

This author's PDF version corresponds to the article as it appeared upon acceptance. Fully formatted PDF versions will be made available soon.

## **Implications of protein malnutrition and inflammatory disorders in the pathophysiology of Alzheimer's disease**

doi: 10.6133/apjcn.202007/PP.0003

Published online: July 2020

**Running title:** Malnutrition & inflammation in Alzheimer's disease

Ingenbleek Yves MD, PhD

University of Strasbourg, Faculty of Pharmacy, Laboratory of Nutrition, France

**Corresponding Author:** Prof Yves Ingenbleek, University of Strasbourg, Faculty of Pharmacy, Laboratory of Nutrition, 15 bis, rue de la Vise, 34540 Balaruc le Vieux, France. Tel: 00-33-467 74 87 17. Email: ingen@unistra.fr

## ABSTRACT

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 tissues 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 Hcy alterations indicate that rising Hcy 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.<sup>1</sup> 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).<sup>2</sup> Globally, approximately 5.6% of mankind that reaches the sixties in age suffers from dementia.<sup>2</sup> A considerable body of scientific publications has appeared in recent decades, focusing careful scrutiny on multiple genetic, immune and dietary corollaries.<sup>3,4</sup> The first review describing that the depletion of N and S from LBM stores might contribute to brain deterioration appeared in 2015.<sup>5</sup> 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,<sup>6</sup> 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,<sup>7</sup> 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.<sup>8</sup> 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,  $\text{NO}_3\text{H}$ ) to -3 (ammonia,  $\text{NH}_4$ ). Through lightnings and other electrical discharges, atmospheric N may undergo oxidizing processes yielding large amounts of  $\text{NO}_3\text{H}$  that are washed down on Earth and carried away by rains in seawaters as highly soluble nitrate salts.<sup>9</sup> Atmospheric N may also be taken up by nodules found on the roots of some plants, and then converted to  $\text{NH}_4^+$  cations by soil microorganisms (*Azotobacter*) living in a symbiotic association with the plant kingdom.<sup>10</sup> The assimilation of N into plant tissues follows complex molecular and transport mechanisms. In contrast to higher animals, plants possess the enzymatic equipment required for the synthesis of all amino acids (AAs), including those fulfilling essential functions (EAAs).<sup>11,12</sup> That of Met is narrowly regulated, occurring as end-product of a retrograde pathway, with cystathionine and Hcy as intermediary compounds.<sup>12</sup>

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.<sup>13</sup> 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 ( $^{39}\text{K}$ ) is intracellularly confined within all living tissues together with minute amounts (0.0117%) of  $^{40}\text{K}$ , a source of natural  $\beta$ -radioactivity.<sup>13</sup> Both  $^{39}\text{K}$  and  $^{40}\text{K}$  isotopes show interchangeable turnover and similar metabolic fate in body tissues, maintaining narrow relationships with nitrogenous compounds assuming molar ratios of approximately 3 mEq  $\text{K}/\text{g}^{-1}\text{N}$ .<sup>14</sup> 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).<sup>13</sup> 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).<sup>15</sup> 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).<sup>16</sup> There exists extensive scientific literature describing the toxicological properties of S and S-containing substrates in living organisms<sup>17</sup> 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.<sup>18</sup>

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.<sup>9</sup> In the Earth's crust, S naturally occurs as a mixture of 4 stable isotopes, the most prevalent being <sup>32</sup>S, accounting for 95.1%.<sup>19</sup> 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.<sup>9</sup> The biological cycle of S is thought to be initiated by chemical and bacterial agents converting inorganic S-salts into gaseous hydrogen sulfide (H<sub>2</sub>S) 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.<sup>20</sup> The progressive retrocession on Earth of the H<sub>2</sub>S biochemical armamentarium has confined the gas transmitter to inhospitable places (swamps) or to coastlands where decomposing

seaweeds may accumulate. This example documents the ambivalent properties exerted by H<sub>2</sub>S manifesting high toxicity after the inhalation of poisonous effluvia<sup>21</sup> but revealing beneficial antioxidative activities induced by tiny concentrations infusing body tissues.<sup>22</sup> Intestinal flora constitutes a notable exception in that it produces considerable amounts of H<sub>2</sub>S rendered innocuous through ready-made oxidation by the colonic epithelium.<sup>23</sup>

The plant kingdom is composed of autotrophic organisms, and the metabolism of S is highly regulated by the oxidation of S-salts into SO<sub>4</sub><sup>2-</sup> prior to their capture from soils by plant roots.<sup>11</sup> 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 (CO<sub>2</sub>) into glucose.<sup>11</sup> Oxyanions undergo complex assimilatory reducing and transfer processes to O-acetylserine 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 most other S-containing compounds, including S-adenosyl-L-methionine (SAM) and glutathione (GSH).<sup>10,12</sup> The bioavailability of SO<sub>4</sub><sup>2-</sup> oxyanions from soils and tap-waters may vary considerably, from less than 10 mg/L to more than 1 g/L, depending on their geohydrological location,<sup>8</sup> working as a limiting factor for protein synthesis and for plant growth.<sup>24</sup>

The animal kingdom consists of heterotrophic organisms that are unable to initiate the synthesis of Met from SO<sub>4</sub><sup>2-</sup> 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.<sup>25</sup> Studies have shown that Cys demonstrates semiessential properties in that it may serve as a sparing factor of Met in biological processes when the EAA is not strictly required.<sup>26</sup> 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.<sup>27</sup> The consumption rate of preformed Met from dietary items works as a limiting factor for protein synthesis and mammalian growth.<sup>27</sup> 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.<sup>28</sup> These last data are in keeping with balance studies performed on pig models showing that, compared with the 7 other EAAs, the withdrawal of Met and Cys from otherwise normal diets causes the greatest LBM depletion, nearly equal to that generated by protein-free regimens.<sup>29</sup>

## **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.<sup>8</sup> 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.<sup>30</sup> 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.<sup>8</sup>

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,<sup>8</sup> 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.<sup>31</sup> 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,<sup>8</sup> consistent with dietary surveys showing that the usual intake of AAs is reduced by 47% in strict vegans.<sup>32</sup> The maintenance of such plant-based dietary regimens from the weaning period throughout the lifespan will result in unachieved LBM replenishment,<sup>33</sup> 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.<sup>34</sup> At all ages, reduced stature appears to be a major and reliable predictor of LBM, accounting for up to 90% of its variance.<sup>35</sup> 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.<sup>36</sup>

## **TRANSTHYRETIN AND ALZHEIMER'S DISEASE**

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.<sup>37</sup> The liver synthesis of TTR occurred much later, approximately 100 million years ago, in most classes of vertebrates.<sup>38</sup> Using electrophoretic methods, researchers were able to identify TTR in human blood<sup>39</sup> and in human CSF<sup>40</sup> 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.<sup>41</sup> 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.<sup>42</sup> 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,<sup>43</sup> thus forming a trimolecular retinol circulating complex (76 kDa as MM) whose components remain attached at a close 1:1:1 stoichiometry.<sup>44</sup>

After birth, plasma TTR concentrations increase linearly without sexual differences during infant growth.<sup>45</sup> 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<sup>46</sup> together with concomitant elevation of TTR plasma values.<sup>47</sup> 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 (Fig. 2). In healthy adults, plasma TTR and RBP concentrations maintain gender-related differences, disclosing plateau levels during the full sexual maturity period.<sup>47</sup> 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,<sup>49</sup> explaining why muscle mass no longer demonstrates sexual differences after the sixties. The serial measurement of TTR values indicates concomitant decline<sup>47</sup> (Fig. 2); therefore, TTR has recently been advocated as a useful biomarker of sarcopenia in aged subjects.<sup>49</sup> Both TTR<sup>40</sup> and RBP<sup>50</sup> molecules produced by the CP and secreted in CSF follow regulatory pathways distinct from those of liver.<sup>51</sup> With increasing age, choroidal production of TTR<sup>52</sup> and RBP<sup>53</sup> displays intrathecal downregulation likely to be genetically programmed.

