Review Article

Implications of protein malnutrition and inflammatory disorders in the pathophysiology of Alzheimer’s disease

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Lean body mass (LBM) agglomerates the bulk of nitrogen (N)-containing molecules following well-identified age and sex evolutionary patterns best appraised in clinical practice using the serial measurement of plasma transthyretin (TTR). Methionine (Met), the sole essential amino acid bearing a sulfur (S) atom, presides at the initiation of protein synthesis while maintaining stable body tissue S:N molar ratios of approximately 1:14.5. In protein-depleted states, N- and Met-deficiencies operate as limiting factors for LBM protein synthesis and accretion, causing growth retardation and subnormal TTR plasma values. In inflammatory disorders, LBM is subjected to cytokine-induced tissue breakdown reflecting the S:N ratio found in healthy tissues whereas the liver secretion of TTR declines in proportion. Both malnutrition and inflammation are characterized by stepwise LBM downsizing and reduced bioavailability of Met body stores setting in motion molecular mechanisms safeguarding Met homeostasis at the expense of augmented homocysteine (Hcy) values in biological fluids. Divergent TTR and Hey alterations indicate that rising Hey values measured in plasma and cerebrospinal fluid should be regarded as the dark side of efficient compensatory processes. As a result, the neuroprotective activities normally exerted by TTR are weakened, whereas the oxidative burden generated by supranormal Hcy concentrations are strengthened. The combination of protein malnutrition and inflammatory disorders of any cause maximizes the risk of incurable neurodegenerative effects.

Key Words: lean body mass, malnutrition, inflammation, transthyretin, homocysteine

INTRODUCTION

Taking Alzheimer’s disease (AD) as an emblematic model, the worldwide ageing of mankind is associated with an increasing prevalence of neurodegenerative disorders entailing major economic and public health impacts. It is estimated that the number of people living with dementia will increase from 50 million in 2018 to 152 million in 2050, meaning a more than threefold increase.¹ East Asia is the region with the most people affected by AD (9.8 million) followed, in order, by Western Europe (7.5 million), South Asia (5.1 million) and North America (4.8 million).² Globally, approximately 5.6% of mankind that reaches the sixties in age suffers from dementia.² A considerable body of scientific publications has appeared in recent decades, focusing careful scrutiny on multiple genetic, immune and dietary corollaries.³,⁴ The first review describing that the depletion of N and S from lean body mass (LBM) stores might contribute to brain deterioration appeared in 2015.⁵ Soon after, a nationwide US survey reported that high concentrations of both elemental S and selenium (Se) in the Earth’s crust were negatively correlated with lowest AD mortality rates,⁶ suggesting that S and Se might exert neuroprotective activities. Whereas substantial gains in knowledge have already been recorded for many aspects of Se metabolism in neural disorders,⁷ no such information is currently available for S. This overlooked approach has prompted us to explore the potential harmful effects of N- and S-deprivation in elderly subjects, taking into account the behavior of both elements showing close interrelationships throughout the human lifespan.⁸ This review is an attempt to throw further insight into the putative roles played by N- and S-deficiencies in the pathophysiology of AD morbidity.

BASIC KNOWLEDGE ON N METABOLIC REQUIREMENTS

N bears the atomic number 7 and constitutes 78% of the Earth’s atmosphere in the form of a rather inert and poorly reactive gaseous substrate. N has 8 oxidation states, ranging from +5 (nitric acid, NO₃H) to -3 (ammonia, NH₃). Through lightnings and other electrical discharges, atmospheric N may undergo oxidizing processes yielding large amounts of NO₃H that are washed down on Earth and carried away by rains in seawaters as highly soluble nitrate salts.⁹ Atmospheric N may also be taken up by nODULES found on the roots of some plants, and then converted to NH₄⁺ cations by soil microorganisms (Azotobacter) living in a symbiotic association with the plant kingdom.¹⁰ The assimilation of N into plant tissues follows complex molecular and transport mechanisms. In contrast

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to higher animals, plants possess the enzymatic equipment required for the synthesis of all amino acids (AAs), including those fulfilling essential functions (EAAs).\textsuperscript{11,12} That of Met is narrowly regulated, occurring as end-product of a retrograde pathway, with cystathionine and Hcy as intermediary compounds.\textsuperscript{12}

