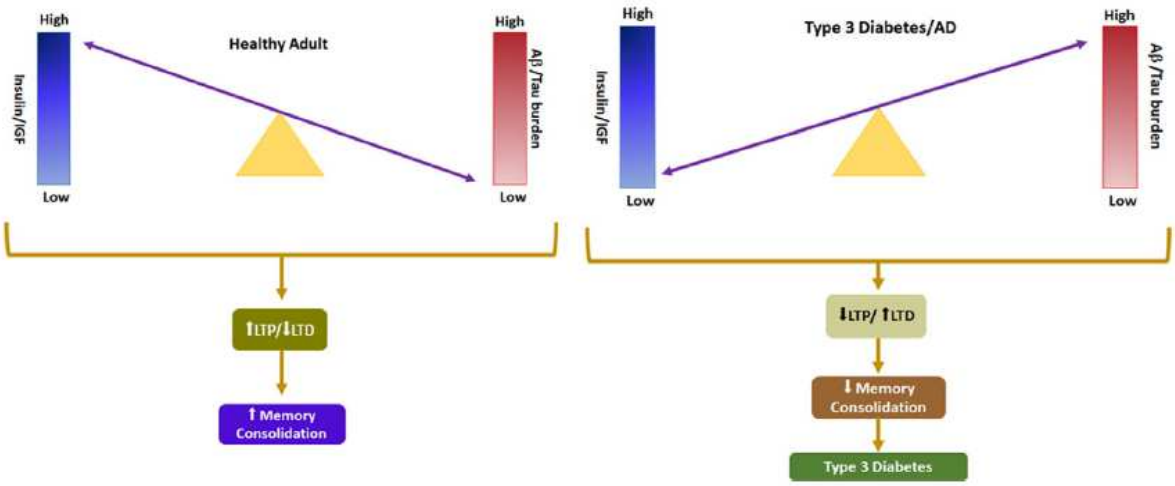
Figure 7. Cellular targets and actions of chronic stress mediated by glucocorticoid receptors.



From Vyas et al. , 2016

GC lead to orexigenic effects and insulin resistance: changes in the hormonal regulation of energy metabolism can then contribute to alteration in body weight. Insulin resistance and AD are linked also by other factors, for example the increased levels of specifically phosphorylated insulin receptor substrate 1, coexpression of AD- and insulin resistance-related genes and insulin-modulating degradation of β amyloid (Diehl et al., 2017). It has been hypothesized that AD may represent a metabolic disease and has subsequently been referred to as ‘‘type 3 diabetes’’ (Kandimalla et al., 2017) (Figure 8).

Figure 8. Insulin signalling pathway in healthy and AD brain.



From Kandimalla et al, 2017

In conclusion, the third mechanism (hypermetabolism) is the most convincing interpretation of weight loss in the initial phases of AD process, but additional factors may play a complementary role.

In a recent study from our group, MCI who lost ≥ 4% of their body weight during follow-up had a 3.4-fold increased risk of dementia and a 3.2-fold increased risk of AD; on average, weight loss was associated with a 2.3 and 2.5 years earlier onset of dementia and AD (Cova et al., 2016b).

This is in agreement with previous findings which showed a 30–40% weight loss in mild to moderate AD patients (White et al., 1996; Gillette-Guyonnet et al., 2000) and with studies suggesting that weight loss begins several years before the diagnosis of AD (Stewart et al., 2005; Johnson et al., 2006). It is also consistent with the results from a population-based study which found that amnestic MCI who lose weight undergo faster functional decline after one year (Besser et al., 2014). So, weight loss could be a predictor of the progression of MCI to dementia and AD.

The measurement of body weight, however, does not allow to distinguish between the various components of the body mass; there are several more precise methods to measure nutritional status. Body mass index (BMI) is the most widely used indicator of overweight and obesity and has a robust correlation with body fat percentage (Lichtash et al., 2013); it is calculated by dividing

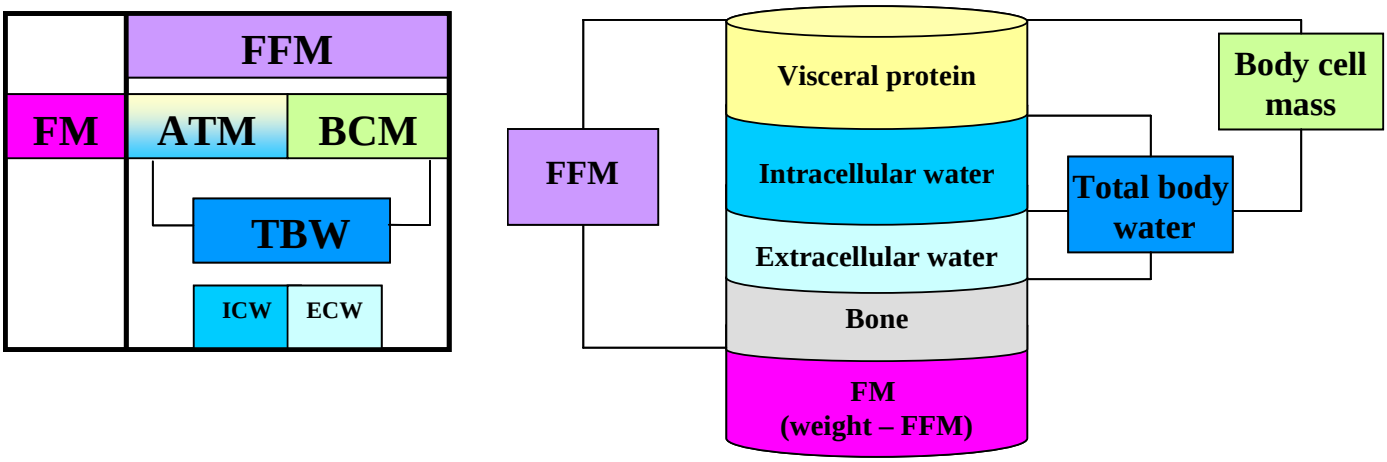
weight (in kilograms) for height squared (in centimeters). BMI also suggests a relationship between body parameters and mortality risk in general population: a lower relative risk of death is described with values between 18.5 and 24.9 (normal weight), a higher risk is present if values are below 18.5 (underweight) and the risk grows almost exponentially with the increase of BMI over values of 25 (overweight and obesity) (Prospective Studies Collaboration et al., 2009). However, BMI has limited accuracy since it does not discriminate the relative contribution of muscle mass and body fat to overall weight; BMI is then used in association with other body measures (Okorodudu et al., 2010). Arm and calf circumferences are useful instruments to evaluate nutritional status: an arm circumference lower than 23 cm in men and 22 cm in women, as well as a calf circumference lower than 31 cm in both sexes increases the risk of malnutrition (Guigoz, 2006).

