Review

Downsizing of Lean Body Mass is a Key Determinant of Alzheimer’s Disease

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Abstract. Lean body mass (LBM) encompasses all metabolically active organs distributed into visceral and structural tissue compartments and collecting the bulk of N and K stores of the human body. Transthyretin (TTR) is a plasma protein mainly secreted by the liver within a trimolecular TTR-RBP-retinol complex revealing from birth to old age strikingly similar evolutionary patterns with LBM in health and disease. TTR is also synthesized by the choroid plexus along distinct regulatory pathways. Chronic dietary methionine (Met) deprivation or cytokine-induced inflammatory disorders generates LBM downsizing following differentiated physiopathological processes. Met-restricted regimens downregulate the transsulfuration cascade causing upstream elevation of homocysteine (Hcy) safeguarding Met homeostasis and downstream drop of hydrogen sulfide (H₂S) impairing anti-oxidative capacities. Elderly persons constitute a vulnerable population group exposed to increasing Hcy burden and declining H₂S protection, notably in plant-eating communities or in the course of inflammatory illnesses. Appropriate correction of defective protein status and eradication of inflammatory processes may restore an appropriate LBM size allowing the hepatic production of the retinol circulating complex to resume, in contrast with the refractory choroidal TTR secretory process. As a result of improved health status, augmented concentrations of plasma-derived TTR and retinol may reach the cerebrospinal fluid and dismantle senile amyloid plaques, contributing to the prevention or the delay of the onset of neurodegenerative events in elderly subjects at risk of Alzheimer’s disease.

Keywords: Alzheimer’s disease, homocysteine, hydrogen sulfide, lean body mass, nutritional assessment, retinoids, transthyretin

INTRODUCTION

Plasma transthyretin (TTR) was initially proposed as an index of protein-depleted states following field surveys undertaken in Senegal (West Africa) on children suffering from varying stages of malnutrition ranging from cachectic marasmus to edematous kwashiorkor [1]. The serum analyte is now widely measured in developing areas for the nutritional follow-up of underprivileged populations [2, 3] and in developed countries to screen hospitalized patients who require dietary management [4, 5]. Several neurological investigations have recently reported the innovative observation that the same TTR biomarker impacts on the outcome of Alzheimer’s disease (AD) [6, 7], raising the basic premise that alterations of protein status might be implicated in neurodegenerative disorders. Preliminary studies have indeed suggested that the reliability of the TTR indicator is based on its accurately identifying loss of lean body mass (LBM) [8] effecting metabolically active tissues in health and disease. The below review describes the unrecognized correlations linking LBM entity to TTR fluctuations and the mechanisms whereby LBM downsizing, as determined by declining TTR plasma concentrations, generates significant public health consequences in neurodeteriorating morbidities, taking AD as exemplary.
MAIN LEAN BODY MASS CHARACTERISTICS

The simplest methodology aiming at assessing body composition and LBM measurement refers to a binary system taking the difference between fat mass and body weight. Dual-energy X-ray absorptiometry measures γ-rays emitted by the naturally occurring radioisotope 40K, 95% of K being intracellular in metabolically active tissues [9]. Dual-energy X-ray absorptiometry, regarded as a gold standard, accurately measures LBM assuming that total body potassium (TBK) values are tightly correlated with total body nitrogen within an average K/N ratio of about 3 mEq K/g N [10]. In healthy adult males, LBM contains the bulk of total body nitrogen (1,800 g or 64 mol) and of TBK (140 g or 3,600 mmol) (Fig. 1) [11]. LBM may be schematically subdivided into the visceral protein compartment, characterized by rapid turnover rates, consisting of the liver, small intestinal mucosa and hematopoietic tissue, and the structural protein compartment, distinguished by slower turnover rates, comprising the muscle mass, skin and connective tissues [12]. Pioneering studies performed by Brožek and Grande on human adults have shown that hepatic tissues manifest an oxygen consumption rate (44 ml O2/Kg) about 20–fold higher than the muscle mass (2.3 ml O2/Kg) [13]. Moreover, the fractional synthesis rate of total liver proteins (stationary and exported) equals approximately 25% of the daily hepatic protein content [14] contrasting with the muscle mass turnover at 1.7%/day [15]. The fractional synthesis rate of the gut mucosa protein is close to 10%/day [16], whereas that of lymphocytes reaches 5% of the whole body protein turnover [17].