The measurement of plasma TTR has been proposed as a sensitive biomarker of protein nutritional status<sup>54</sup> 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.<sup>55</sup> Rat experiments have shown that the restriction of dietary AA supply leading to protein malnutrition is accompanied by depressed hepatic production of TTR mRNA,<sup>56</sup> decreased abundance of TTR nuclear transcripts,<sup>57</sup> 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<sup>58-60</sup> and to be inversely correlated with disease progression;<sup>59</sup> hence, plasma TTR was recommended as a candidate marker identifying AD severity.<sup>60</sup> 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.<sup>61</sup> The healthy brains of unaffected subjects contain amyloid precursor proteins (APPs) which may undergo proteolytic cleavage, regulated by  $\beta$ - and  $\gamma$ -secretase enzymes to release smaller amyloid  $\beta$  ( $A\beta$ ) 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.<sup>62</sup> The irreversible evolutionary pattern of AD toward brain atrophy appears to result from multifactorial conditions. The sequestration of  $A\beta_{1-42}$  peptides within the gray matter precedes cognitive impairment, allowing the identification of preclinical AD states.<sup>63</sup>  $A\beta$  peptides may undergo several stages of polymerization to generate soluble oligomers characterized by heterogeneity in size, function and toxicity.<sup>64</sup> 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.<sup>65</sup> Some oligomeric aggregates may diffuse within the CSF, undergo intraneuronal uptake or bind to cellular membranes.<sup>66</sup> Many aspects remain to be clarified i.e. neuroprotective properties of the TTR tetramer requiring its molecular stability<sup>67</sup> as opposed to its proteolytic cleavage yielding monomers and peptide derivatives;<sup>68</sup> respective roles of TTR tetramer vs monomeric TTR species on  $A\beta$  aggregation processes;<sup>69</sup> TTR control of the oligomeric nucleation of  $A\beta$  peptides;<sup>70</sup> and the intriguing role of RBP as a possible inhibitory factor of TTR-mediated  $\beta$ -amyloid aggregation.<sup>71</sup>

CSF is a complex and well-regulated homeostatic milieu providing several nutrients, growth factors, hormones, vitamins and carrier-proteins to brain tissues.<sup>72</sup> 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,<sup>73</sup> 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.<sup>74</sup> 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.<sup>75</sup> Some intrathecal components such as RBP actively cross the BBB using specific membrane receptors<sup>50</sup> 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.<sup>76</sup> These alterations are associated with modified expression of a variety of CP gene transcripts explaining the decreased CSF production.<sup>77</sup> Some AD patients may show reduced CSF concentrations of thyroxine, evoking the occurrence of mild brain hypothyroidism,<sup>78</sup> a condition stimulating gene expression of APP proteins and derived products, thereby contributing to AD pathogenesis.<sup>79</sup> Using transgenic mouse AD models, Japanese researchers have shown that intraperitoneal injections of vitamin A decrease A $\beta$  deposition and tau-phosphorylation, attenuate neuronal degeneration, and improve spatial learning and memory.<sup>80</sup> The 3 physiologically active retinoid molecules (retinol, retinal, 13-cis retinoic acid) may disaggregate preformed in vitro A $\beta$  fibrils, revealing nevertheless more efficient splitting capacity with retinol.<sup>81</sup> Evidence for defective retinoid transport and function is a well-established issue in AD patients, paving the way for novel therapeutic strategies.<sup>82,83</sup>

The seminal observation by Schwarzman et al. showing that TTR was able to inhibit A $\beta$  amyloid formation<sup>84</sup> remains central to many ongoing investigations. TTR demonstrates neuroregenerative activities in mouse models<sup>85</sup> together with the suppression of A $\beta$ -induced behavioral and neurotoxic damage.<sup>86</sup> 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.<sup>87</sup> The molecular events occurring during neural AD processes demonstrate substoichiometric molar ratios with unaltered TTR concentrations,<sup>70</sup>

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,<sup>52,53</sup> several working teams have documented that the decreasing tendency is accelerated in the course of AD morbidity.<sup>88-90</sup>

## **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), transsulfuration (TS) and remethylation (RM) with details given elsewhere.<sup>25</sup> The nutritional story of the Met↔Hcy cycle in protein-depleted states started with an investigation undertaken in a rural area of West Africa.<sup>91</sup> The aim of the field study was to compare the respective clinical usefulness of TTR<sup>54</sup> vs free EAAs 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.<sup>91</sup> 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,<sup>91</sup> although the putative explanation remained elusive at that time. A second comparable investigation was performed in Central Africa,<sup>92</sup> focusing more specific attention on the 3 hydrosoluble B vitamins regulating RM and TS pathways.<sup>25</sup> 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.<sup>92</sup> These findings do not match the overall consensus that vitamin B12-deficiency is a worldwide scourge in vegan population groups mainly found in Asian countries<sup>93,94</sup> but also in Western vegetarian subjects.<sup>95</sup> Indian HHcy patients subjected to pharmacological doses of oral B12<sup>94</sup> 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,<sup>94</sup> 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.<sup>96</sup> 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- $\beta$ -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).<sup>97</sup> 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  $\rightarrow$  Met remethylation processes.<sup>97</sup> 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.<sup>98</sup> 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.<sup>99</sup>

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.<sup>100</sup> 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,<sup>100</sup> indicating a very close correlation with the S:N ratio typical of mammalian tissues.<sup>8</sup> 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".<sup>100</sup> 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.<sup>101</sup> 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).<sup>102</sup> Interleukin-6 (Il-6) is the major regulator of any stress disorder inducing the hepatic overproduction of APRs<sup>103</sup> endowed with specific kinetic and functional properties governing most immune, defense and repair processes.<sup>104</sup> 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.<sup>105</sup> These findings are consistent with the view that protein breakdown processes predominate over repair syntheses,<sup>106</sup> leading to the concept of massive

LBM losses.<sup>33</sup> It is worth stressing the point that Il-6 also abrogates the liver production of TTR in animal<sup>107</sup> and in clinical<sup>108</sup> 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,<sup>25</sup> plasma Met concentrations remain unaltered, whereas the levels of most other AAs are reduced by 10 to 30%.<sup>109</sup> The data confirm that helpful Met safeguarding mechanisms operate in inflammatory morbidities.<sup>98</sup>

In agreement with Cuthbertson's study,<sup>100</sup> large excretions of urinary S and N catabolites were reported in septic patients 110 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 molecules<sup>111</sup> confined within body tissues although GSH<sup>112</sup> and Cys<sup>113</sup> storage sites may have contributed substantially owing to the key participation of the liver in all inflammatory processes.<sup>31</sup> After the initial description of HHcy states in intensive care patients,<sup>114</sup> supranormal fluctuating Hcy plasma values were reported in a great range of morbid circumstances specifically inflicting any body organ, regardless of sex and age, as shown in children suffering from acute leukemia<sup>115</sup> or in elderly persons enduring inflammation ailments.<sup>116</sup> HHcy values are negatively correlated to LBM downsizing and to the drop in plasma TTR,<sup>117</sup> reflecting the severity and duration of the morbid process and serving as a prognostic indicator of outcome.<sup>118</sup> 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.<sup>119</sup> 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 inhibitor<sup>120</sup> 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,<sup>121</sup> 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.<sup>98</sup> 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.<sup>122</sup> 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 malnutrition,<sup>117</sup> the low brain anchoring of

neuroprotective H<sub>2</sub>S,<sup>123</sup> the contribution of inflammatory biomarkers<sup>124</sup> and of vascular alterations,<sup>125</sup> and the Hcy-induced exacerbation of  $\beta$ -amyloid and tau-pathologies.<sup>126</sup> The oxidative injury associated with AD progression implicates the disruption of the BBB,<sup>127</sup> dysfunction of mitochondria and neuronal toxicity,<sup>128</sup> together with generation of noxious derivatives leading to epigenetic dysregulation of gene expression.<sup>129</sup>