Waist circumference is directly related to visceral fat tissue. (WHO, 2008). The muscular tissue is directly proportional to the lean mass and reveals the basal metabolism of each subject, namely the energy spent at rest (i.e. 45-75% of the total energy expenditure). Basal metabolism can be calculated with predictive equations or by calorimetric (direct and indirect) and non calorimetric methods. Direct calorimetry evaluates the energy expenditure by measuring the heat dispersion of a subject within a metabolic chamber; it is the most accurate method but it is very expensive and not applicable at outpatient level due to its complexity. Indirect calorimetry measures energy expenditure through variations in oxygen and carbon dioxide concentrations in respiratory gases and calculates the oxidation of energy substrates; it is rather imprecise since it does not detect the differences between glucidic and lipidic substrates.

The Dual Energy X-ray Absorptiometry (DEXA) can be used to calculate the various components of the body mass: mineral bone density, lean mass, cellular mass and, indirectly, fat mass. Hydrostatic Weighing is another expensive exam able to detect the displacement of water generated by a submerged body to measure its density and from that figure calculate percentage body fat. Plicometry is a simpler but unreliable method to measure the fat mass through skin folds (Walter-Kroker et al., 2011).

Bio-Impedance Analysis (BIA) is a non-invasive technique which discriminates between lean and fat mass (as percentages); it is based on the principle that different tissues express a specific electrical conductivity and offer a dissimilar resistance to the passage of current: adipose tissue proffers a high resistance while muscular tissue a low resistance (since its highest content of water) (Walter-Kroker et al., 2011). Different resistances are detected and transformed through appropriate equations in the parameters shown in Figure 9.

Figure 9. Analysis of the 2-compartmental body composition.



Fat Free Mass (FFM); Fat Mass (FM); Body Cell Mass (BCM); Extracellular Mass (ECM); Total Body Water (TBW); Intracellular Water (ICW); extracellular water (ECW); Lean Body Mass (LBM).

Empirical formulas of BIA are obtained by means of statistical calculations from healthy subjects but are based on the hypothesis that hydration of lean mass is a fixed percentage (estimated at 73%); any condition associated with a different hydration can thus introduce a distortion in compartmental

estimations with an unpredictable propagation of the error affecting the entire body composition analysis, especially in the elderly and/or in pathological subjects.

A variant of the BIA, called bioelectrical impedance vector analysis (BIVA) (Piccoli et al., 1994), is a more accurate method because it does not require the use of predictive equations. It is a stand-alone procedure based on patterns of direct impedance measurements (impedance vectors). Impedance (Z vector) is a combination of Resistance (R), which expresses the opposition of intra- and extracellular body fluids to flow of an alternating current, and Reactance (Xc) which consists in the capacitative component of tissues. R negatively correlates with the quantity of ionic solutions, Xc is directly related to the amount of soft tissue structures. The vectors of each subject are compared with a reference population and described as percentiles of a normal distribution of a probabilistic bivariate graph. Unlike BIA, BIVA method does not estimate any body compartment; the length and position of the Z vector supplies information about the state of hydration and body cell mass. The length of the vector indicates the level of hydration, from fluid overload (shorter vector: decreased resistance) to bodily dehydration (longer vector: increased resistance). Lateral vector displacements due to high or low reactance denotes an increase or a decrease of dielectric mass (membranes and tissue interfaces) of soft tissues. The phase shift of the tissue interfaces, called phase angle (PA), represents both the quantity and quality of soft tissue and can be calculated directly as arctan (Xc/R). Clinically it is the most important impedance parameter, predicting morbidity and mortality in a variety of diseases; higher values of PA correspond to a higher cellularity and a better cell membrane integrity; PA decreases with age and is significantly lower in women, due to the (physiologic) lower amount of body muscle (Norman et al., 2012).

Table 3 summarizes the studies focusing on the analysis of body composition in AD and healthy controls through various methods, including the two studies using the BIVA detailed below (Buffa et al., 2010, 2014).

Table 3 Case-control studies (AD vs. HC) evaluating the composition of body mass with different methods.

Studies	Population	Methods	Results
Renvall et al., 1993	28 HC 23 institutionalized AD	BMI BIA Hydrostatic weighting in 6 HC	♀AD vs. HC: < BMI, > % FFM, < % FM, > % TCW ♂AD vs. HC: < BMI (no differences in body composition)
Wolf-Klein et al., 1995	7 HC 5 mild-moderate AD 4 severe institutionalized AD 5 severe institutionalized multi-infarct dementia (MID)	Indirect calorimetry	Mild-moderate AD vs. HC: > energy requirement, different pattern FFM Non institutionalized AD vs. MID: > weight loss, > energy requirement
Donaldson et al., 1996	75 HC 25 non institutionalized AD	Indirect calorimetry DEXA	AD vs. HC: no differences in basal metabolism, FFM, FM
(Spindler et al., 1996)	23 HC 17 AD	Computer program (Body Composition II, version 1.0, 1987)	♀AD vs. HC: < BMI, > % FFM ♂AD vs. HC: no differences in BMI / body composition
(Poehlman et al., 1997)	103 HC 30 AD	Indirect calorimetry DEXA	AD vs. HC: <FFM No differences in FM e basal metabolism
(Gillette-Guyonnet et al., 2000)	32 ♀ HC 32 ♀ AD	DEXA	No differences in FFM (trend < FFM in AD)
Burns et al., 2010	70 HC 70 mild AD	DEXA	AD vs. HC: <FFM
Buffa et al., 2010	468 HC 83 mild-moderate AD 9 institutionalized AD	BIVA Mini Nutritional Assessment	AD vs. HC: < PA In severe AD: > Z
Buffa et al., 2014	560 HC 70 AD	Specific BIVA Mini Nutritional Assessment	AD vs. HC: < BCM, > ECW/ICW, > % FM No differences in MNA between AD and HC

HC= healthy controls; AD = Alzheimer's disease patients; BCM=Body Cell Mass; ECW=Extracellular Water; FFM=Fat Free Mass; FM=Fat Mass; PA=Phase Angle; TCW=Total Body Water; Z=Impedance.