The data imply that, on a ponderal basis, the protein turnover in visceral organs is about 10 to 20-times faster than that of the skeletal muscle mass. However, comparing the liver mass (2.6% of body weight [9] at 1.7 Kg) with that of the musculature (37% of body weight [18] or 26 Kg), both components contribute equally to the basal metabolic activities daily estimated at 26.4% and 25.6%, respectively [13]. These studies conducted nearly 60 years ago are confirmed by a more recent clinical investigation showing that liver and musculature, principal determinants of resting energy expenditure, together generate 50% of the basal metabolic expenditure [19].

A good example of the dichotomous LBM partitioning into visceral and structural activities is provided by patients undergoing chronic renal failure. The concomitant measurement of LBM using TBK-40 method and creatinine generation rate in kidney patients resulted in a systematic underestimation of the latter technique when compared to the former [20]. This discrepancy lies in the creatinine generation rate method only identifying the structural component of LBM but failing to measure the metabolically active visceral component. This comparative peritoneal dialysis study led to the conclusion that creatinine generation rate is an inappropriate parameter to appraise LBM.
status in renal patients [20]. Most kidney studies indicate that plasma TTR concentrations have better predictive reliability in assessing outcome [21]. The concept of compartmental specificity is also documented in long-lasting wasting disorders characterized by greater magnitude of TBK over total body nitrogen losses, pointing to a more rapid reduction in skeletal muscle mass and a relative resistance of visceral tissues [22].

MAIN TTR CHARACTERISTICS

TTR is a highly conserved protein in vertebrate species secreted by the choroid plexus (CP) of reptiles that has remained confined within the cerebrospinal fluid (CSF) for 300 million years [23]. Liver synthesis of TTR occurred much later, about 100 million years ago, in birds and eutherian mammals [24]. The sole intracerebral site of TTR synthesis is confined to the epithelial cells lining the ventricular surface of the CP [25, 26]. TTR was identified in human CSF in 1942 [27] whereas the liver-secreted TTR was discovered in human serum in 1956 [28]. TTR is a tryptophan-rich molecule [28] displaying tetrameric conformation, each subunit having a sequence of 127 amino acids (AAs) aggregated within a nonglycosylated edifice of 55 kDa as molecular mass (MM) [29]. TTR is the third specific binding protein that transports thyroid hormones [30] besides serum-albumin and thyroxine-binding globulin. One of the four monomers constituting the tetrameric TTR edifice carries a small companion protein displaying a single binding site for a molecule of all-trans-retinol [31] (retinol-binding protein, RBP, 21 kDa as MM) thus forming together a trimolecular retinol-carrying complex (RCC) totaling 76 kDa as MM [31, 32]. The International Nomenclature Committee has recommended the denomination of trans-thyr-retin to emphasize the dual conveying roles played by TTR in human serum [33]. Despite different biological half-lives (2 days for TTR [34] versus half a day for RBP [35]), the three RCC components are bound at 1:1:1 stoichiometry. Apo-RBP molecules devoid of retinol ligand disclose a significantly reduced T1/2 and undergo rapid glomerular leakage, tubular disintegration and subsequent recycling of the released AA residues [35]. The data indicate that TTR protects RBP from premature urinary output and serves as a limiting factor for the delivery of retinoid compounds to target tissues [36]. Animal experiments have shown that the liver is the main site for TTR degradation, followed by muscle mass, skin, and kidneys [37].