In a reference healthy man weighing 70 kg, N occupies the fourth rank position, totaling 64 mol (1,800 g), which is mainly sequestered in the form of total body N (TBN) within the LBM.\textsuperscript{13} The size and evolutionary patterns of LBM throughout the human lifespan were investigated following the discovery that at least 95% of the naturally occurring nonradioactive potassium (39K) is intracellularly confined within all living tissues together with minute amounts (0.0117%) of 40K, a source of natural $\beta$-radioactivity.\textsuperscript{13} Both 39K and 40K isotopes show interchangeable turnover and similar metabolic fate in body tissues, maintaining narrow relationships with nitrogenous compounds assuming molar ratios of approximately 3 mEq K/g N.\textsuperscript{14} The collected results allowed to measure total body K (TBK) making possible the calculation of LBM in both sexes from birth to very old age (Figure 1).\textsuperscript{13} These pioneering surveys constitute the core of our present knowledge on body composition. Further studies have described LBM as a composite agglomeration of organs and fat free tissues that may be metabolically subdivided into two exchangeable pools: a visceral compartment comprising tissues characterized by rapid metabolic turnover rates (liver, intestinal mucosa, thymus, leukocytes) and a structural compartment made up of organs with slower turnover rates (skeletal muscle mass, skin, joints, connective tissues, appendages).\textsuperscript{13} These body distribution patterns give an account of the two main catabolites recovered in the urinary output: the excretion of urea represents approximately 90% of all N end-products, reflecting the level of deamination / transamination reactions occurring in all bodily tissues, prevailing particularly in the liver and other tissues endowed with high turnover activities. The urinary excretion of creatinine usually amounts to 5-7% of all N-catabolites, allowing the evaluation of N fluctuations in the skeletal musculature in health and disease.

**BASIC KNOWLEDGE ON S METABOLIC REQUIREMENTS**

Sulfur (S) bears the atomic number 16 in the periodic table of elements. It is the seventh most abundant element in the human body and is confined within the total body S (TBS) pool, amounting to 140 g (4,400 mmol) in the adult reference man, the same concentration as that of TBK (3,600 mmol).\textsuperscript{16} There exists extensive scientific literature describing the toxicological properties of S and S-containing substrates in living organisms\textsuperscript{17} but little has been reported about S-deprivation conditions until now. S appears to be the forgotten element, and this is all the more regrettable as several other micronutrients confined at lower degrees of magnitude within bodily tissues are held responsible for well-defined nutritional deficiencies.\textsuperscript{18}

There is geological evidence that S was present early in primordial compounds as products arising from volcanic lava flows. Soils and drinking waters surrounding eruptive areas reveal the highest concentrations of S present in free form or combined with other elements.\textsuperscript{9} In the Earth’s crust, S naturally occurs as a mixture of 4 stable isotopes, the most prevalent being 32S, accounting for

![Figure 1. Body accretion of TBK values during the lifespan of healthy human subjects. The whole body assessment of TBK levels was achieved using dual-energy X-ray absorptiometry (DXA) technology which allows the measurement in biological tissues of the naturally occurring $\beta$-radioactivity of $^{40}$K. The pioneering devices were assembled at the University of Rochester, New York, USA, under the guidance of G.B. Forbes \textsuperscript{13} in close collaboration with the International Atomic Energy Agency (IAEA), Vienna, Austria. Figure 1 compiles seven different clinical investigations performed in healthy subjects from birth until very old age. The results are plotted against age in double-logarithmic coordinates. The bulk of TBK (95%) sequestered within metabolically active tissues is tightly correlated with total body N (TBN), making this last parameter a reliable tool to assess LBM values in health and disease. The data shows a linear progression without sexual differences from birth until the onset of puberty, abrupt S-shaped rising trajectory partially obliterated during adolescence owing to altered graduations of the abscissa scale. Occurrence of gender dimorphism displays plateau levels during adulthood and disappearance of sexual differences after the sixth decade.](image-url)
Most S-compounds are water-soluble and may be washed out during rainy seasons, explaining that the soil and groundwater richness in S-compounds decreases with geographical remoteness from native sites. The biological cycle of S is thought to be initiated by chemical and bacterial agents converting inorganic S-salts into gaseous hydrogen sulfide (H₂S) at the origin of life approximately 3.8 billion years ago, working as a major source of energy in support of primitive respiratory mechanisms in early organisms. The progressive retrocession on Earth of the H₂S biochemical armamentarium has confined the gasotransmitter to inhospitable places (swamps) or to coastlands where decomposing seaweeds may accumulate. This example documents the ambivalent properties exerted by H₂S manifesting high toxicity after the inhalation of poisonous effluvia but revealing beneficial antioxidative activities induced by tiny concentrations infusing body tissues. Intestinal flora constitutes a notable exception in that it produces considerable amounts of H₂S rendered innocuous through ready-made oxidation by the colonic epithelium.