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.<sup>4</sup> 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 A $\beta$  burden, which was seemingly induced by the upregulation of TTR expression,<sup>130</sup> an observation sustaining the TTR sequestering concept.<sup>84</sup> The promising therapeutic roles played by retinoids<sup>81-83</sup> deserve to be more intensively investigated. The well-known neuroprotective effects elicited by H<sub>2</sub>S in cerebral tissues<sup>131</sup> might counteract AD brain deficits.<sup>123</sup> Exogenous administration of H<sub>2</sub>S is indeed able to ameliorate mitochondrial dysfunctions in HHcy animals<sup>132</sup> and BBB disruption in AD mice model.<sup>133</sup> Last but not least, taking into account that high concentrations of elemental S may lessen AD mortality rates,<sup>6</sup> inhibit enzymatic activities<sup>134</sup> and stimulate the functioning of biological systems,<sup>135</sup> 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 in 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 (Fig. 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 ( $r=0.52$ ).<sup>136</sup> In protein-depleted states, the restriction of both dietary protein and Met downregulates TTR hepatic production.<sup>56,57</sup> In inflammatory disorders, cytokines abrogate the synthesis of TTR<sup>107,108</sup> 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.<sup>137</sup>

In addition to the multiple biological properties assumed by TTR in human beings,<sup>138,139</sup> 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.<sup>140</sup> 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.<sup>140</sup> The lower limit of plasma TTR normalcy ( $\sim 200$  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,<sup>141</sup> cardiology,<sup>142</sup> neurosurgery,<sup>143</sup> and oncology.<sup>144</sup> Reaching the critical threshold of 100 mg TTR/L bears a significant ominous prognosis as described by investigators working in intensive care units,<sup>145</sup> nephrology,<sup>146</sup> and oncology.<sup>147</sup> TTR operates as an alarm signal, indicating that elderly persons characterized by supranormal 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.<sup>98</sup> These last observations are consistent with the stepwise increased relative risk (RR) of lethality associated with declining TTR concentrations.<sup>148</sup>

## AUTHOR DISCLOSURE

The corresponding author declares having no sources of funding neither from industrial nor pharmaceutical companies. He also declares having no conflict of interest with academic or scientific institutions.

## REFERENCES

1. World Health Organization. Global action plan on the public health response to dementia 2017-2025. Geneva: WHO Report; 2017.
2. Prince M, Wimo A, Guerchet M, Ali GC, Wu YT, Prina M. The global impact of dementia. An analysis of prevalence, incidence, cost and trends. *Alzheimer's Disease International*, London: World Alzheimer's Report; 2015.
3. International Genomics of Alzheimer's Disease Consortium (IGAP) with 186 collaborators. Convergent genetic and expression data implicate immunity in Alzheimer's disease. *Alzheimers Dement* 2015;11: 658-71.
4. Román GC, Jackson RE, Gadhia R, Román AN, Reis J. Mediterranean diet: The role of long-chain  $\omega$ -3 fatty acids in fish; polyphenols in fruits, vegetables, cereals, coffee, tea, cacao and wine; probiotics and vitamins in prevention of stroke, age-related cognitive decline, and Alzheimer disease. *Rev Neurol (Paris)*. 2019;175:724-41. doi: 10.1016/j.neurol.2019.08.005.
5. Ingenbleek Y, Bernstein LH. Downsizing of lean body mass is a key determinant of Alzheimer's disease. *J Alzheimer Dis*. 2015;44:745-54. doi: 10.3233/jad-141950.
6. Sun H. Associations of spatial disparities of Alzheimer's disease mortality rates with soil selenium and sulfur concentrations and four common risk factors in the United States. *J Alzheimer Dis*. 2017;58: 897-907. doi: 10.3233/jad-170059.
7. Loef M, Schrauzer GN, Walach H. Selenium and Alzheimer's disease: a systematic review. *J Alzheimer Dis*. 2011;26:81-104. doi: 10.3233/jad-2011-110414.
8. Ingenbleek Y. The nutritional relationship linking sulfur to nitrogen in living organisms. *J Nutr*. 2006; 136(Suppl 6):S1641-51. doi: 10.1093/jn/136.6.1641s.
9. Holland HD. Aspects of the geological history of seawater. *Orig Life*. 1974;5:87-91.
10. Mitchell SC. Biological interactions of sulfur compounds. London: Taylor & Francis; 1996.
11. Mohr H, Schopfer P. Plant physiology. Berlin: Springer; 1994.
12. Amir R, Hacham Y. Methionine metabolism in plants. In: Jez JM, editor. Sulfur, a Missing Link between Soils, Crops, and Nutrition. Monograph N° 50. Madison: American Society of Agronomy, Crop Science of America, Soil Science Society of America; 2008. pp. 251-79.
13. Forbes G.B. Human body composition, growth, aging, nutrition, and activity. Berlin: Springer; 1987.
14. Cohn SH, Vartsky D, Yasumura S, Vaswani AN, Ellis KJ. Indexes of body cell mass: nitrogen versus potassium. *Am J Physiol*. 1983;244: E305-10. doi :10.1152/ajpendo.1983.244.3.E305.

15. Ingenbleek Y, Young VR. Significance of transthyretin in protein metabolism. *Clin Chem Lab Med*. 2002;40:1281-91. doi: 10.1515/cclm.2002.222.
16. Forbes GB. Body composition. In: Ziegler EE, Filer IJ, editors. *Present Knowledge in Nutrition*, 7th edition, Washington DC: ILSI Press; 1996. pp. 7-12.
17. Komarnisky LA, Basu TK. Biological and toxicological considerations of dietary sulfur. In: Preedy VR, Watson RR, editors. *Reviews in Food and Nutrition Toxicity*, vol. 4. Boca Raton: CRC Press; 2005. pp. 85-104.
18. Erdman JW Jr, MacDonald IA, Zeisel SH. *Present knowledge in nutrition*, 10th edition, Washington DC: ILSI Press; 2012.
19. Ault WP, Kulp JL. Isotopic geochemistry of sulfur. *Geochim Cosmochim Acta*. 1959;16:201-35.
20. Olson KR, Straub KD. The role of hydrogen sulfide in evolution and the evolution of hydrogen sulfide in metabolism and signaling. *Physiology (Bethesda)*. 2016;31:60-72. doi: 10.1152/physiol.00024.2015.
21. Warencya MW, Goodwin LR, Benishin CG, Reiffenstein RJ, Francom DM, Taylor JD, Dieken FP. Acute hydrogen sulfide poisoning. Demonstration of selective uptake of sulfide by the brainstem by measurement of brain sulfide levels. *Biochem Pharmacol*. 1989;38:973-81. doi: 10.1016/0006-2952(89)90288-8.
22. Kimura H, Shibuya N, Kimura Y. Hydrogen sulfide is a signaling molecule and a cytoprotectant. *Antioxid Redox Signal*. 2012;17:45-57. doi: 10.1089/ars.2011.4345.
23. Linden DR. Hydrogen sulfide signaling in the gastrointestinal tract. *Antioxid Redox Signal*. 2014;20: 818-30. doi: 10.1089/ars.2013.5312. doi:10.1089/ars.2013.5312.
24. Jez JM. Sulfur: A missing link between soils, crops and nutrition. Monograph N° 50. Madison: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America; 2008.
25. Ingenbleek Y, Kimura H. Nutritional essentiality of sulfur in health and disease. *Nutr Rev*. 2013;71:413-32. doi: 10.1111/nure.12050.
26. Ball RO, Courtney-Martin G, Pencharz PB. The in vivo sparing of methionine by cysteine in sulfur amino acid requirements in animal models and adult humans. *J Nutr*. 2006;136(Suppl 6):S1682-93. doi: 10.1093/jn/136.6.1682s.
27. Young VR, Borgonha S. Nitrogen and amino acid requirements: the Massachusetts Institute of Technology amino acid requirement pattern. *J Nutr*. 2000;130:S1841-9. doi: 10.1093/jn/130.7.1814s.
28. Rudland PS, Clark BFC. Polypeptide chain initiation and the role of a methionine tRNA. In: Bosch L, editor. *The mechanism of protein synthesis and its regulation*. Amsterdam: North Holland Publishing Co; 1972. pp 55-131.
29. Fuller MF, McWilliam R, Wang TC, Giles LR. The optimum dietary amino acid pattern for growing pigs. 2. Requirements for maintenance and for tissue protein accretion. *Br J Nutr*. 1989;62:255-67. doi: 10.1079/bjn19890028.