Newborn plasma TTR concentrations reach roughly two thirds those of their mothers, increasing linearly without sexual difference during infant growth [38]. When puberty begins, major hormonal and metabolic alterations lead to increased height velocity, weight gain, and redistribution of body tissues [39], especially
muscle mass constituting the main LBM component by weight [18]. The result is an S-shaped growth of LBM greater in male adolescents than the blunted curve documented in teen-aged girls [9]. The gender dimorphism, an evolutionary pattern observed during adolescence for TTR values [40, 41], matches the growth of LBM [9]. During the full sexual maturity period, healthy adults reveal sex-related difference in plasma TTR concentrations stabilized at plateau levels of 300-330 mg/L in males and around 250-270 mg/L in females [40, 41] (Fig. 2), whereas RBP plasma values are likewise around 63 mg/L and 52 mg/L [40], respectively. The muscle mass begins downsizing in the sixties, with a steeper slope in elderly men [9, 18] accounting for the concomitant decline in LBM [9] and TTR-RBP values [40]. The serial measurement of plasma TTR concentrations allows grading the level of protein wasting, 180 mg/L, constituting a boundary predicting serious complications [42] and the threshold of 100 mg/L, presumably reflecting the exhaustion of LBM resources, bearing ominous prognostic significance [43]. Early detection and aggressive nutritional support provided to high risk patients significantly improves their prognostic outcome while alleviating the financial burden of hospitalization [44].

LEAN BODY MASS: TTR INTERRELATIONSHIPS

The above observations demonstrate for the first time the striking similarities linking LBM and TTR evolutionary patterns throughout the lifetime of human subjects, stressing the functional uniqueness of the plasma biomarker. Due to its short biological half-life, TTR appears as a sensitive responder to any alteration of protein status [45, 46] allowing rapid identification of LBM stores. LBM downsizing may be the consequence of either chronic dietary restriction leading to unachieved protein replenishment [47] or to inflammatory disorders causing excessive urinary N-losses [48]. The former condition is described in animal models [49, 50] and human subjects [51, 52] experiencing methionine (Met)-deprived regimens. The latter condition is reported in liver experiments [53] and clinical studies [54, 55] characterized by cytokine-induced inflammatory events. These events are associated with an up and down TTR fluctuations, showing that LBM may undergo N recovery or depletion processes influencing the liver secretory rate of plasma TTR that emerges as the ultimate signal of LBM reserves. We postulate the existence of many intersected linkages and centrally-mediated regulatory mechanisms governing the balance between protein accretion and protein breakdown as well as between inter-organ energy and AA fluxes feeding LBM components. The data indicate that any nutritional or inflammatory causal factor generates comparable LBM downsizing and shrinking of Met body pools, operating nevertheless throughout unrelated physiological mechanisms. To make short a long story described with details [47], reduced Met bioavailability causes the greatest catabolic losses, depressing protein syntheses nearly as deeply as when protein-free regimens are provided [56]. Maintenance of Met homeostasis is therefore of survival importance as appropriate dietary provision of Met plays crucial roles in the preservation of N body stores. Met-depleted animals set in motion adaptive mechanisms aiming at adjusting to reduced activity of cystathionine-β-synthase (CBS, EC 4.2.1.22) enzyme [57, 58] along the transsulfuration (TS) cascade. The blockade promotes an upstream accumulation of homocysteine (Hcy) values in body fluids [49-51], serving as a precursor pool readily driven into efficient Hcy → Met remethylation (RM) pathways. The final result affords unmodified Met baseline levels whereas Hcy did manifest gradually rising values as protein status worsens [51] explaining why Hcy and TTR plasma values are inversely correlated and manifest mirror image of each other [8]. Taken together, the data show that, in the case of inadequate Met dietary intake, upsurge of Hcy values operate as the dark side of beneficially stimulated RM processes allowing a safeguard of Met homeostasis. Inhibition of CBS activity generates another important biological effect, namely the downstream synthesis of cysteine (Cys) and glutathione (GSH) reductant molecules [52, 59], accounting for the depressed enzymatic and non-enzymatic physiological production of hydrogen sulfide (H2S) in body tissues [47]. Met is the main provider of S by the way of dietary items and the content of this indispensable AA (IAA) holds, on a ponderable basis, significant advantage in animal over vegetable kingdoms. S and N are indeed closely correlated within stable molar ratios turning around 1:1.4.5 in mammalian tissues [60] whereas plant products are characterized by lesser N and S contents revealing molar ratios usually ranging from 1:20 to 1:35 [60] which do not optimally fulfill human tissue requirements. The data explain why strict vegan subjects incur the risk of permanent N and S deficiencies leading to LBM shrinking [47] and upgraded Hcy production [61, 62]. Plant products based upon cereals cannot meet human IAA needs unless at least 30% of total protein is provided by animal sources [63].
THE TTR: LEAN BODY MASS COUPLE IN ALZHEIMER’S DISEASE