Buffa et al., are the first group which has used the BIVA method to study nutritional status in AD subjects (Buffa et al., 2010, 2014). In their studies they have shown that AD had a significant lower PA, namely a lower BCM; no other group have so far replicated their data. No body composition studies in MCI subjects are yet available; it is likely that a different assessment of body compartments can be detectable before a clinical evident AD, namely at the stage of MCI due to AD. In such a case, the use of a simple screening tool such as BIVA could identify subjects with MCI most at risk of progressing to AD.

Aims of the study

Cross-sectional part

To analyze nutritional and body composition differences between AD, MCI and HC in order to identify a possible AD diagnostic marker.

Longitudinal part

To verify if any differences in nutritional and/or body composition parameters suggested from the cross-sectional part of the study could serve also as a staging biomarker of AD.

To verify if any differences in nutritional and/or body composition parameters suggested from the cross-sectional part of the study could serve also as a prognostic biomarker of MCI.

Materials and Methods

Subjects and design

This study was carried out in the Center for Research and Treatment on Cognitive Dysfunctions of the Luigi Sacco Hospital, University of Milan.

The study protocol was approved by the Sacco Hospital Ethics Committee and informed written consent from all subjects was obtained by the principal researcher, after neuropsychological assessment of the patients' capacity to provide a consent.

We enrolled outpatients consecutively admitted from December 2014 to January 2016 with a diagnosis of MCI (by NIA-AA criteria (Albert et al., 2011) or mild to moderate probable AD with increased level of certainty with a documented decline (by NIA-AA criteria (McKhann et al., 2011). Cognitively healthy controls (HC) were enrolled in the same period and consisted of patients'

spouses or relatives of Neurology department inpatients (hospitalized due to acute disease of CNS, such as stroke) and outpatients.

Subjects were excluded if aged < 65 years, if they had pacemakers, heart defibrillators or other electrical implants and if they were suffering from a known active cancer.

All study participants underwent an evaluation following a standardized protocol. Collected data included demographic characteristics, medical history, present and previous pharmacological treatments. MCI and AD participants were also evaluated with an extensive neuropsychological assessment (Cova et al., 2016a), clinical and neurological examination, standard laboratory blood tests and neuroimaging (MRI or CT scan). In our center, AD patients are usually requested to return every 6 months for clinical follow-up visits to monitor cognitive status, level of functioning based on information from caregivers and subsequently adjust therapies. MCI are follow-up annually by repetition of neuropsychological tests.

HC underwent cognitive screening with Mini Mental State Examination (MMSE) (Folstein et al., 1975) and mood evaluation with 30-items Geriatric Depression Scale (GDS) (Yesavage et al., 1982).

Nutritional evaluation

Nutritional evaluation was performed at baseline for all the subjects and repeated at follow-up in AD patients by means of anthropometry, Mini Nutritional Assessment (MNA) and bioelectrical impedance vector analysis

Anthropometric measurements were taken by the principal researcher following standard criteria (Timothy G, Lohman, Alex F. Roche, Reynaldo Martorell, 1988). Height (cm) was measured with an anthropometer and weight (kg) with a mechanical beam scale; body mass index (BMI) (kg/m^2) was hence calculated. Body circumferences (waist, mid arm and calf) were obtained with an inelastic plastic-fiber tape measure (to the nearest 1 cm); the waist was measured midpoint between the lowest rib and the upper border of the iliac crest; the mid arm was measured at the midpoint

between the lateral tip of the acromion and the most distal point on the olecranon; the calf was measured at the maximum girth (Al-Gindan et al., 2014).

MNA (Guigoz, 2006) as previously described, is an 18-item tool used to assess nutritional risk in elderly, grouped in 4 rubrics: anthropometric assessment (BMI, weight loss, arm and calf circumferences; items B, F, Q and R); general assessment (lifestyle, medication, mobility and presence of signs of depression or dementia; items C, D, E, G, H and I); short dietary assessment (number of meals, food and fluid intake, and autonomy of feeding; items A, J, K, L, M and N); and subjective assessment (self perception of health and nutrition; items O and P). Each answer has a numerical value and contributes to the final score, which reaches a maximum of 30; AD patients were helped to complete it by their caregivers. MNA screening (sum of items from A to F), global (sum of items from G to R) and total score were collected.

Bioimpedance measurements were carried out in subjects fasting for at least three hours. The bioelectrical variables of resistance (R, Ohm) and reactance (Xc, Ohm) were measured with a single frequency impedance analyzer (EFG-ElectroFluidGraph; AKERN-Srl, Florence, Italy).

The accuracy was checked with a calibration circuit of known impedance (R: 383 Ohm, Xc: 45 Ohm, 1% error). Moreover, test–retest reliability over a time interval of 14 days was checked in a group of 15 healthy controls.

Whole body impedance measurements were taken using the standard positions of outer and inner electrodes on the right hand and foot (Anon, 1996). The length of the impedance vector (Z) was calculated by the equation $Z= (R^2+Xc^2)^{0,5}$ and the phase angle (PA) by arctan (Xc/R). The R, Xc and Z values were divided by the subject's height (H) to remove the effect of conductor length (Piccoli et al., 1994).

Impedance measurements standardized by height were represented as bivariate vectors with their confidence intervals, which are ellipses in the R-Xc plane.