The plant kingdom is composed of autotrophic organisms, and the metabolism of S is highly regulated by the oxidation of S-salts into SO₄²⁻ prior to their capture from soils by plant roots. The main source of plant energy is solar radiation which stimulates the photosynthetic processes occurring in chloroplastic organelles and leads to the conversion of carbon dioxide (CO₂) into glucose. Oxyanions undergo complex assimilatory reducing and transfer processes to O-acetylslerine molecules, which may exchange a C atom for an S atom to become cysteine (Cys), which is regarded as a precursor substrate yielding Met and other S-containing compounds, including S-adenosyl-L-methionine (SAM) and glutathione (GSH). The bioavailability of SO₄²⁻ oxyanions from soils and tap-waters may vary considerably, from less than 10 mg/L to more than 1 g/L, depending on their geo-hydrological location, working as a limiting factor for protein synthesis and for plant growth.

The animal kingdom consists of heterotrophic organisms that are unable to initiate the synthesis of Met from SO₄²⁻ sources, hence they are depend on the intake of plant and animal tissues to fulfill the requirements for this EAA with ensuing production of all S-containing molecules. Studies have shown that Cys demonstrates semi-essential properties in that it may serve as a sparring factor of Met in biological processes when the EAA is not strictly required. The daily requirements for both Met and Cys are estimated to range from 13 to 16 mg/kg/day, meaning approximately 910 to 1,120 mg/day in human adult nutrition. The consumption rate of preformed Met from dietary items works as a limiting factor for protein synthesis and mammalian growth. It is also worth remembering that bioavailable Met controls the ribosomal initiation of protein synthesis which starts with the attachment of a free Met molecule to initiator transfer RNA to yield formyl-methionyl-tRNA launching the process of mRNA translation. These last data are in keeping with balance studies performed on pig models showing that, compared with the 7 other EAs, the withdrawal of Met and Cys from otherwise normal diets causes the greatest LBM depletion, nearly equal to that generated by protein-free regimens.

**N AND S INTERRELATIONSHIPS**

The two main S-containing AAs (SAAs) are characterized by striking disparities in tissue distribution patterns between the plant and animal kingdoms. The mean protein concentration of plants currently consumed in developing countries reaches 9.8 g% (1.56 g N%) with Met and Cys equally shared into two proportions of 1.27% and 1.23%, respectively, thus totaling 2.49% per g protein. These SAAs represent at least 90% of all protein-bound S-molecules. The remaining balance is made up of several minor free fractions that include Met, Cys, and GSH; this last compound is mainly confined to chloroplastic cells. Fruits and vegetables may provide more than 50% of dietary GSH. Taken together, plant products reveal great heterogeneity in terms of energy density, protein and SAA content. Most vegetable items used as staple foods are characterized by a molar S:N ratio between 1:20 and 1:35.

The mean protein concentration of animal products is nearly twice as high as that of plant products, reaching 16.5 g% (2.64 g N%). The content of both SAAs is 4.48% with a significantly greater proportion of Met (3.17%) than of Cys (1.31%). Most animal foodstuffs display large homogeneity in terms of energy, density and SAA values, yielding narrowly fluctuating S:N ratios ranging from 1:13 to 1:18, very close to mammalian tissue composition. The liver is by far the organ containing the largest concentrations of GSH, being regarded as storage site for Cys. Taken together, the above data indicate that animal tissue requirements for N and S are not optimally fulfilled by the exclusive intake of plant products, as shown in strict vegan subjects who incur the risk of persistent N- and S-deficiencies. On a weight basis, plant products indeed provide hardly half the abundance of N and S than animal-based dishes, consistent with dietary surveys showing that the usual intake of AAs is reduced by 47% in strict vegans. The maintenance of such plant-based dietary regimens from the weaning period throughout the lifespan will result in unachieved LBM replenishment, clinically identifiable by lower body weight (BW) and shorter height. The data are sustained by nutritional studies showing that vegan patients reveal highly significant (p<0.001) LBM downsizing.