AD is a common and devastating form of dementia affecting a growing proportion of individuals over the age of 65 years and characterized by the harmful accumulation in the brain of two types of histopathological deposits (1) neurofibrillary tangles formed by aggregates of hyperphosphorylated tau proteins and (2) senile amyloid plaques resulting from self-aggregation of amyloid-β (Aβ) peptides with Aβ1-40 monomers predominating over the most aggressive Aβ1-42 species [64]. Both inflammatory events and generation of reactive oxidative species (ROS) participate in the breakdown of basal lamina, increasing permeability of the vascular blood-brain barrier (BBB) [65]. The AD plaque is a localized form of amyloid fibril deposit remaining confined to cerebral tissues that has not been identified in other organs of the body [66]. Clear distinction must be made between the cleavage of amyloid-β protein precursor into Aβ peptides found in AD brain and the autosomal dominant amyloidogenic process resulting from the substitution of several enzymes implicated in the development of Hcy-mutation process has been first recognized by Andrade [67] in 1952 under the clinical denomination of familial amyloidotic polyneuropathy (FAP) characterized by anomalous protein misfolding and aggregation. At least 100 different mutated variants have been enumerated up to now [68] liable to generate structural and functional damages in most body tissues [66]. Some studies have shown that FAP patients may undergo several stages of protein malnutrition characterized by subnormal TTR plasma values [69]. This clinical anomaly must be regarded as the secondary consequence of FAP-induced liver dysfunction in contrast to the primary role played by protein deficiency in the occurrence of AD disorders. The different conformation and properties distinguishing FAP from AD amyloidic processes is documented by tissue studies failing to report immunochemical similarities or antigenic cross-reactivity [70, 71].

Several recent investigations performed on matrix metalloproteinases (MMP) enzymes in neurodegenerative processes shed new light in that research domain. MMP-2 and MMP-9 are the two main zinc-dependent endoproteases manifesting ambivalent properties under the regulatory control of a family of four tissue inhibitors of MMPs (TIMPs) [72, 73]. Under physiological circumstances, the highly integrated MMP-TIMP balance maintain normal cell signaling processes, growth promoting activities, release of extracellular matrix, wound healing and angiogenesis of neural tissue [72, 73]. In neuro-inflammatory disorders, the MMP-TIMP balance is disrupted, promoting the formation of neurofibrillary tangles and senile amyloid plaques [74] with the participation of mitochondrial cytochrome C oxidase [75] and caspase-3 [76]. Both MMP-2 and MMP-9 enzymes may conversely exert beneficial effects such as degradation of Aβ1-40 [74] and Aβ1-42 [77] peptides.

The strong anti-oxidative properties normally elicited by H2S against the deleterious effects of Hcy burden [47] are shown to be impaired in AD patients owing to their severely depressed intracerebral CBS activity releasing reduced amounts of the gasotransmitter from Cys [78]. Therapeutic proof of the defect is provided by exogenous administration of H2S that protects neurons from ROS stress [79], allows to redress MMP-TIMP imbalance [80] and to mitigate Hcy-induced cerebrovascular dysfunction, memory deficits and inflammatory remodeling [81]. Most H2S molecules decompose into sulfide anions (S2−) [82] shown to inhibit the activity of several enzymes implicated in the development of Hcy-induced ROS damages [47]. For example, MMP-2 and MMP-9 in human breast cancer cells are downregulated in a dose-dependent manner by the addition of highly purified S in the culture media [83]. Likewise, human malignant keratinocytes incubated with elemental S causes the reduction of cytochrome C oxidase and caspase-3 activities in a dose- and time-related manner with subsequent growth inhibition and apoptosis of cancer cells [84]. We assume that elemental S plays the role of cofactor of several enzyme activities critically involved in the regulation of Hcy-dependent oxidative stress [47].

