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TRANSTHYRETIN (PREALBUMIN) IN HEALTH AND DISEASE: Nutritional Implications

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CONTENTS

INTRODUCTION

Transthyretin (TTR), formerly called prealbumin, is a plasma protein secreted mainly by the liver and involved in the transport of both thyroid hormones. TTR is also indirectly implicated in the carriage of vitamin A through the

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mediation of the retinol-binding protein (RBP). A number of recent reviews encompass all behavioral aspects of RBP in health and disease (27, 99, 217, 227). To our knowledge, no such information exists concerning TTR, with the notable exception of one survey published in 1987 in French (41). The present article aims to fill a gap in the Anglo-Saxon literature and to provide within a historical perspective a general overview of much that is known about TTR. Hence, this paper is an attempt to collect the most fundamental and clinical data currently available. We focus more specific attention on nutritional aspects because TTR is regarded as one of the most common biochemical indicators of protein-depleted states both in developed and developing countries. This review marks the twentieth anniversary of the first publication of TTR as a marker of nutritional status (125).

ANALYTICAL PROCEDURES

TTR was first identified in 1942 in concentrated cerebrospinal fluid (CSF) using the Tiselius moving boundary electrophoresis technique (148) and later by immunoelectrophoresis (88). The presence of TTR was soon confirmed in human plasma using several electrophoretic systems based on varying migrating media, such as paper sheet (278), starch gel (274), polyacrylamide gel (241), or reverse-flow electrophoresis with several buffering systems (122, 231). At the same time, pioneer groups working in the field of thyroid studies devised an alternative approach that assessed the maximal binding capacity of TTR for radiothyroxine (124, 207). The principle of the method was based on the addition of increasing amounts of labeled material in the incubation medium until the saturation of TTR binding sites was achieved. Under normal conditions, the plateau level recorded by maximal radiohormonal binding determines the TTR concentration of the unknown blood sample. More recent procedures measure TTR with affinity chromatography by the mediation of RBP coupled with sepharose (163) or by reversed-phase high-performance liquid chromatography (36).

The isolation of human TTR in a purified form (63, 224) and the preparation of specific antibodies gave impetus to the development of a large spectrum of immunological methods. Radial immunodiffusion (275), the oldest and simplest immune method, is still the most widely employed, followed by electroimmunoassay (164), immunofluorescence (95), turbidimetry (48), nephelometry (96), enzyme-linked immunosorbent assay (152), and radioimmunoassay (56). The recent advent of monoclonal antibodies (51) has boosted immunochemical studies in living tissues, making it possible to identify molecular variants of TTR.

The measurement of TTR either by its maximal binding capacity for radiothyroxine or by an immunological technique yields highly positively correlated

results (129). However, this observation is valid only when the blood concentration of thyroxine-binding globulin (TBG), the most important of the three carrier proteins of thyroid hormones, remains within its normal dispersion range. The specific values are determined by gender and ethnical factors. The former approach becomes clearly irrelevant in the case of subnormal TBG levels, as seen in protein malnutrition (126). Under these circumstances, a spuriously high peak of radioactive thyroxine is displaced toward and accumulates in the prealbuminic zone, thus leading to an overestimation of TTR values (128). From these divergent results, one may conclude that when a genetic or acquired TBG deficit is likely or suspected, only an immunological method will reliably estimate the true TTR concentrations.

The isolation and purification of rat TTR (28, 196) has paved the way for the development of a useful animal model designed to provide further insight into thyroid, retinoid, and nutritional investigations. However, results obtained through these experimental studies must be interpreted with caution because in contrast to the human situation, TTR is the main transport vehicle of thyroid hormones in the rat. TBG plays a relatively minor role, circulating in the bloodstream during a limited period of the animal's growth (247).

CONFORMATIONAL STUDIES OF TRANSTHYRETIN

TTR appears as a nonglycosylated tetrameric holoprotein displaying tetrahedral symmetry. It consists of 4 identical subunits whose primary sequence of 127 amino acids (AAs) has been established, and it has a molecular mass (MM) of 54,980 Daltons (Figure 1, 150). The secondary and tertiary structures of each monomer have been studied using X-ray diffraction analysis of the crystalized protein at a resolution of 1.8 Å (25). Approximately 55% of the AA residues in each monomer are engaged in the formation of two extended eight-pleated sheets, each of which comprises four strands with antiparallel orientation. Only 5% of the AA residues are situated in one segment of α-helix (25). Two subunits aggregate to form a dimer by the interaction of two internal planes of the β-pleated sheets. The assembly of two dimers occurs through noncovalent links between two interplaying sites that belong to the edge strands of the sheets and that involve three hydrogen bonds and five AA residues (25).