At all ages, reduced stature appears to be a major and reliable predictor of LBM, accounting for up to 90% of its variance. The issue of imbalanced vegan diets has been addressed three decades ago by workers of the Massachusetts Institute of Technology who did express the recommendations, which are still valid currently, that plant-based dietary regimens should comprise at least 30% animal proteins to meet appropriate human tissue requirements.

**TRANSTHYRETIN AND ALZHEIMER'S DISEASE**

Transferrin (TTR) is a highly conserved protein in animal species first secreted by the choroid plexus (CP) and diffusing within the cerebrospinal fluid (CSF) of reptiles for 300 million years. The liver synthesis of TTR occurred much later, approximately 100 million years ago, in most classes of vertebrates. Using electrophoretic
methods, researchers were able to identify TTR in human blood\(^3\) and in human CSF\(^4\) in 1942. In addition to the formerly known serum-albumin (Alb) and thyroxine-binding globulin (TBG), this recently discovered TTR molecule was recognized as the third carrier-protein conveying thyroid hormones in the bloodstream.\(^4\) TTR is a tetrameric protein made up of 4 identical subunits (each comprising 127 AAs) that coalesce noncovalently to generate a nonglycosylated edifice, having a molecular mass (MM) of 55 kDa.\(^4\) One of the monomers transports a small retinol-binding protein (RBP, 21 kDa as MM) displaying a single binding site for one molecule of all-trans-retinol,\(^4\) thus forming a trimolecular retinol circulating complex (76 kDa as MM) whose components remain attached at a close 1:1:1 stoichiometry.\(^4\)

After birth, plasma TTR concentrations increase linearly without sexual differences during infant growth.\(^4\) Human puberty is characterized by major hormonal and metabolic alterations, leading to substantial redistribution of body tissues with a strong androgen-induced development of skeletal musculature in male teenagers\(^6\) together with concomitant elevation of TTR plasma values.\(^5\) A similar upsurge of TTR values is observed in female adolescents but with less amplitude owing to the weaker hormonal stimulation caused by estrogenic impregnation. As a result, a significantly higher S-shaped elevation of TTR is recorded in male adolescents than in their female counterparts for whom the TTR curve is blunted (Figure 2). In healthy adults, plasma TTR and RBP concentrations maintain gender-related differences, disclosing plateau levels during the full sexual maturity period.\(^5\) Normal TTR values peak at about 300-330 mg/L in adult men and at 250-270 mg/L in adult women, whereas RBP concentrations manifest comparable gender differences at 63 mg/L and 52 mg/L, respectively. Starting from the age of 60 years, muscle mass undergoes stepwise shrinking leading to a stage of sarcopenia, but with a steeper slope in elderly men,\(^4\) explaining why muscle mass no longer demonstrates sexual differences after the sixties. The serial measurement of TTR values indicates concomitant decline\(^7\) (Figure 2); therefore, TTR has recently been advocated as a useful biomarker of sarcopenia in aged subjects.\(^8\) Both TTR\(^9\) and RBP\(^10\) molecules produced by the CP and secreted in CSF follow regulatory pathways distinct from those of liver.\(^11\) With increasing age, choroidal production of TTR\(^3\) and RBP\(^9\) displays intrathecal downregulation likely to be genetically programmed.

The measurement of plasma TTR has been proposed as a sensitive biomarker of protein nutritional status\(^12\) owing to its short biological half-life (2 days), its confinement within the intravascular space and its unusual richness in tryptophan. Soon after, plasma RBP was regarded as an equally informative tool, allowing to follow-up protein-depleted patients.\(^13\) Rat experiments have shown that the restriction of dietary AA supply leading to protein malnutrition is accompanied by depressed hepatic production of TTR mRNA,\(^14\) decreased abundance of TTR nuclear transcripts,\(^7\) and corresponding reduced exportation of native TTR molecules into the bloodstream. The measurement of plasma TTR values has been reported to be diminished in AD patients\(^9\) and to be inversely correlated with disease progression;\(^9\) hence, plasma TTR was recommended as a candidate marker identifying AD severity.\(^9\) The decrease in plasma TTR values is likely explained by the downsizing of LBM resources throughout the dementia process (see below).