30. Jones DP, Coates RJ, Flagg EW, Eley JW, Jones DP, Gunter EW. Glutathione in foods listed in the National Cancer Institute's health habits and history food frequency Questionnaire. *Nutr Cancer*. 1992; 17:57-75. doi : 10.1080/01635589209514173.
31. Higashi T, Tateishi N, Naruse A, Sakamoto Y. A novel physiological role of liver glutathione as a reservoir of L-cysteine. *J Biochem*. 1977;82:117-24. doi: 10.1093/oxfordjournals.jbchem.a9131659.
32. Schmidt, JA, Rinaldi S, Scalbert A, Ferrari P, Achaintre DE, Gunter MJ, Appleby PN, Key TJ, Travis RC. Plasma concentrations and intakes of amino acids in male meat-eaters, fish-eaters, vegetarians and vegans: a cross-sectional analysis in the EPIC-Oxford cohort. *Eur J Clin Nutr*. 2016;70:306-12. doi: 10.1038/ejcn.2015.144.
33. Ingenbleek Y, Young VR. Transthyretin (prealbumin) in health and disease: Nutritional implications. *Annu Rev Nutr*. 1994;14:495-533. doi: 10.1146/annurev.nu.14.070194.002431.
34. Vanacore D, Messina G, Lama S, Bitti G, Ambrosio P, Tenore G et al. Effect of restriction vegan diet's on muscle mass, oxidative status, and myocytes differentiation: A pilot study. *J Cell Physiol*. 2018;233: 9345-53. doi: 10.1002/jcp.26427.
35. Forbes GB. Stature and lean body mass. *Am J Clin Nutr*. 1974;27:595-602. doi: 10.1093/ajcn/27.6.595.
36. Young VR, Pellett PL. Current concepts concerning amino acids in adults and their implications for international planning. *Food Nutr Bull*. 1990;12:289-300.
37. Schreiber G. The evolution of transthyretin synthesis in the choroid plexus. *Clin Chem Lab Med*. 2002; 40:1200-10. doi: 10.1515/cclm.2002.210.
38. Richardson SJ. Evolutionary changes to transthyretin: evolution of transthyretin biosynthesis. *FEBS Lett*. 2009;276:5342-56. doi: 10.1111/j.1742-4658.2009.07244.x.
39. Siebert FB, Nelson JW. Electrophoretic study of the blood protein response in tuberculosis. *J Biol Chem*. 1942;143:29-38.
40. Kabat EA, Moore DH, Landow H. An electrophoretic study of the protein components in cerebrospinal fluid and their relationship to serum proteins. *J Clin Invest*. 1942;21:571-7. doi: 10.1172/jci.101335.
41. Ingbar SH. Pre-albumin: a thyroxine binding protein of human plasma. *Endocrinology*. 1958;63:256-9. doi: 10.1210/endo-63-2-256.
42. Kanda Y, Goodman DS, Canfield RE, Morgan FJ. The amino acid sequence of human plasma prealbumin. *J Biol Chem*. 1974;249:6796-805.
43. Kanai M, Raz A, Goodman DS. Retinol-binding protein: the transport protein for vitamin A in human plasma. *J Clin Invest*. 1968;47:2025-44. doi: 10.1172/jci105889.
44. Monaco HL. The transthyretin-retinol binding protein complex. In: Richardson SJ, Cody V, editors. *Recent advances in transthyretin evolution, structure and biological functions*. Berlin: Springer; 2009. pp. 123-42.
45. Vahlquist A, Rask L, Peterson PA, Berg T. The concentrations of retinol-binding protein, prealbumin, and transferrin in the sera of newly delivered mothers and children of various ages. *Scand J Clin Lab Invest*. 1975;35:569-75.

46. Veldhuis JD, Roemmich JN, Richmond EJ, Rogol AD, Lovejoy JC, Sheffield-Moore M, Mauras N, Bowers CY. Endocrine control of body composition in infancy, childhood and puberty. *Endocr Rev.* 2005;26:114-46. doi: 10.1210/er.2003-0038.
47. Bienvenu J, Jeppson JO, Ingenbleek Y. Transthyretin & retinol-binding protein. In: Ritchie RF, Navolotskaia O, editors. *Serum Proteins in Clinical Medicine*. Scarborough: Foundation for Blood Research; 1996, pp. 9011-18.
48. Gallagher D, Visser M, De Meersman RE, Sepúlveda D, Baumgartner RN, Pierson RN, Harris T, Heymsfield SB. Appendicular skeletal muscle mass: effects of age, gender and ethnicity. *J Appl Physiol.* 1997;83:229-39. doi: 10.1152/jappl.1997.83.1.229.
49. Ingenbleek Y. Plasma transthyretin as a biomarker of sarcopenia in elderly subjects. *Nutrients* 2019;11:895-913. doi: 10.3390/nu11040895.
50. MacDonald PN, Bok D, Ong DE. Localization of cellular retinol-binding protein and retinol binding protein in cells comprising the blood-brain barrier of rat and human. *Proc Natl Acad Sci USA.* 1990;87:4265-9. doi: 10.1073/pnas.87.11.4265.
51. Dickson PW, Aldred AR, Marley PD, Bannister D, Schreiber G. Rat choroid plexus specializes in the synthesis and the secretion of transthyretin (prealbumin). Regulation of transthyretin synthesis in choroid plexus is independent from that of liver. *J Biol Chem.* 1986;261:3475-8.
52. Chen RL, Athauda SB, Kassem NA, Zhang Y, Segal MB, Preston JE. Decrease of transthyretin synthesis at the blood-cerebrospinal fluid barrier of old sheep. *J Gerontol A Biol Sci Med Sci.* 2005;60:852-8. doi: 10.1093/gerona/60.7.852.
53. Chen CP, Chen RL, Preston JE. The influence of ageing in the cerebrospinal fluid concentrations of proteins that are derived from the choroid plexus, brain, and plasma. *Exp Gerontol.* 2012;47:323-8. doi: 10.1016/j.exger.2012.01.008.
54. Ingenbleek Y, De Visscher M, De Nayer P. Measurement of prealbumin as index of protein-calorie malnutrition. *Lancet.* 1972;2:106-9. doi: 10.1016/s0140-6736(72)91596-6.
55. Ingenbleek Y, Van den Schrieck HG, De Nayer P, De Visscher M. The role of retinol-binding protein in protein-calorie malnutrition. *Metabolism.* 1975;24:633-41. doi: 10.1016/0026-0495(75)90143-2.
56. de Jong FA, Schreiber G. Messenger RNA levels of plasma proteins in rat liver during protein depletion and refeeding. *J Nutr.* 1987;117:1795-800. doi: 10.1093/jn/117.10.1795.
57. Straus DS, Marten NW, Hayden JM, Burke EJ. Protein restriction specifically decreases the abundance of serum albumin and transthyretin nuclear transcripts in rat liver. *J Nutr.* 1994;124:1041-51. doi: 10.1093/jn/124.7.1041.
58. Elovaara I, Maury CP, Palo J. Serum amyloid A protein, albumin and prealbumin in Alzheimer's disease and demented patients with Down's syndrome. *Acta Neurol Scand.* 1986;74:245-50. doi: 10.1111/j.1600-0404.1986.tb07863.x.
59. Han SH, Jung ES, Sohn JH, Hong HJ, Hong HS, Kim JW et al. Human serum transthyretin levels correlate inversely with Alzheimer's disease. *J Alzheimers Dis.* 2011;25:77-84. doi: 10.3233/jad-2011-102145.