A growing body of neurobiological studies indicates that plasma TTR, RBP, and retinol are effector molecules flowing across the blood-brain barrier and diffusing in CSF to blend together with TTR molecules intrathecally produced. Intracerebral TTR participates in memory [85], cognitive [86], behavioral [87], regenerative [88], and neuroprotective [89] processes. The brain CP manifests declining intracerebral TTR secretory potential with increasing age [90], a regressive process likely to be genetically programmed and poorly responsive to therapeutic manipulation, at variance with plasma-derived TTR and retinoids. The data support the concept that the production of TTR in the liver and in the CP is regulated independently [91], suggesting that the brain might escape most harmful events affecting overall body economy in the course of...
protein-depleted and/or inflammatory states. The first evidence that TTR is capable of preventing Aβ amyloid formation and toxicity was described in experimental studies [92]. TTR works as a rate-limiting determinant of the plasma transport of retinol [36] which in turn operates as a rate-limiting determinant of both physiologically active cis and trans retinoic acid (RA) derivatives [93]. Under normal ageing process, the concentration of RA compounds [94] and expression of RA receptors [95] are downregulated in cerebral tissues. In adult rat brains, depletion of RAs causes deposition of Aβ peptides, favoring the formation of senile amyloid plaques [96]. Conversely, exogenous administration of RAs restores the expression of retinoid receptors and the control of amyloidogenic pathways [97]. In addition, retinol may disaggregate preformed Aβ fibrils more efficiently than do RAs [98], indicating that the three retinoid molecules operate synergistically to prevent the deterioration of cerebral tissues. Finally, cerebral dysfunctions such as depression [106], schizophrenia [107], and Guillain-Barré syndrome [108] are reportedly characterized by comparable CSF decline in TTR and RBP values.

Fig. 3. Total plasma Hcy (A) and mean TTR (B) values in elderly persons aged 65 to 100 years. A) Bates et al. [109] studied free-living British elderly persons (375 men and 373 women totaling 748 subjects) stratified into three groups aged 65–74, 75–84, and 85 years or more. Because gender exerted only minor influence, Hcy concentrations for both sexes were combined, submitted to logarithmic transformation before regression analysis. The study shows the 3rd, 10th, 50th, 90th, and 97th reference centiles calculated for the physiological elevation of Hcy in process of age. B) Bienvenu et al. [8,41] investigated 17,645 healthy US elderly persons (5,796 men and 11,849 women) subdivided into four groups aged 61–70, 71–80, 81–90, and 91–100 years and combining both sexes. The study shows the physiological lowering of TTR values with increasing age, the mean value being indicated in bold characters in each of the four categories.
tendency suggests that women are disproportionally affected by AD [111, 114], especially after the age of 80-85 years [115]. Figure 3 outlines the divergent relationships linking the physiological lowering of the LBM-TTR couple with the physiological elevation of Hcy values in healthy elderly persons and the likelihood to develop cerebral disability therefrom. Any causally impairing normal LBM replenishment as seen in protein malnutrition [52, 116] or depleting LBM stores as documented in acute or chronic inflammatory diseases [48, 55, 117] further distort both LBM/Hcy relationships while keeping unaltered the mirror image of each other. As a result of LBM shrinking, evidence has accumulated in acute or chronic inflammatory diseases [48, 52, 116] or depleting LBM stores as documented in protein malnutrition [52, 116] or depleting LBM stores as documented in acute or chronic inflammatory diseases [48, 55, 117] further distort both LBM/Hcy relationships while keeping unaltered the mirror image of each other.

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