Covariates

Potential confounders included personal data, such as age, sex, years of education, MMSE score, GDS score, smoking habits (previous smoker, actual smoker, no smoker). Somatic comorbidities were quantified using the Modified Cumulative Illness Rating Scale (CIRS) (Salvi et al., 2008). The modified CIRS includes 14 categories assessing the impairment of each organ system, with a score ranging from 0 to 4. The total score was calculated by adding the scores from each of the 14 individual system scores. The "CIRS comorbidity index", based on the sum of CIRS items with scores ≥ 2 (indicating moderate disability or morbidity and/or requirement of first line therapy) was also calculated. We evaluated comorbidities with particular emphasis on disease and treatments which could play a role in body composition, such as diabetes mellitus, dysthyroidism (hypothyroidism or hyperthyroidism), depression (clinical depression with/without treatment and/or GDS score ≥ 11), use of antihypertensives, especially diuretics, oral hypoglycemic agents, insulin, levothyroxine and antidepressants.

Statistical Analysis

Subjects' characteristics among the three groups of participants (HC, MCI and AD subjects) were compared separately for males and females as previously proposed (Anon, 1996; Buffa et al., 2010) using univariate ANOVA test for continuous variables and Pearson's χ^2 test for categorical variables. Post hoc analyses with Bonferroni correction were performed when appropriate. Multivariate analyses with general linear models were then carried out, with nutritional indicators, anthropometric and bioelectrical variables as the dependent variable, the dementia diagnostic category (normal, MCI, and AD) as group and other significant demographic and psycho-functional variables emerged in previous univariate analyses as potential confounding variables as covariates. Three general linear models (GLM) were carried out: unadjusted, partially adjusted (for sociodemographic variables such as age, gender and education) and fully adjusted (for sociodemographic variables and psycho-functional status).

The differences between the mean impedance vectors in AD, MCI and HC groups were assessed with Hotelling's T^2 test, a multivariate extension of the univariate t-test and graphically with 95% probability confidence ellipses. Non-overlapping 95% confidence ellipses correspond to statistically significant difference between mean vector displacements on the R-Xc plane ($P < 0.05$, which corresponds to a significant Hotelling's T^2 test, that is equivalent to a significant difference in R, Xc or both parameters).

Mahalanobis D distance (D) among mean vectors, which uses within-groups variation (elliptical shape) as a yardstick for differences between means, was also calculated.

Nutritional variables of AD group at baseline and follow-up were analysed by Wilcoxon test and linear regression analysis within gender.

MCI subjects' characteristics at baseline were compared by outcome using the Mann-Whitney test for continuous variables and Pearson' s χ^2 test for categorical variables.

All p values <0.05 were regarded as statistically significant. SPSS for Windows (version 23.0) was used for statistical analyses. BIVA was performed with an open source specific software (Piccoli and Pastori, 2002).

Results

Cross-sectional part of the study

Table 1 and 2 show psycho-functional, anthropometric, multidimensional and bioelectrical variables in healthy controls (HC), Mild Cognitive Impairment (MCI) and Alzheimer's disease (AD), respectively in men and in women.

Table 1. Descriptive and comparative statistics for the psycho-functional, anthropometric, multidimensional and bioelectrical variables in healthy controls (HC), Mild Cognitive Impairment (MCI) and Alzheimer's disease (AD) men.

	HC (N=29)	**MCI** (N=14)	**AD** (N=24)	**F**	**Post Hoc §**
Demographic variables					
Age (y)	74.3 ± 5.3	78.6 ± 4.4	77.5 ± 8.2	2.85	
Education (y)	10.2 ± 3.9	10.1 ± 4.8	9.7 ± 3.8	0.11	
Psycho-functional indicators					
MMSE score	29.2 ± 0.9	25.1 ± 2.5	19.7 ± 4.4	68.78***	AD<MCI <HC
GDS score[a]	6.6 ± 3.6	5.1 ± 3.6	11.8 ± 5.1	7.34***	HC=MCI<AD
ADL (functions lost)	0.0 ± 0.0	0 ± 0	1.6 ± 1.8	16.73***	AD<MCI=HC
IADL (functions lost)	0.0 ± 0.0	0.7 ± 0.1	3.5 ± 1.7	159.29***	AD<MCI=HC
Nutritional indicators					
MNA screening score	13.9 ± 0.4	12.9 ± 1.3	11.9 ±1.2	24.78***	AD< MCI<HC
MNA global score	14.2 ±1.1	14.2 ± 1.3	12.2 ± 1.4	19.06***	AD<MCI=HC
MNA total score	28.0 ± 1.1	27.1 ± 1.9	24.1 ± 2.3	33.04***	AD<MCI=HC
Anthropometric variables					
BMI (kg/m^2)	26.6 ± 2.5	26.5 ± 3.0	24.8 ± 3.0	3.12	
Arm circumference (cm)	26.9 ± 2.8	26.1 ±2.0	24.0 ± 3.0	7.96**	AD<HC
Calf circumference (cm)	35.2 ± 2.8	33.6 ± 3.1	32.9 ± 2.3	4.86*	AD<HC
Waist circumference (cm)	96.7 ± 7.9	99.1 ± 9.4	90.4 ±10.6	4.86*	AD<MCI
Bioelectrical variables					
Rz/h (Ω/m)	231.8 ± 23.8	261.0 ± 34.2	260.6 ± 35.4	7.29**	HC<MCI=AD
Xc/h (Ω/m)	26.7 ± 3.2	29.5 ± 6.5	27.2 ± 4.8	1.77	
PA (°)	6.6 ± 0.7	6.4 ± 0.7	5.9 ± 0.6	6.39**	AD<HC
Z/h (Ω/m)	233.3 ± 23.9	262.0 ± 34.6	262.1 ± 35.6	7.20**	HC<MCI=AD

Values are expressed as mean ± standard deviation

[§]Only significant differences are shown [a] available for HC, MCI and 28 % AD group. * $p<0.05$ ** $p<0.01$ *** $p< 0.001$

Table 2. Descriptive and comparative statistics for the psycho-functional, anthropometric, multidimensional and bioelectrical variables in healthy controls (HC), Mild Cognitive Impairment (MCI) and Alzheimer's disease (AD) women.