60. Velayudhan I, Killick R, Hye A, Kinsey A, Güntert A, Lynham S et al. Plasma transthyretin as a candidate marker for Alzheimer's disease. *J Alzheimers Dis.* 2012;28:369-75. doi: 10.3233/jad-2011-110611.
61. Musicco M. Gender differences in the occurrence of Alzheimer's disease. *Funct Neurol.* 2009;24:89-92.
62. Cline EN, Bicca MA, Viola KL, Klein WL. The amyloid- $\beta$  oligomer hypothesis: Beginning of the third decade. *J Alzheimers Dis.* 2018;64:S567-610. doi: 10.3233/jad-179941.
63. Falcon C, Tucholka A, Monté-Rubio GC, Cacciaglia R, Operto G, Rami L, Gisbert JD, Molinuevo JL. Longitudinal structural cerebral changes related to core CSF biomarkers in preclinical Alzheimer's disease: A study of two independent datasets. *Neuroimage Clin.* 2018;19:190-201. doi: 10.1016/niel.2018.04.016.
64. Breydo L, Uversky VN. Structural, morphological, and functional diversity of amyloid oligomers. *FEBS Lett.* 2015;589:2640-8. doi: 10.1016/j.febslet.2015.07.013.
65. Viola KL, Klein WL. Amyloid  $\beta$  oligomers in Alzheimer's disease pathogenesis, treatment, and diagnostic. *Acta Neuropathol.* 2015;129:183-206. doi: 10.1007/s00401-015-1386-3.
66. Laferla FM, Green KN, Oddo S. Intracellular amyloid-beta in Alzheimer's disease. *Nat Rev Neurosci.* 2007;8:499-509. doi: 10.1038/nrn2168.
67. Alemi M, Silva SC, Santana I, Cardoso I. Transthyretin stability is critical in assisting beta-amyloid clearance - Relevance of transthyretin stabilization in Alzheimer's disease. *CNS Neurosci Ther.* 2017;23:605-19. doi: 10.1111/cns.12707
68. Silva CS, Eira J, Ribeiro CA, Oliveira A, Sousa MM, Cardoso I, Liz MA. Transthyretin neuroprotection in Alzheimer's disease is dependent on proteolysis. *Neurobiol Aging.* 2017;59:10-14. doi: 10.1016/j.neurobiolaging.2017.07.002
69. Du J, Murphy RM. Characterization of the interaction of  $\beta$ -amyloid with transthyretin monomers and tetramers. *Biochemistry.* 2010;49:8276-89. doi: 10.1021/bi.101280t.
70. Nilsson L, Pamrén A, Islam T, Brännström K, Golchin SA, Petterson N, Iakovleva I, Sandblad L, Gharibyan AL, Olofsson A. Transthyretin interferes with A $\beta$ -amyloid formation by redirecting oligomeric nuclei into non-amyloid aggregates. *J Mol Biol.* 2018;430:2722-33. doi: 10.1016/j.jmb.2018.06.005.
71. Mangrolia P, Murphy RM. Retinol-binding protein interferes with transthyretin-mediated  $\beta$ -amyloid aggregation inhibition. *Biochemistry.* 2018;57:5029-40. doi: 10.1021/acs.biochem.8b00517.
72. Spector R, Keep RF, Robert Snodgrass S, Smith QR, Johanson CE. A balanced view of choroid plexus structure and function: Focus on human adults. *Exp Neurol.* 2015;267:78-86. doi: 10.1016/j.expneurol.2015.02.032.
73. Gorlé N, Van Cauwenberghe C, Libert C, Vandenbroucke RE. The effect of aging on brain barriers and the consequences for Alzheimer's disease development. *Mamm Genome.* 2016;27:407-20. doi: 10.1007/s60335-016-9637-8.
74. Peterson PA, Berggård I. Isolation and properties of a human retinol-transporting protein. *J Biol Chem.* 1971;246:25-33.

75. Kassem NA, Deane R, Segal MB, Preston JE. Role of transthyretin in thyroxine transfer from cerebrospinal fluid to brain and choroid plexus. *Am J Physiol Regul Integr Comp Physiol.* 2006;291:R1310-15. doi :10.1152/ajpregu.00789.2005.
76. Kaur C, Rathnasamy G, Ling EA. The choroid plexus in healthy and diseased brain. *J Neuropathol Exp Neurol.* 2016;75:198-213. doi: 10.1093/jnen/nlv030.
77. Kant S, Stopa EG, Johanson CE, Baird A, Silverberg GD, Blennow K, Zetterberg H, Svensson J. Choroid plexus genes for CSF production and brain homeostasis are altered in Alzheimer's disease. *Fluids Barriers CNS.* 2018;15:34 doi: 10.1186/s12987-018-0120-7.
78. Johansson P, Almqvist EG, Johansson JO, Mattson N, Hansson O, Wallin A, Blennow K, Zetterberg H, Svensson J. Reduced cerebrospinal fluid level of thyroxine in patients with Alzheimer's disease. *Psychoneuroendocrinology.* 2013;38:1058-66. doi: 10.1016/j.psyneuen.2012.10.012.
79. O'Barr SA, Oh JS, Ma C, Brent GA, Schultz JJ. Thyroid hormone regulates endogenous amyloid-beta precursor protein gene expression and processing in both in vitro and in vivo models. *Thyroid.* 2006;16:1207-13. doi: 10.1089/thy.2006.16.1207.
80. Ono K, Yamada M. Vitamin A and Alzheimer's disease. *Geriatr Gerontol Int.* 2012;12:180-8. doi: 10.1111/j.1447-0594.2011.00786.x.
81. Ono K, Yoshiike Y, Takashima A, Hasegawa K, Naiki H, Yamada M. Vitamin A exhibits potent anti-amyloidogenic and fibril-destabilizing effects in vitro. *Exp Neurol.* 2004;189:380-92. doi: 10.1016/j.expneurol.2004.05.035.
82. Lerner AJ, Gustaw-Rothenberg K, Smyth S, Casadesus G. Retinoids for treatment of Alzheimer's disease. *Biofactors.* 2012;38:84-9. doi: 10.1002/biof.196.
83. Das BC, Dasgupta S, Ray SK. Potential roles of retinoids for prevention of neuroinflammation and neurodegeneration in Alzheimer's disease. *Neural Regen Res.* 2019;14:1880-92. doi:10.4103/1673-5374.259604.
84. Schwarzman AL, Gregori L, Vitek MP, Lyubski S, Strittmatter WJ, Enghilde JJ et al. Transthyretin sequesters amyloid beta protein and prevents amyloid formation. *Proc Natl Acad Sci USA.* 1994;91:8368-72. doi: 10.1073/pnas.91.18.8368.
85. Fleming CE, Saraiva MJ, Sousa MM. Transthyretin enhances nerve regeneration. *J Neurochem.* 2007;103:831-9. doi: 10.1111/j.1471-4159.2007.04828.x.
86. Buxbaum JN, Ye Z, Reixach N, Friske L, Levy C, Das P, Golde T, Masliah E, Roberts AR, Bartfai T. Transthyretin protects Alzheimer's mice from the behavioral and biochemical effects of A $\beta$ -toxicity. *Proc Natl Acad Sci USA.* 2008;105:2681-6. doi: 10.1073/pnas.0712197105.
87. Stein TD, Anders NJ, DeCarli C, Chan SL, Mattson MP, Johnson JA. Neutralization of transthyretin reverses the neuroprotective effects of secreted amyloid precursor protein (APP) in APPSW mice resulting in tau phosphorylation and loss of hippocampal neurons: Support for the amyloid hypothesis. *J Neurosci.* 2004;24:7707-17. doi: 10.1523/jneurosci.2211-04.2004.
88. Riisøen H. Reduced prealbumin (transthyretin) in CSF of severely demented patients with Alzheimer's disease. *Acta Neurol Scand.* 1988;78:455-9. doi: 10.1111/j.1600-0404.1988.tb03687.x.