Alzheimer’s disease is a neurodegenerative disorder affecting predominantly female subjects.\(^15\) The healthy

![Figure 2](image.png)  
**Figure 2.** Evolutionary patterns of plasma TTR concentrations throughout healthy human lifespan. TTR concentrations were measured in the blood samples of 68,720 healthy U.S. citizens from birth until very old age using immunoturbidimetric analysis.\(^5\) Plasma TTR values identify the accretion and/or losses of N in bodily tissues, conferring to the biomarker the unique property to reflect the fluctuations of LBM stores in health and disease. The more pronounced elevation of TTR values in adolescent males is in keeping with the androgen-induced development of a larger skeletal musculature. Sexual dimorphism and TTR plateau levels observed during adulthood disappear after the sixties, indicating that elderly men and women move towards comparable sarcopenia stages with no longer sexual differences.\(^5\)\(^6\) The last four decades of life (60-100 years) collect concentrations measured in 17,645 subjects, showing that healthy centenarians maintain levels situated well above the cut-off line of 200 mg TTR / L regarded as the lower limit of normalcy. Downward decline of plasma TTR concentrations into the subnormal area works as an alarm signal pointing to the reduced LBM capacity to properly face the nutritional and inflammatory challenges associated with AD.\(^9\)\(^16\) The data show that the increased severity of AD processes is not a problem of age but of LBM downsizing.
brains of unaffected subjects contain amyloid precursor proteins (APPs) which may undergo proteolytic cleavage, regulated by β- and γ-secretase enzymes to release smaller amyloid β (Aβ) proteins that may be eliminated by clearing processes. Cline et al. have provided a recent comprehensive overview covering our present knowledge on AD pathophysiological processes. The irreversible evolutionary pattern of AD toward brain atrophy appears to result from multifactorial conditions. The sequestration of Aβ1-42 peptides within the gray matter precedes cognitive impairment, allowing the identification of preclinical AD states. Aβ peptides may undergo several stages of polymerization to generate soluble oligomers characterized by heterogeneity in size, function and toxicity. Soluble oligomeric species may evolve into toxic fibrils and tissue deposits taking the form of extracellular senile plaques or intracellular tangles made up of hyperphosphorylated tau-neurofibrils. Some oligomeric aggregates may diffuse within the CSF, undergo intraneuronal uptake or bind to cellular membranes. Many aspects remain to be clarified i.e. neuroprotective properties of the TTR tetramer requiring its molecular stability as opposed to its proteolytic cleavage yielding monomers and peptide derivatives; respective roles of TTR tetramer vs monomeric TTR species on Aβ aggregation processes; TTR control of the oligomeric nucleation of Aβ peptides; and the intriguing role of RBP as a possible inhibitory factor of TTR-mediated β-amyloid aggregation.

CSF is a complex and well-regulated homeostatic milieu providing several nutrients, growth factors, hormones, vitamins and carrier-proteins to brain tissues. CSF is confined within cerebral ventricles, which are protected by a cellular bilayer associating endothelial cells belonging to the blood microvasculature and epithelial cells pertaining to the CP, thereby forming the brain-blood barrier (BBB). The relative protein composition of CSF is significantly different from that of blood, as the total protein content of CSF in adult mammals is only 0.5% compared to that found in plasma (350 mg/L vs 70 g/L). The concentration of TTR in CSF is approximately 15-25 mg/L whereas that of RBP reaches 0.31-0.40 mg/L. Most plasma RBP molecules coalesce with TTR but circulate in CSF in free and unbound forms, suggesting distinct albeit complementary functional pathways. TTR is secreted by the CP into CSF and takes up thyroid hormones from the blood, sustaining the view that the distribution of thyroxine from CSF to the brain is mediated by TTR, indicating that the carrier-protein releases its hormonal ligand within the ventricular system in support of neurogenic processes. Some intrathecal components such as RBP actively cross the BBB using specific membrane receptors whereas others (Alb) diffuse passively within the CSF space. The roles played by thyroid and retinoid compounds are largely underestimated in AD patients, although these hormonal fractions are well identified in cerebral tissues. As an effect of age and disease, the CP may undergo atrophy of the epithelial cells and a thickening of the basement membrane. These alterations are associated with modified expression of a variety of CP gene transcripts explaining the decreased CSF production. Some AD patients may show reduced CSF concentrations of thyroxine, evoking the occurrence of mild brain hypothyroidism, a condition stimulating gene expression of APP proteins and derived products, thereby contributing to AD pathogenesis. Using transgenic mouse AD models, Japanese researchers have shown that intraperitoneal injections of vitamin A decrease Aβ deposition and tau-phosphorylation, attenuate neuronal degeneration, and improve spatial learning and memory. The physiologically active retinoid molecules (retinol, retinal, 13-cis retinoic acid) may disaggregate preformed in vitro Aβ fibrils, revealing nevertheless more efficient splitting capacity with retinol. Evidence for defective retinoid transport and function is a well-established issue in AD patients, paving the way for novel therapeutic strategies.