	HC (N=29)	MCI (N=20)	AD (N=35)	F	Post Hoc §	F ♯
Demographic variables						
Age (y)	75.1 ± 6.4	76.9 ± 4.5	82.1 ± 4.8	14.82***	HC<AD; MCI<AD	
Education (y)	8.0 ± 3.4	6.5 ± 3.5	6.1 ± 3.3	2.46		3,81*
Psycho-functional indicators						
MMSE score	29.2 ± 0.9	25.9 ± 2.5	18.9 ± 4.9	75.42***	AD<MCI; MCI<HC	50,12***
GDS score[a]	7.6 ± 4.0	10.7 ± 5.6	7.6 ± 5.0	2.56		1,96
ADL (functions lost)	0.0 ± 0.0	0.0 ± 0.0	1.9 ± 1.8	27.09***	HC=MCI<AD	21,87***
IADL (functions lost)	0.0 ± 0.0	0.3 ± 0.6	5.0 ± 2.0	104.93***	HC<MCI<AD	102,38***
Nutritional indicators						
MNA screening score	12.7 ± 1.4	12.4 ± 2.4	11.8 ± 1.4	2.24		2,02
MNA global score	14.4 ± 1.1	13.0 ± 1.4	11.6 ± 1.7	28.80***	AD<MCI <HC	19,90***
MNA total score	27.1 ± 4.5	25.4 ± 3.1	23.4 ± 2.7	16.62***	AD<HC	12,11***
Anthropometric variables						
BMI (kg/m^2)	27.2 ± 4.5	25.8 ± 4.3	25.1 ± 3.9	1.87		1,88
Arm circumference (cm)	26.9 ± 3.2	25.4 ± 2.8	25.0 ± 3.1	3.02		3,07*
Calf circumference (cm)	33.9 ± 2.8	33.1 ± 2.7	30.9 ± 3.5	7.68**	AD<HC=MCI	5,58**
Waist circumference (cm)	93.0 ± 12.2	86.4 ± 9.5	86.4 ± 10.5	3.48		2,37
Bioelectrical variables						
Rz/h (Ω/m)	286.8 ± 34.3	303 ± 38.2	311.9 ± 30.9	4.40*	HC<AD	3,30*
Xc/h (Ω/m)	30.2 ± 4.1	29.3 ± 3.4	29.5 ± 5.0	0.30		4,71**
PA (°)	6.1 ± 0.6	5.6 ± 0.6	5.4 ± 0.7	6.39*	AD<HC	17,54***
Z/h (Ω/m)	288.4 ± 34.4	305 ± 38.2	313.4 ± 31.0	7.20*	HC<AD	3,21*

Values are expressed as mean ± standard deviation.

§only significant differences are shown [a] available for HC, MCI and 14,3 % AD group * p<0.05 ** p<0.01 *** p< 0.001

♯ multivariate general linear model (adjusted for age)

Sociodemographic variables did not differ among AD, MCI and HC, except for females' age (HC <AD; MCI <AD), therefore age was used as covariate in a multivariate GLM (Table 2). AD were enrolled after 68.1 ± 12 months and MCI after 64.1 ± 43.5 months from the onset of cognitive impairment.

AD, MCI and HC were similar in terms of factors potentially confounding the relationship between body composition and dementia process (smoking habit, diabetes mellitus, use of oral hypoglycemic agents/insulin, dysthyroidism, use of levothyroxine, use of diuretics/other antihypertensives, use of antidepressants), with the exception of clinical depression which was more prevalent among women with MCI than HC (Pearson's χ^2 test $p<0.05$; post hoc: HC<MCI; AD<MCI) and CIRS total score which was higher in MCI with respect to AD and HC (0.5 ± 0.2 vs. 0.3 ± 0.2 vs. 0.4 ± 0.2, p= 0.003; post hoc: HC<MCI; AD<MCI).

MNA global and total score were lower in AD than in HC in both sexes; MCI did not differ from HC except for a lower MNA screening score in men and for a lower global score in women.

Interestingly, when analyzing each MNA subitems, hydration (item M) resulted significantly reduced in AD with respect to MCI and HC (Pearson's χ^2 test; men $p<0.05$, women $p<0.001$).

With regards to anthropometric measurements, AD of both sexes showed significantly lower arm and calf circumferences with respect to HC (women's arm circumferences in multivariate GLM corrected for age: F 3.07, $p<0.05$; AD< MCI=HC). AD men had smaller waist circumferences than HC and MCI.

The phase angle (PA), ratio of reactance to height (R/h) and ratio of impedance to height (Z/h) were significantly different between AD and HC in both sexes; AD women showed also a significantly lower ratio of reactance to height (Xc/h) than HC in the multivariate GLM with age used as a covariate (F 4.71, $p<0.01$). A higher ratio of reactance to height (R/h) and ratio of impedance to height (Z/h) was found in men with MCI with respect to HC.

No statistically significant differences in bioelectrical parameters (PA, R/h, Xc/h, Z/h) were found between MCI women with and without depression.

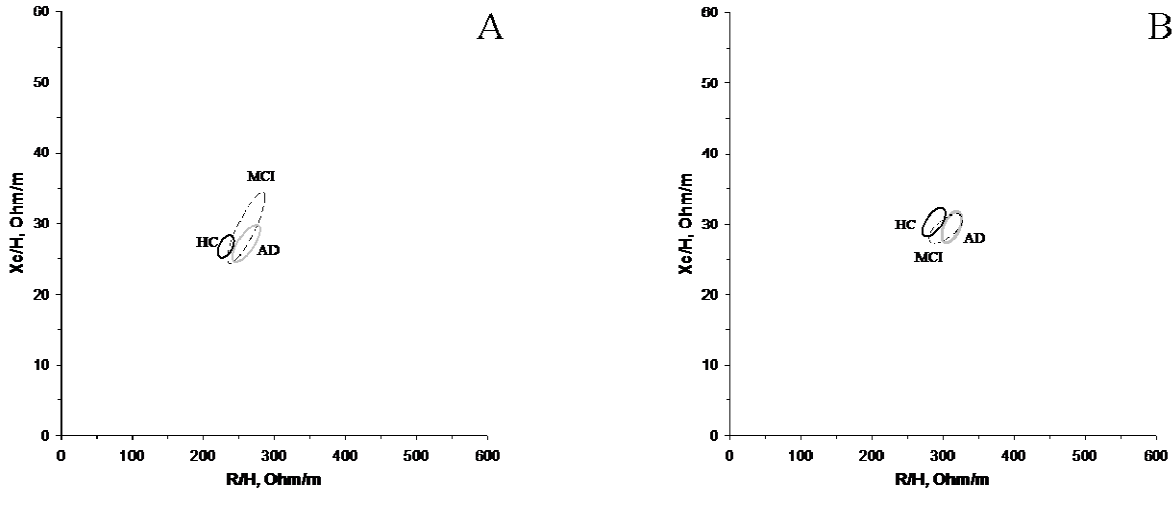
Unadjusted, partially adjusted and fully adjusted GLM showed overlapping results.