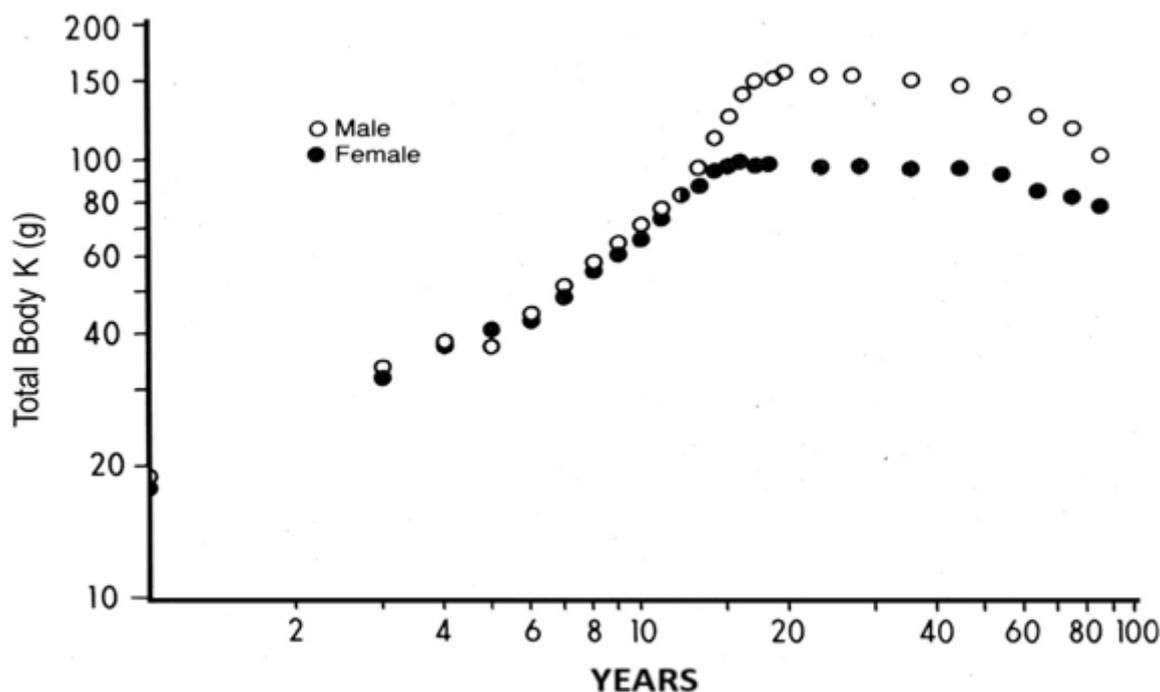
89. Isobe C, Murata T, Sato C, Terayama Y. Increase of total homocysteine concentration in cerebrospinal fluid of patients with Alzheimer's disease and Parkinson's disease. *Life Sci.* 2005;77:1836-43. doi: 10.1016/j.lfs.2005.02.014.
90. Gloeckner SF, Meyne F, Wagner F, Heinemann U, Krasnianski A, Meissner B, Zerr I. Quantitative analysis of transthyretin, tau and amyloid-beta in patients with dementia. *J Alzheimers Dis* 2008;14:17-25. doi: 10.3233/jad-2008-14102.
91. Ingenbleek Y, Barclay D, Dirren H. Nutritional significance of alterations in serum amino acid patterns in goitrous patients. *Am J Clin Nutr.* 1986;43:310-9. doi: 10.1093/ajcn/43.2.310.
92. Ingenbleek Y, Hardillier E, Jung L. Subclinical protein malnutrition is a determinant of hyperhomocysteinemia. *Nutrition.* 2002;18:40-6. doi: 10.1016/s0899-9007(01)00783-3.
93. Hung CJ, Huang PC, Lu SC, Li YH, Huang HB, Lin BF, Chang SJ, Chou HF. Plasma homocysteine levels in Taiwanese vegetarians are higher than those of omnivores. *J Nutr.* 2002;132:152-8. doi: 10.1093/jn/132.2.152.
94. Yajnik CS, Lubree HG, Thuse NV, Ramdas LV, Despanthe SS, Despanthe VU et al. Oral vitamin B12 supplementation reduces plasma total homocysteine concentration in women in India. *Asia Pac J Clin Nutr.* 2007;16:103-9.
95. Obersby D, Chappell DC, Dunnett A, Tsiami AA. Plasma total homocysteine status of vegetarians compared with omnivores: A systematic review and meta-analysis. *Br J Nutr.* 2013;109:785-94. doi: 10.1077/s000711451200520.x.
96. Finkelstein JD, Martin JJ. Methionine metabolism in mammals. Distribution of homocysteine between competing pathways. *J Biol Chem.* 1984;259:9508-13.
97. Finkelstein JD. Pathways and regulation of homocysteine metabolism in mammals. *Semin Thromb Hemost.* 2000;26:219-25. doi: 10.1055/s-2000-8466.
98. Ingenbleek Y. Lean body mass harbors sensing mechanisms that allow safeguarding of methionine homeostasis. *Nutrients.* 2017;9:1035-47. doi: 10.3390/nu9091035.
99. Elshorbagy AK, Valdivia-Garcia M, Refsum H, Smith AD, Mattocks DA, Perrone CE. Sulfur amino acids in methionine-restricted rats: Hyperhomocysteinemia. *Nutrition.* 2010;26:1201-4. doi: 10.1016/j.nut.2009.09.017.
100. Cuthbertson DP. The distribution of nitrogen and sulphur in the urine during conditions of increased catabolism. *Biochem J.* 1931;25:236-44. doi: 10.1042/bj0250236.
101. Powanda MC, Beisel WR. Metabolic effects of infection on protein and energy status. *J Nutr.* 2003;133:S322-7. doi: 10.1093/jn/133.1.322s.
102. Bienvenu J, Monneret G, Fabien N, Revillard JP. The clinical usefulness of the measurement of cytokines. *Clin Chem Lab Med.* 2000;38:267-85. doi: 10.1515/cclm.2000.040.
103. Castell JV, Gómez-Lechón MJ, David M, Andus T, Geiger T, Trullenque R, Fabra R, Heinrich PC. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. *FEBS Lett.* 1989;242:237-9. doi: 10.1016/0014-5793(89)80476-4.

104. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med*. 1999;340:448-54. doi: 10.1056/nejm.199902113400607.
105. Lustgarten MS, Fielding RA. Metabolites associated with circulating interleukin-6 in older adults. *J Gerontol A Biol Sci Med Sci*. 2017;72:1277-83. doi: 10.1093/gerona/glw039.
106. Arnold J, Campbell IT, Samuels TA, Devlin JC, Green CJ, Hipkin LJ, MacDonald IA, Scrimgeour CM, Smith K, Rennie MJ. Increased whole body protein breakdown predominates over increased whole body synthesis in multiple organ failure. *Clin Sci (Lond)*. 1993;84:655-61. doi: 10.1042/cs0840655.
107. Murakami T, Ohnishi S, Nishiguchi S, Maeda S, Araki S, Shimada K. Acute-phase response of mRNAs for serum amyloid P component, C-reactive protein and prealbumin (transthyretin) in mouse liver. *Biochem Biophys Res Commun*. 1988;155:554-60. doi:10.1016/s0006-291x(88)80530-8.
108. Banks RE, Forbes MA, Storr M, Higginson J, Thompson D, Raynes J, Illingworth JM, Perren TJ, Selby PJ, Whicher JT. The acute phase protein response in patients receiving subcutaneous IL-6. *Clin Exp Immunol*. 1995;102:217-23. doi: 10.1111/j.1365-2249.1995.tb06659.x.
109. Vente JP, von Meyenfeld MF, van Eijk HM, van Berlo CL, Gouma DJ, van der Linden CJ, Soeters PB. Plasma amino acid profiles in sepsis and stress. *Ann Surg*. 1989;209:57-62. doi: 10.1097/0000658-198901000-00009.
110. Breitzkreuz R, Holm S, Pittack N, Beichert M, Babylon A, Yodoi J, Dröge W. Massive loss of sulfur in HIV infection. *AIDS Res Hum Retroviruses* 2000;16:203-9. doi: 10.1089/088922200309296.
111. Malmezat T, Breuillé D, Pouyet C, Buffière C, Denis P, Mirand PP, Obled C. Methionine transsulfuration is increased during sepsis in rats. *Am J Physiol Endocrinol Metab*. 2000;279:E1391-7. doi: 10.1152/ajpendo.2000.279.6.E1391.
112. Malmezat T, Breuillé D, Capitan P, Mirand PP, Obled C. Glutathione turnover is increased during the acute phase of sepsis in rats. *J Nutr* 2000;130:1239-46. doi: 10.1093/jn/130.5-1239.
113. Malmezat T, Breuillé D, Pouyet C, Mirand PP, Obled C. Metabolism of cysteine is modified during the acute phase of sepsis in rats. *J Nutr* 1998;128:97-105. doi: 10.1093/jn/128.1.97.
114. Schindler K, Zauner C, Buchmayer H, Födinger M, Wöfl G, Bieglmayer C, Heinz G, Wilfing A, Hörl WH, Sunder-Plassmann G. High prevalence of hyperhomocysteinemia in critically ill patients. *Crit Care Med*. 2000;28:991-5. doi:10.1097/00003246-200004000-00013.
115. Ruud E, Holmstrøm H, Brosstad F, Wesenberg F. Children with acute lymphoblastic leukaemia have high plasma levels of total homocysteine at time of diagnosis. *Scand J Clin Lab Invest*. 2006;66:67-78. doi: 1080/00365510500453003.
116. Gori AM, Corsi AM, Fedi S, Gazzini A, Sofi F, Bartali B, Bandinelli S, Gensini SF, Abbate R, Ferrucci L. A proinflammatory state is associated with hyperhomocysteinemia in the elderly. *Am J Clin Nutr*. 2005;82:335-41. doi: 10.1093/ajcn.82.2.335
117. Ingenbleek Y, McCully KS. Vegetarianism produces subclinical malnutrition, hyperhomocysteinemia and atherogenesis. *Nutrition*. 2012;28:148-53. doi: 10.1016/j.nut.2011.04.009.

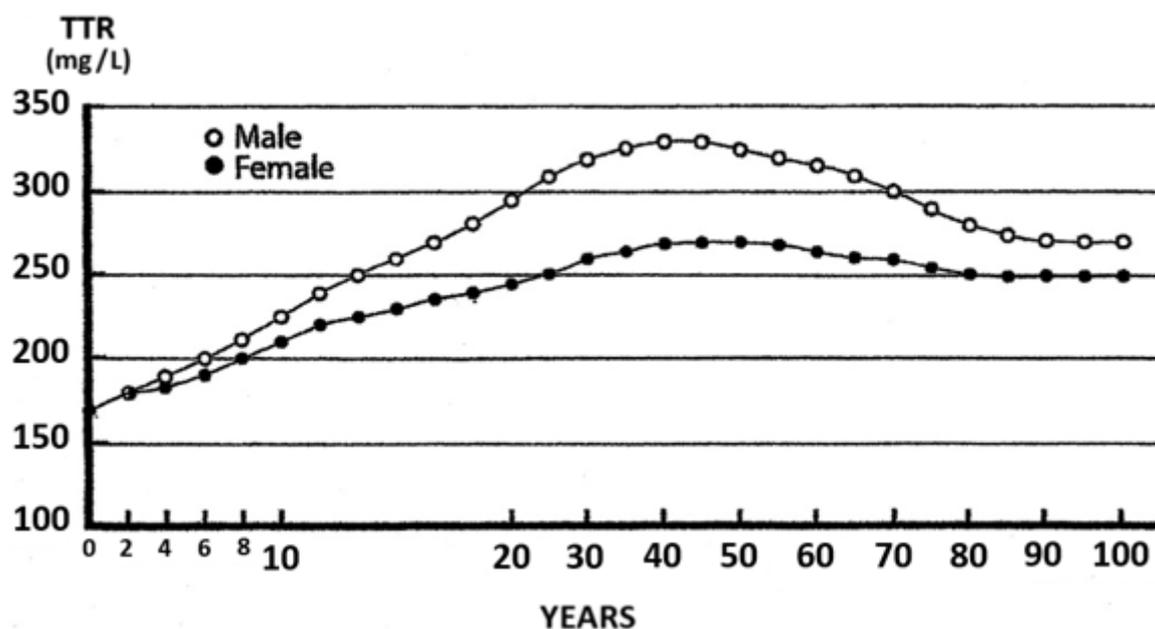
118. Sun CF, Haven TR, Wu TL, Tsao KC, Wu JT. Serum total homocysteine increases with the rapid proliferation rate of tumor cells and decline upon cell death: a potential new tumor marker. *Clin Chim Acta*. 2002;321:55-62. doi: 10.1016/s0009-8981(02)00092-x.
119. McKeever MP, Weir DG, Molloy A, Scott JM. Betaine-homocysteine methyltransferase: organ distribution in man, pig and rat and subcellular distribution in the rat. *Clin Sci (Lond)*. 1991;81:551-6. doi: 10.1042/cs0810551.
120. Strakova J, Williams KT, Gupta S, Schalinske KL, Kruger WD, Rozen R, Jiracek J, Li L, Garrow TA. Dietary intake of S-( $\alpha$ -carboxybutyl)-DL-homocysteine induces hyperhomocysteinemia in rats. *Nutr Res*. 2010;30:492-500. doi: 10.1016/j.nutres.2010.06.017.
121. Stead LM, Brosnan ME, Brosnan JT. Characterization of homocysteine metabolism in the rat liver. *Biochem J*. 2000;350:685-92.
122. Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, Wilson PW, Wolf PA. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med*. 2002;346:476-83. doi: 10.1056/nejmoa011613.
123. Eto K, Asada T, Arima K, Makifuchi T, Kimura H. Brain hydrogen sulfide is severely decreased in Alzheimer's disease. *Biochem Biophys Res Commun*. 2002;293:1485-8. doi: 10.1016/s0006-291x(02)00422-9.
124. Popp J, Oikonomidi A, Tautvydaitė D, Dayon L, Bacher M, Migliavacca E et al. Markers of neuroinflammation associated with Alzheimer's disease pathology in older adults. *Brain Behav Immun*. 2017;62:203-11. doi: 10.1016/j.bbi.2017.01.020.
125. Price BR, Wilcock DM, Weekman EM. Hyperhomocysteinemia as a risk factor for vascular contributions to cognitive impairment and dementia. *Front Aging Neurosci*. 2018;10:350-361. doi: 10.3389/fnagi.2018.00350.
126. Li JG, Chu J, Barrero C, Merali S, Praticó D. Homocysteine exacerbates  $\beta$ -amyloid pathology, tau pathology, and cognitive deficit in a mouse model of Alzheimer disease with plaques and tangles. *Ann Neurol*. 2014;75:851-63. doi: 10.1002/ana.24145.
127. Kamath AF, Chauhan AK, Kisucka J, Dole VS, Loscalzo J, Handy DE, Wagner DD. Elevated levels of homocysteine compromise blood brain barrier integrity in mice. *Blood*. 2006;107:591-3. doi: 10.1182/blood-2005-06-2506
128. Baloyannis SJ. Mitochondrial alterations in Alzheimer's disease. *J Alzheimers Dis*. 2006;9:119-26. doi: 10.3233/jad-2006-9204.
129. Jakubowski H. Homocysteine thiolactone: metabolic origin and protein homocysteinylation in humans. *J Nutr*. 2000;130 (Suppl.2):S377-81. doi: 10.1093/jn/130.2.377s.
130. Costa DA, Cracchiolo JR, Bachstetter AD, Hughes TF, Bales KR, Paul SM, Mervis RF, Arendash GW, Potter H. Enrichment improves cognition in AD mice by amyloid-related and unrelated mechanisms. *Neurobiol Aging*. 2007;28:831-44. doi: 10.1016/neurobiolaging.2006.04.009.