The seminal observation by Schwarzman et al. showing that TTR was able to inhibit Aβ amyloid formation remains central to many ongoing investigations. TTR demonstrates neuroregenerative activities in mouse models together with the suppression of Aβ-induced behavioral and neurotoxic damage. In addition, the administration of anti-TTR antibodies abrogates the neuroprotective effects of TTR and causes greater neuronal loss and higher tau-phosphorylation in mouse models. The molecular events occurring during neural AD processes demonstrate substoichiometric molar ratios with unaltered TTR concentrations, confirming that the synthesis of intrathecal TTR, contrary to hepatic TTR, is unresponsive to inflammatory burden hence remaining a stable biomarker of current AD brain status. In addition to the aforesaid physiological CSF downregulation of TTR and RBP with increasing age, several working teams have documented that the decreasing tendency is accelerated in the course of AD morbidity.

**HOMOCYSTEINE AND ALZHEIMER'S DISEASE**

Under well-balanced dietary regimens, most ingested Met molecules are incorporated into the biosynthesis of body proteins. The remaining Met fraction may undergo 3 distinct metabolic pathways: transmethylation (TM), transulfuration (TS) and remethylation (RM) with details given elsewhere. The nutritional story of the Met→Hcy cycle in protein-depleted states started with an investigation undertaken in a rural area of West Africa. The aim of the field study was to compare the respective clinical usefulness of TTR vs free EAsAs advocated as potential biomarkers of protein status. Using WHO criteria, a total of 105 adult vegetarian volunteers were recruited and subjected to fasting conditions for blood sampling, allowing the measurement of 8 EAAs and 21 non-EAA residues. As expected, TTR and 7 EAAs manifested a stepwise decline as the health state worsened. Surprisingly, Met revealed unaltered levels whereas Hcy values demonstrated gradual elevation toward hyperhomocysteinemic (HHcy) states. The data raised the hypothesis that Met should benefit from some homeostatic mechanism developed at the expense of Hcy, although the putative explanation remained elusive at that time. A second comparable investigation was performed in Central Africa, focusing more specific attention on the 3 hydrosoluble B vitamins regulating RM and TS pathways. Folate and pyridoxine values of these patients were satisfactory, whereas cobalamin levels were at the lowest threshold of
normalcy and regarded unlikely to be responsible for augmented Hcy values.92 These findings do not match the overall consensus that vitamin B12-deficiency is a worldwide scourge in vegan population groups mainly found in Asian countries93,94 but also in Western vegetarian subjects.95 Indian HHcy patients subjected to pharmacological doses of oral B1294 demonstrated that cobalamin fortification was efficient in reducing but not normalizing plasma Hcy concentrations. Additional B12 administration to those HHcy patients confirmed the refractoriness of some cobalamin-replete subjects,94 lending credence to coexisting undetected malnutrition. Under physiological circumstances, healthy adults who consume well-balanced diets with appropriate Met intake levels are subjected to the partition of the TS and RM pathways into nearly equivalent proportions regulated by the hepatocyte concentrations of SAM assuming switch functions between competing channels.96 In response to low Met intake, SAM concentration decreases in liver cells, causing allosteric alterations of the Michaelis constant (Km) for Hcy, thereby impairing the activity of cystathionine-β-synthase (CBS, EC 4.2.1.22) governing the first step of the TS cascade while enhancing those of betaine-Hcy-methyltransferase (BHMT, EC 2.1.1.5) and methionine synthase (MS, EC 2.1.1.13).97 As a result, the downregulation of CBS entails the upstream accumulation of Hcy in biological fluids whereas concomitant overstimulation of BHMT and MS promotes Hcy → Met remethylation processes.97 These adaptive changes have survival value, allowing the safeguarding of Met homeostasis in Met-deprived conditions, yet at the expense of an undesirable overflow of Hcy molecules in the extracellular space.98 An animal experiment subjecting rat strains to Met-restricted regimens has validated the impairment of the TS pathway and the upsurge of HHcy states at the end of the deprivation period.99