Given the small study sample, a sensitivity analysis with non-parametric tests (Kruskal-Wallis) was also performed and confirmed the results.

No significant correlation emerged between AD disease duration (from disease onset) and nutritional assessment with MNA or bioelectrical parameters.

Mean impedance vector and confidence ellipses are shown in Fig. 1A and 1B; statistical comparison of groups with Hotelling's T^2 test, with the corresponding p value and Mahalanobis distance D are also reported.

Fig. 1 A Distribution of confidence ellipses of men with Alzheimer's dementia (AD), Mild Cognitive Impairment (MCI) and healthy controls (HC). B Distribution of confidence ellipses of women with Alzheimer's dementia (AD), Mild Cognitive Impairment (MCI) and healthy controls (HC).



Men	T^2	P	D
AD vs. HD	18.2	<0.001	1.20
AD vs. MCI	3.6	0.18	0.64
MCI vs. HD	10.6	<0.05	1.06

Women	T^2	P	D
AD vs. HD	16.9	<0.001	1.03
AD vs. MCI	0.9	0.63	0.27
MCI vs. HD	7.9	<0.05	0.82

T2, Hotelling's statistic; P, p- value; D, Mahalanobis' generalized distance

The 95% confidence ellipses significantly differed between HC and AD (Hotelling's T^2 test: men = 18.2; women = 16.9; $p < 0.001$). The ellipses of AD shifted toward the inferior region of the RXc

graph, corresponding to low body cell mass (Fig 1). The ellipses of MCI were closer to AD than HC, and significantly differed from HC (Hotelling's T^2 test: men = 10.6; women= 7.9; $p < 0.05$).

The effect size (Anon, 2013) computed for groups (AD vs. HC), by adjusting the calculation of the pooled standard deviation with weights for the sample sizes are reported in table 3.

Table 3. Effect size (CI 95%) of bioelectrical variables for Alzheimer's disease (AD) patients with respect to healthy controls (HC).

Groups	Bioelectrical variables	Effect size (95% CI)
AD vs. HC (men)	PA(°)	1.066 (0.488 – 1.643)
	Z/h (Ω/m)	-0.9 (-1.539 – -0.397)
AD vs. HC (women)	PA(°)	1.066 (0.539 – 1.592)
	Z/h (Ω/m)	-0.767 (-1.277 – -0.258)

Longitudinal part of the study

AD

After 8.7 ± 3.6 months, AD showed overall a significant worsening of MiniMental State Examination (MMSE) (19.4 ± 4.5 vs.18.3 ± 5.2, $p = 0.04$), Clinical Dementia Rating Scale (CDR) (1.7 ± 0.1 vs. 2.1 ± 0.1, $p < 0.001$), Activity of Daily Living (ADL) scores (4.0 ± 0.3 vs. 3.6 ± 0.3, $p = 0.02$). Anthropometric and bioelectrical variables did not significantly change during follow-up except for women's arm circumference (24.2 ± 2.7 vs. 23.6 ± 2.7, $p = 0.049$) Table 4 and 5 show clinical, functional and nutritional variables at baseline and follow-up in AD for males and females respectively.

Table 4. Clinical, functional and nutritional variables in 15 men with AD at baseline (T0) and follow-up (T1).

	AD T0	AD T1	p
MMSE	20.0 ± 4.6	18.7 ± 5.0	ns
ADL (lost)	2.4 ± 1.8	1.3 ± 1.8	ns
IADL (lost)	4.1 ± 1.5	4.1 ± 1.6	ns
CDR	1.8 ± 2.1	2.1 ± 0.7	ns
BMI (kg/m^2)	25.1 ± 3.4	25.1 ± 3.2	ns
Arm circumference (cm)	24.1 ± 3.2	24.7 ± 3.7	ns
Calf circumference (cm)	32.7 ± 2.4	32.5 ± 2.7	ns
Waist circumference (cm)	91.8 ± 10.7	91.7 ± 9.0	ns
Rz/h (Ω/m)	256.5 ± 36.3	250.5 ± 36.3	ns
Xc/h (Ω/m)	26.0 ± 4.5	25.8 ± 4.9	ns
PA (°)	5.7 ± 0.5	5.8 ± 0.7	ns
Z/h (Ω/m)	257.8 ± 36.4	251.8 ± 36.5	ns

Values are expressed as means ± standard deviation

Table 5. Clinical, functional and nutritional variables in 25 women with AD at baseline (T0) and follow-up (T1).

	AD T0	AD T1	p
MMSE	19.2 ± 4.4	18.1 ± 5.4	ns
ADL (lost)	1.8 ± 1.8	2.5 ±1.8	0.003
IADL (lost)	5.0 ± 2.2	5.6 ± 1.6	0.042
CDR	1.6 ± 0.7	2.1 ± 0.7	0.002
BMI (kg/m^2)	24.2 ± 3.3	23.9 ± 3.5	ns
Arm circumference (cm)	24.2 ± 3.3	23.9 ± 2.7	0.038
Calf circumference (cm)	30.8 ± 3.0	30.4 ± 3.2	ns
Waist circumference (cm)	83.5 ± 9.5	82.7 ± 10.3	ns
Rz/h (Ω/m)	321.1 ± 32.3	323.9 ± 39.8	ns
Xc/h (Ω/m)	31.1 ± 5.1	32.0 ± 5.0	ns
PA (°)	5.6 ± 0.8	5.6 ± 0.7	ns
Z/h (Ω/m)	322.7 ± 32.4	326.2 ± 39.2	ns

Values are expressed as means ± standard deviation

A linear regression model with phase angle as dependent variable and time of follow-up and MMSE score change over time as independent variables did not yield significance.