The quaternary structure of TTR has the shape of a globular protein whose overall size is 70 Å × 55 Å × 50 Å. The two dimers are slightly rotated in relation to each other along the y axis. This positioning gives the molecule outstanding resistance to most alkali and acid solutions as well as to usual denaturating agents such as urea, guanidine-HC1, and sodium-dodecyl-sulfate (30, 31). The four subunits delineate through the molecule an open channel whose internal wall is formed by inner β-sheets and where two binding sites for thyroid hormones are located (25, 201). These binding sites are interrelated

NH_2-Gly-Pro-Thr-Gly-Thr-Gly-Glu-Ser-Lys-Cys-
Pro-Leu-Met-Val-Lys-Val-Leu-Asp-Ala-Val-Arg-Gly-Ser-
Pro-Ala-Ile-Asn-Val-Ala-Val-His-Val-Phe-Arg-Lys-Ala-Ala-
Asp-Asp-Thr-Trp-Glu-Pro-Phe-Ala-Ser-Gly-Lys-Thr-Ser-
Glu-Ser-Gly-Glu-Leu-His-Gly-Leu-Thr-Thr-Glx-Glx-Gln-
Phe-Val-Glu-Gly-Ile-Tyr-Lys-Val-Glu-Ile-Asp-Thr-Lys-Ser-
Tyr-Trp-Lys-Ala-Leu-Gly-Ile-Ser-Pro-Phe-His-Glu-His-
Ala-Glu-Val-Val-Phe-Thr-Ala-Asn-Asp-Ser-Gly-Pro-Arg-
Arg-Tyr-Thr-Ile-Ala-Ala-Leu-Leu-Ser-Pro-Tyr-Ser-Tyr-
Ser-Thr-Thr-Ala-Val-Val-Thr-Asn-Pro-Lys-Glu-COOH.

Figure 1 The AA sequence of each TTR monomeric subunit (150).

by the phenomenon of negative cooperativity (10, 78), which implies that when one binding site is occupied by a first thyroid molecule, the association constant (Ka) for the second thyroid molecule is markedly reduced. Under normal conditions, the Ka of the two TTR binding sites for thyroxine (T4) has been estimated at 7×10^7 M^{-1} and 6.7×10^5 M^{-1}, and that for triiodothyronine (T3) around 1.4×10^7 M^{-1} and 5.5×10^5 M^{-1} (47, 233). The phenolic group of the thyroid molecule stands in front of the hydrophilic group of the TTR tetramer in the narrowest part of the channel, whereas the opposite alanyl moiety floats in its wide-mouthed opening (25).

The external surface of the TTR tetramer is depressed by the presence of two deep concavities whose centers are ~ 33 Å apart, or the same order of magnitude as that separating the two DNA helices (25). The observation of a three-dimensional building structure complementary to the DNA edifice prompted Blake & Oatley to postulate that the plasma TTR could be the remnant of a phylogenetically related nuclear binding protein, which could serve as a model for thyroid hormone receptor studies (26).

TTR possesses one binding site for RBP, the specific carrier protein for one molecule of *all-trans*-retinol (Figure 2, 149). RBP is a monomeric chain comprising 182 AA residues (226) and totaling 21,000 Daltons as MM (27, 99, 149, 217, 226, 227). The conformational structure of RBP and its binding site for retinol have been determined by X-ray crystallography (198). In most instances, RBP is transported in the bloodstream in the form of a saturated holo-RBP protein equimolarly attached to TTR, which means that the resulting trimolecular retinol circulating complex (RCC) has an overall MM of ~ 76,000 Daltons (27, 99, 149, 198, 217, 226, 227). The Ka for the TTR-RBP binding site has been estimated at 1.2×10^6 M^{-1} (296). The TTR-RBP binding domain is still not entirely identified but rather is considered stereochemically unrelated to the AA sequence responsible for the antigenic response of the TTR molecule. This antigenicity has been localized in a midregion fragment of the