The first balance studies on the roles played by N and S in inflammatory disorders were carried out by Cuthbertson upon adult subjects suffering from bone fractures who revealed negative urinary N and S balances proportionate to the severity of impact, reflecting the magnitude of tissue proteolysis.100 The mean total S measured in 8 hospitalized patients was 1.18 g/day whereas the mean N excretion amounted to 17.53 g/day,100 indicating a very close correlation with the S:N ratio typical of mammalian tissues.8 The Scottish researcher anticipated - 50 years before the discovery of cytokines - that the urinary spillover of N- and S-catabolites should result from the “direct poisoning of the tissue cells such as might be supposed to take place in febrile conditions and in tissue injury”.100 These findings indicate that TBN and TBS stores undergo concomitant breakdown. More recent studies have confirmed these initial data, enlarging the scope by the urinary measurement of urea and creatinine but also of some minor compounds such as 3-methyl-histidine, creatine, ammonia, hydroxyproline, uric acid and free AAs, showing that most organs belonging to both visceral and structural compartments participate.101 Stressful disorders of any cause stimulate leukocytes that release proinflammatory cytokines working as autocrine, paracrine and endocrine molecules regulating the overproduction of acute-phase reactants (APRs).102 Interleukin-6 (IL-6) is the major regulator of any stress disorder inducing the hepatic overproduction of APRs103 endowed with specific kinetic and functional properties governing most immune, defense and repair processes.104 The prevailing role played by IL-6 in LBM proteolysis is documented by the identification of 36 metabolites positively or negatively associated with log IL-6.105 These findings are consistent with the view that protein breakdown processes predominate over repair syntheses,106 leading to the concept of massive LBM losses.33 It is worth stressing the point that IL-6 also abrogates the liver production of TTR in animal107 and in clinical108 experiments. Taken together, the last data indicate that declining TTR values throughout breakdown processes match those of and occur in parallel with declining LBM recorded during inflammatory disorders. Despite the great metabolic upheaval affecting septic and injured patients,25 plasma Met concentrations remain unaltered, whereas the levels of most other AAs are reduced by 10 to 30%.109 The data confirm that helpful Met safeguarding mechanisms operate in inflammatory morbidities.98

In agreement with Cuthbertson’s study,100 large excretions of urinary S and N catabolites were reported in septic patients100 as long as cytokine-induced LBM breakdown was maintained. These amounts of S and N output were thought to stem from the decay of Met-containing molecules111 confined within body tissues although GSH112 and Cys113 storage sites may have contributed substantially owing to the key participation of the liver in all inflammatory processes.31 After the initial descriptive of HHcy states in intensive care patients,114 supranormal fluctuating Hcy plasma values were reported in a great range of morbid circumstances specifically infecting any body organ, regardless of sex and age, as shown in children suffering from acute leukemia115 or in elderly persons enduring inflammation ailments.116 HHcy values are negatively correlated to LBM downsizing and to the drop in plasma TTR,117 reflecting the severity and duration of the morbid process and serving as a prognostic indicator of outcome.118 The molecular anomaly underlying these metabolic disturbances was cleared up by animal experiments showing the defective activity of the BHMT enzyme in mammalian LBM tissues.119 The data show that Hcy → Met conversion proceeds normally under healthy and relaxed conditions but fail to succeed in the case of superimposed inflammatory burden. The molecular mechanism explaining these impaired responses has been unraveled in rat models using a specific chemical inhibitor120 that mimics the roles played by cytokines in human disease, imposing significant increase in Hcy levels in the extracellular space. A part of this upstream HHcy flooding is removed by hepatocytes,121 yielding nascent Met molecules by-passing the BHMT blockade and restoring damaged tissue losses. CBS exert regulatory controls upon these adaptive mechanisms described in detail elsewhere.98 The search for the molecular mechanisms underlying neurodegenerative disorders was initiated by clinical studies pointing to HHcy as a strong and independent risk factor for AD.122 The deleterious effects caused by HHcy on brain activities appear to result from the combined roles played by the plasma drop in antioxidative molecules such as GSH and Cys in preexisting malnutri-
tion,117 the low brain anchoring of neuroprotective H2S,123 the contribution of inflammatory biomarkers124 and of vascular alterations,125 and the Hcy-induced exacerbation of β-amyloid and tau-pathologies.126 The oxidative injury associated with AD progression implicates the disruption of the BBB,127 dysfunction of mitochondria and neuronal toxicity,128 together with generation of nutritious derivatives leading to epigenetic dysregulation of gene expression.129