MCI

Forty-three MCI (28 females, 15 males) were recruited and followed up for 14.4 ± 8.6 months; 8 (6 females, 2 males) of them progressed to AD, amongst them 3 (2 females, 1 male) also showed a mild vascular encephalopathy at MRI; no other forms of dementia have been diagnosed during follow-up. Due to the limited number of males progressed, we have only explored bioelectrical characteristics at baseline of females MCI (Table 6).

Table 6. Baseline clinical and bioelectrical variables in the whole sample of MCI, in stable MCI at follow-up and in MCI progressed to AD (females).

	MCI stable (N = 22)	**MCI progressed to AD (N = 6*)**	**p**
Age	78.3 ± 5.0	76.5 ± 3.0	n.s.
Education	5.5 ± 2.4	10.0 ± 4.1	0.006
MMSE score	26.1 ± 2.5	24.3 ± 2.9	n.s.
IADL lost	0.1 ± 0.4	0.8 ± 1	n.s. (0.064)
CIRS total score	0.45 ± 0.23	0.54 ± 0.25	n.s.
CIRS 2	2.9 ± 1.6	3.5 ± 1.6	n.s.
BMI	25.1 ± 5.1	24.6 ± 2.4	n.s.
CB	24.8 ± 2.9	24.5 ± 3.5	n.s.
CP	32.1 ± 3.6	31.5 ± 3.1	n.s.
CA	83.3 ± 11.8	87.8 ± 7.4	n.s.
Rz/h	305.7 ± 38.4	326.4 ± 42.9	n.s.
Xc/h	31.5 ± 5.6	29.8 ± 3.3	n.s.
PA	5.9 ± 1.0	5.2 ± 0.6	n.s. (0.069)
Z/h	307.3 ± 38.4	327.8 ± 42.9	n.s.

*2 of them received a diagnosis of AD associated with mild vascular encephalopathy

The effect size computed for groups (MCI vs. HC), by adjusting the calculation of the pooled standard deviation with weights for the sample sizes, are reported in table 6.

Table 7. Effect size (CI 95%) of bioelectrical variables for Mild Cognitive Impairment (MCI) subjects with respect to healthy controls (HC).

Groups	Bioelectrical variables	Effect size (CI 95%)
MCI vs. HC (men)	PA(°)	0.286 (-0.335 – 0.926)
	Z/h (Ω/m)	-1.034 (-1.709 – -0.360)
MCI vs. HC (women)	PA(°)	0.833 (0.240 – -1.426)
	Z/h (Ω/m)	-0.461 (-1.038 – -0.116)

Discussion

Patients with mild-moderate AD showed a significantly different nutritional status with respect to cognitively HC in anthropometric measurements and bioelectrical parameters of BIVA; MCI subjects demonstrated an intermediate pattern of BIVA vectors between mild-moderate AD and HC and, in particular, female MCI who progressed to clinically evident AD had a lower PA (which is the most important impedance parameter indicating soft tissue) than stable MCI. Bioelectrical parameters appear to be stable when BIVA was repeated during AD follow-up (8.7 ± 3.6 months).

While our findings in AD patients are consistent with available literature, to our knowledge this is the first study which has analysed body composition by BIVA in subjects with MCI. In 2010 Buffa et al. (Buffa et al., 2010) first applied BIVA to AD patients and found significantly lower PA in patients with mild-moderate AD of both sexes with respect to controls; furthermore they found that women with severe AD showed reduced tissue mass and dehydration when compared with AD patients with mild-moderate disease severity; no longitudinal data were available.

In 2012 the same group (Saragat et al., 2012) detected higher impedance values (Z/h and R/h) in AD patients than in controls and suggested an increase of fat component with respect to the muscle mass along with psycho-functional decline: this hypothesis was supported by replication of these findings

with the technique of “specific” BIVA (Buffa et al., 2014), where “specific” values (resistivity [Rsp] and reactivity [Xcsp], $\Omega \cdot cm$) were obtained by multiplying R and Xc by a correction factor which includes an estimate of the cross-sectional area of the body.

We have noticed that nutritional status of our HC and AD population completely differs from Sardinian population enrolled in the studies of Saragat and Buffa (Saragat et al., 2012; Buffa et al., 2014), since we detected significantly lower impedance values (R/H, Xc/H and Z/h) in both populations. This finding underlines the importance of recruiting a local control population in nutritional studies, because differences in body composition and hydration status can be conspicuous even among regions of the same country and may be linked to different dietary habits.

Recently, it has been suggested that BIVA could reflect dementia-related changes in body composition better than BIA in a study which involved men with (undefined subtype of) dementia (Camina Martín et al., 2015). This statement could be mainly due to the fact that fat free mass has not the same hydration percentage (73%) as conventional BIA presupposes. This is the reason why we preferred to avoid calculating fat mass (FM) and fat free mass (FFM) in the present study. Our findings of such alteration of electrical properties of tissues in AD patients support the hypothesis of their lower muscle mass and consequently higher fat mass. During aging process, reduction of body weight, height and FFM, associated with an increase in FM is well documented (Doherty, 2003). However, body composition of elderly subjects with AD differs from that of cognitively healthy elderly subjects (Renvall et al., 1993): lower arm and calf circumferences and bioelectrical differences in AD patients with respect to controls in the present study corroborate this hypothesis. Right side displacement of impedance vector in AD group indicates lower values of body cell mass (Withers et al., 1999) and therefore worse nutritional parameters.

Nutritional status found with BIVA in AD tends to remain relatively stable during follow-up (after a mean follow-up of 9 months); no previous studies have investigated the presence of an eventual variability of BIVA parameters during AD follow-up. This result could be interpreted in two ways: on the one hand follow-up might have been too short to identify any significant difference, but on the other hand a possible alternative explanation is that body composition change may occur earlier,

namely in a prodromal phase (MCI due to AD) and then remain stable or progress slowly. So even if BIVA pattern appears able to distinguish AD from HC, it does not seem useful as a marker of disease progression (staging biomarker), at least in a short time interval suitable for change analysis in clinical trials. Moreover, body composition differences in AD may appear during early phase of disease (MCI) and then decline slowly: the different BIVA pattern shown by Buffa et al. is the result of a cross-sectional comparison.