131. Chen WL, Niu YY, Jiang WZ, Tang HL, Zhang C, Xia QM, Tang XQ. Neuroprotective effects of hydrogen sulfide and the underlying signaling pathways. *Rev Neurosci*. 2015;26:129-42. doi: 10.1515/revneuro-2014-0051.
132. Kumar M, Ray RS, Sandhir R. Hydrogen sulfide attenuates homocysteine-induced neurotoxicity by preventing mitochondrial dysfunctions and oxidative damage: In vitro and in vivo studies. *Neurochem Int*. 2018;120:87-98. doi: 10.1061/j.neuint.2018.07.010.
133. Kamat PK, Kyles P, Kalani A, Tyagi N. Hydrogen sulfide ameliorates homocysteine-induced Alzheimer's disease-like pathology, blood brain barrier disruption, and synaptic disorder. *Mol Neurobiol*. 2016;53:2451-67. doi: 10.1007/s12035-015-9212-4.
134. Conner J, Russell PJ. Elemental sulfur: A novel inhibitor of adenylate kinase. *Biochem Biophys Res Commun*. 1983;113:348-52. doi: 10.1016/0006-291x(83)90472-2.
135. Lee J, Lee HJ, Park JD, Lee SK, Lee SI, Lim HD et al. Anti-cancer activity of highly purified sulfur in immortalized and malignant human oral keratinocytes. *Toxicol in Vitro*. 2008;22:87-95. doi: 10.1016/j.tiv.2007.08.016.
136. Sergi G, Coin A, Enzi G, Volpato S, Inelmen EM, Buttarello M, Peloso M, Mulone S, Marin S, Bonometto P. Role of visceral proteins in detecting malnutrition in the elderly. *Eur J Clin Nutr*. 2006;60:203-9. doi:10.1038/sj.ejcn.1602289.
137. Brosnan JT, Brosnan ME, Bertolo RF, Brunton JA. Methionine: A metabolically unique amino acid. *Livestock Sci*. 2007;112:2-7.
138. Buxbaum JN, Reixach N. Transthyretin: the servant of many masters. *Cell Mol Life Sci*. 2009;66:3095-101. doi: 10.1007/s00018-009-0109-0.
139. Vieira M, Saraiva MJ. Transthyretin: a multifaceted protein. *Biomol Concepts*. 2014;5:45-54. doi: 10.1515/bmc-2013-0038.
140. Ingenbleek Y, Bernstein LH. Plasma transthyretin as a biomarker of lean body mass and catabolic states. *Adv Nutr*. 2015;6:572-80. doi: 10.3945/an.115.008508.
141. Rambod M, Kovesdy CP, Bross R, Kopple JD, Kalantar-Zadeh K. Association of serum prealbumin and its changes over time with clinical outcomes and survival in patients receiving hemodialysis. *Am J Clin Nutr*. 2008;88:1485-94. doi: 10.3945/ajcn.2008.25906.
142. Yu PJ, Cassiere HA, Dellis SL, Manetta F, Kohn N, Hartman AR. Impact of preoperative prealbumin on outcomes after cardiac surgery. *J Parenter Enter Nutr* 2015;39:870-4. doi: 10.1177/0148607114536735.
143. Salvetti DJ, Tempel ZJ, Goldschmidt E, Colwell NA, Angriman F, Panczykowski D, Agarwal N, Kanter AS, Okonkwo DO. Low preoperative serum prealbumin levels and the postoperative surgical site infection risk in elective spine surgery. a consecutive series. *J Neurosurg Spine*. 2018;29:549-52. doi: 10.3171/2018.3.spine171183.
144. Liao YY, Teng CL, Peng NF, Jia RR, Cui J, Chen K, Ma L, Xiang BD, Zhong JH, Li LQ. Serum prealbumin is negatively associated with survival in hepatocellular carcinoma patients after hepatic resection. *J Cancer*. 2019;10:3006-11. doi: 7150/jca.30903.

145. Isono N, Imamura Y, Ohmura K, Ueda N, Kawabata S, Furuse M, Kuroiwa T. Transthyretin in acute stroke patients predict convalescent rehabilitation. *J Stroke Cerebrovasc Dis.* 2017;26:1375-82. doi: 10.1016/j.jstrokecerebrovasdis.2017.02.020.
146. Perez-Valdivieso JR, Bes-Rastrollo M, Monedero P, de Irala J, Lavilla FJ. Impact of prealbumin levels on mortality in patients with acute kidney injury: an observational cohort study. *J Ren Nutr* 2008;18:262-8. doi: 10.1053/j.jrn.2007.11.003.
147. Ho SY, Guo HR, Chen HH, Peng CJ. Nutritional predictors of survival in terminally ill cancer patients. *J Formos Med Assoc.* 2003;102:544-50.
148. Chertow GM, Goldstein-Fuchs DJ, Lazarus JM, Kaysen GA. Prealbumin, mortality, and cause-specific hospitalization in hemodialysis patients. *Kidney Int.* 2005;68:2794-800. doi: 10.1111 /j.1523-1755.2005.00751.x.



**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  $^{40}\text{K}$ . The pioneering devices were assembled at the University of Rochester, New York, USA, under the guidance of G.B. Forbes<sup>13</sup> in close collaboration with the International Atomic Energy Agency (IAEA), Vienna, Austria. Fig. 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.



**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.<sup>47</sup> 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.<sup>48,49</sup> 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.<sup>49,140</sup> The data show that the increased severity of AD processes is not a problem of age but of LBM downsizing.