It is beyond the scope of the present review to provide detailed developments on the preventive and therapeutic strategies that might help to contain the progression of the process of dementia. Current dietary recommendations emphasize the beneficial impact of antioxidative molecules and hydrosoluble B vitamins implicated in the Hcy → Met cycle.4 An original approach using AD mouse strains subjected to enriched environmental and behavioral stimulation has improved cognitive and synaptic functions, causing a large reduction in Aβ burden, which was seemingly induced by the upregulation of TTR expression,130 an observation sustaining the TTR sequestering concept.84 The promising therapeutic roles played by retinoids81-83 deserve to be more intensively investigated. The well-known neuroprotective effects elicited by H2S in cerebral tissues131 might counteract AD brain deficits.123 Exogenous administration of H2S is indeed able to ameliorate mitochondrial dysfunctions in HHcy animals132 and BBB disruption in AD mice model.133 Last but not least, taking into account that high concentrations of elemental S may lessen AD mortality rates,6 inhibit enzymatic activities134 and stimulate the functioning of biological systems,135 implementation of S in regions where soils and tap-waters are S-deficient deserves to be open to debate. Ongoing observations indeed show that population groups living in the vicinity of volcano craters enjoy better neuroprotection status than dwellers from remote areas (unpublished).

CONCLUDING REMARKS
Contrary to plants, the lifespan of animals proceeds along 3 successive periods initially distinguished after birth by growth and tissue accretion rates, followed during adulthood by stable body composition, and terminated in old age by progressive downsizing of the metabolically active organs until death ensues. These evolutionary patterns are well documented by Forbes’ pioneering studies (Figure 1), indicating that N appears to be the cornerstone of body building. The bulk of N-containing molecules aggregates within the LBM whose evolving configuration over time is closely identified by plasma TTR (Figure 2). This statement is sustained by studies comparing the respective usefulness of biomarkers currently measured in elderly subjects, showing that TTR disclosed the highest positive correlation with LBM (r=0.64), whereas RBP had a medium correlation (r=0.57) and Alb had the least correlation (r = 0.52).136 In protein-depleted states, the restriction of both dietary protein and Met downregulates TTR hepatic production.56,57 In inflammatory disorders, cytokines abrogate the synthesis of TTR107,108 with concomitant spillover of LBM stores entailing urinary overflow of N and SAA compounds. Both malnutrition and inflammation morbidities constitute distinct nosological entities attributable to specific etiological factors but nevertheless displaying similar metabolic responses with respect to Met tissue requirements. In both conditions, reduced bioavailability of Met works as a triggering factor setting up the adaptive machinery safeguarding Met homeostasis. As a result, helpful restoration of Met stores in damaged tissues confers unequal survival benefits to this EAA molecule. A similar conclusion was drawn by Canadian researchers after investigating most aspects of AA metabolism in several animal models.137

In addition to the multiple biological properties assumed by TTR in human beings,138,139 TTR values also reveal the unique capacity of integrating the magnitude of N depletion resulting either from unachieved LBM replenishment in protein malnutrition or from the massive LBM losses generated by cytokine-induced inflammatory burden.140 This means that, whatever the sex, age, or disease state, TTR reflects at any time the residual LBM aptitude to surmount the metabolic, immune and nutritional challenges associated with morbid processes.140 The lower limit of plasma TTR normalcy (~200 mg/L) constitutes the turning point defining LBM competence below which patients incur increasing risks of complications, as reported by clinical teams representing several medical specialties such as nephrology,141 cardiology,142 neurosurgery,143 and oncology.144 Reaching the critical threshold of 100 mg TTR/L bears a significant ominous prognosis as described by investigators working in intensive care units,145 nephrology,146 and oncology.147 TTR operates as an alarm signal, indicating that elderly persons characterized by age-related normal values will likely escape the process of dementia, whereas those entering the subnormal area become liable to the harmful consequences of the AD morbid processes, a peril proportionate to the drop of TTR values.98 These last observations are consistent with the stepwise increased relative risk (RR) of lethality associated with declining TTR concentrations.148

AUTHOR DISCLOSURES
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REFERENCES