MCI could be a preclinical phase of AD or other type of dementia since it has a progression rate around 10–15% per year in memory clinics (Petersen et al., 2014). Our previous works showed that a low BMI, as well as weight loss, could predict progression of MCI and several biologically plausible hypotheses have been previously proposed (Cova et al., 2016a, 2016b). In the cross-sectional part of the present study we found significantly different confidence ellipses in RXc graph with respect to controls, meaning decreased conductive tissue mass (tendency towards sarcopenia); this suggests that soft tissue mass could decrease with cognitive impairment independently from aging process. Analysis of body composition with BIVA could then detect early changes in body composition which could reflect early systemic manifestation of the AD process (Morris et al., 2014) at MCI stage of disease, before anthropometric change becomes evident. Indeed, after a follow-up of a mean of 14 months, female MCI who progressed to clinically evident AD showed a lower PA than stable MCI (which tend to be significant). This last finding represents a very preliminary result, since we know that MCI subjects should be followed up longer to capture all cases which will develop dementia (Petersen, 2004).

This result further supports the hypermetabolic hypothesis of weight loss in AD (see above) (Sergi et al., 2013). Moreover, in our cohort of AD we have found approximately a 60% of insulin resistance (Homeostasis Model Assessment of IR [HOMA] index 2 > 1,4) (Geloneze et al., 2009) in available blood samples (16 patients). HOMA index is a validated method to measure insulin resistance from fasting glucose and insulin. Few HOMA index were available for our MCI cohort (only for 5 subjects, 4 of them showed insulin resistance). High insulin concentrations are implicated in the neuropathological mechanisms underlying the neuronal damage of AD

(Kandimalla et al., 2017). In a study of 2015, subjects affected by amnestic MCI who converted to AD showed higher insulin sensitivities indexes than stable MCI (Fiammetta et al., 2015): MCI due to AD is proposed as an "early biochemical active disease stage" where hyperinsulinemia, glycoxidation and pro-amyloidogenic status are at the highest rate, whereas clinically manifest AD could represent a final stage of a glycoxidative cascade, a process which possibly began two decades earlier.

There is growing evidence that brain insulin levels and insulin-like growth factor (IGF) resistance and mediated metabolic imbalance may be considered as critical etiologic factors in AD; this suggests that these indices and their consequences (i.e. oxidative stress, neuro-inflammation, and reduced neuronal plasticity) should be included in biomarker panels for AD (Lee et al., 2013). Our study suggested a possible role of BIVA parameters in this context.

It is also clear that dietary habits influence glucose-insulin homeostasis, pathways of weight regulation, visceral adiposity, the gut microbiota as well as oxidative stress, and inflammation (Solfrizzi et al., 2017). Recent findings suggest that AD patients have a higher detection threshold for all tastes and a higher recognition threshold for sweet (Sakai et al., 2016); this result may explain their common increased desire for sweet taste, which is known also to grow when body requires more energy (due to a hypermetabolic state). Moreover, dorsolateral prefrontal cortex dysfunction in AD could be involved in sugar cravings. Different nutritional "trajectories" may be postulated in different subtypes of AD and perhaps from other dementia types, due to different shares of each potential pathological stressors (Mravec et al., 2017)and which become apparent in body composition changement. Diet is one of the main factor which can influence ageing of the brain and subsequent age-related diseases. It is well known that certain nutrients, such as polyphenolic compounds contained in fruits and vegetables (Dai et al., 2006), polyunsaturated fatty acids, some vitamins (Luchsinger and Mayeux, 2004) may play a protective role in the ageing brain and in pathogenesis of AD. Also voluptuary habits, such as alcohol, coffee and chocolate consumption, due to their content in neuroactive substances, may interfere with cognitive processes. Alcohol may impair blood glucose control: if a malnourished or a fasting

person drink alcohol, hypoglycemia can arise and subsequently a depletion of stored sugar needed for energetic cellular functions occurs (Patel, 1989). The neuroprotective effect of methylxanthines (caffeine, theobromine and theophylline) is well-known (Oñatibia-Astibia et al., 2017) so much that xanthine derived-drugs have been studying for AD therapy. It is very complex to understand the long-life effect of a complex diet, but it is unreasonable not to suppose its key role in the pathogenesis of several disease such as AD. Although a healthy diet may provide bioactive nutrients able to preserve biological functions and potentially to prevent disease development, different food processing and cooking methods are also important elements to considered. Whereas dietary recommendations for many years focused on single vascular risk factors prevention and treatment (e.g., hypertension, blood cholesterol, etc.) and current nutritional discussions often concern total calories and obesity, the full health impact of diet extends far beyond these themes, opening the way to consider a diet-related prevention of dementia and AD.

Our results should be interpreted within the context of the limitations of the study. First, the sample size was small; therefore, further studies with a larger sample size are required to confirm our data. Second, our study lacks other putative biomarkers' investigation; however, the subjects classified as MCI respected international core clinical criteria of 2011 (Albert et al., 2011).

The strengths of our study are the clinical setting where the study took place, which allows an optimal characterization of subjects with MCI and AD from a psychometric point of view; furthermore, this setting facilitated detailed baseline collection of several potential confounding variables (comorbidities – particularly with regard to metabolic disorders and depression – and treatments). Finally, participants of this study are representative of those who routinely consult memory clinics.

Increasing the cohort of MCI subjects and their longitudinal observation will provide further information to allow generalization to populations of MCI attending memory clinics and to understand if a BIVA pattern indicating a worse nutritional status could be an early and sensitive marker of progression to dementia or specifically to AD in MCI subjects. Further studies will be needed to evaluate nutritional status and bioimpedance analysis patterns in other types of dementia than Alzheimer's.

Considering the differences found in MNA scores among different cognitive groups, we suggest implementing clinical practice of cognitively impaired patients with such a simple questionnaire, also to address nutritional advice when malnutrition is suspected.

In summary, since little is known about nutritional status of MCI subjects, our work contributes to the growing research interest in this area. In any cross-sectional study, we cannot discriminate the direction of causality; longitudinal data provided are preliminary. Our finding should be considered tentative until future studies confirm or disprove our observations.

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