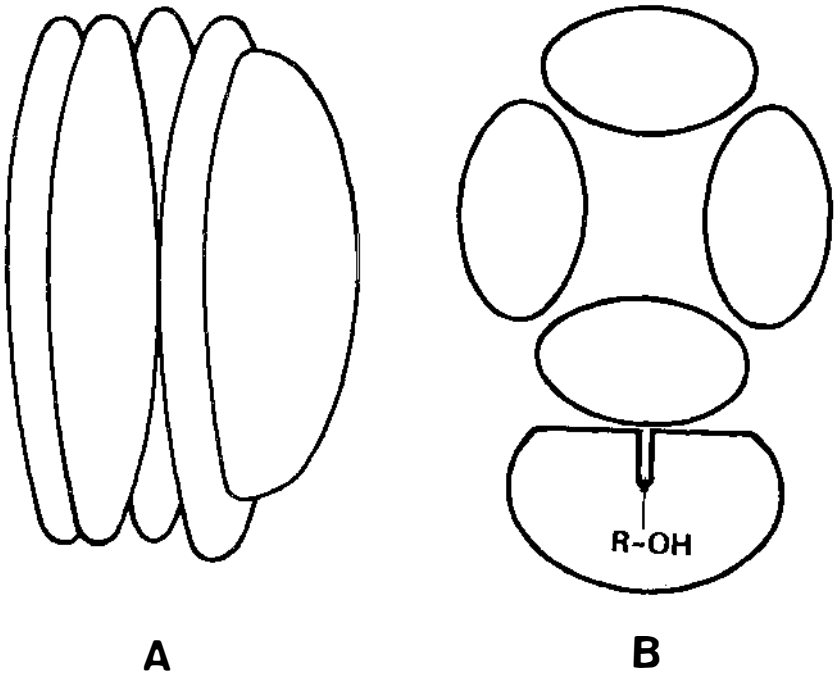

Figure 2 Schematic configuration of the retinol circulating complex (RCC).

A. Lateral view showing the tetrameric structure of TTR (54,980 Daltons as MM). Under normal conditions, a single RBP molecule (21,000 Daltons as MM) is bound to one of the four subunits.

B. Transversal view displaying the open channel delineated by the tetramer, which contains two binding sites for thyroid hormones. The inner part of the small RBP molecule has a cleft comprising one specific binding site for one retinol (R-OH) molecule.

monomeric subunit and corresponds to the 35–103 AA sequence, which forms at least two epitopes (51).

TTR has been identified in at least 15 vertebrate species (163). Most animal TTRs exhibit striking similarities to the human homolog, in particular to the tetrameric structure devoid of a carbohydrate load. The complete AA sequence of the TTR subunit has been clarified in three rodent species, namely rat (196), mouse (305), and rabbit (283), amounting to 127 residues like in human beings. Nevertheless, some minor interspecies differences in the AA sequencing hinder the cross-reaction between the specific animal antibodies and human TTR.

TRANSTHYRETIN GENE EXPRESSION

The nucleotide sequence of the entire human TTR gene, including the 5′ (transcription initiating site) and the 3′ (untranslated region) flanking regions,

has been determined (244, 290). The human gene has been localized in the long arm of chromosome 18 (306). The TTR subunit is encoded by a 6.9-kilobase (kb) gene composed of 4 exons and 3 introns (244, 290). Exon 1 contains 95 basepairs (bp) and 26 bp 5′ untranslated and codes for a 20-AA leader peptide and AAs 1–3 of the mature protein. Exons 2 (131 bp), 3 (136 bp), and 4 (253 bp) possess the coding sequences for AAs 4–47, AAs 48–92 and AAs 93–127 of the monomer, respectively (244, 290). A common sequence upstream to the 5′ cap site is highly conserved in different species and seems to play a critical role in gene expression (54). In addition, a liver-specific nuclear receptor has been discovered that can bind a distant enhancer element (55).

The TTR-mRNA spans ~ 0.7 kb and contains a 5′-untranslated region (26–27 nucleotides), a coding region (441 nucleotides), and a 3′-untranslated region (145–148 nucleotides) preceding the poly(A)tail (182, 272). Human (182, 306), rat (67, 283), and mouse (54, 305) coding regions exhibit a considerable degree of homology (~ 85%). This similarity suggests a phylogenetically preserved modulating role in gene expression. As predicted by the cDNA nucleotide sequence (182), TTR-mRNA encodes the pro-TTR-monomer (pre-TTR), a polypeptide comprising 147 AA residues whose N-terminal region is a hydrophobic signal peptide consisting of 20 AAs (142, 272). Pre-TTR undergoes a cleaving process during its migration through the endoplasmic reticulum bilayer to yield the native TTR-monomer after leakage of the signal peptide (142, 272). During the secretory process, four identical subunits agglomerate to build the mature tetrameric molecule (150).

To our knowledge, no ethnic differences are reported for TTR, in contrast to the genetically high and low TBG serum levels described in African (126) and Australian (311) populations, respectively. Some authors have reported that TTR may exhibit microheterogeneity (218). Elderly people may be subjected to amyloid organ deposits principally confined to the myocardial tissue (52). Normal TTR participates in the senescent process (314). The mechanisms whereby nonmutated TTR takes part in this systemic amyloidotic deposit are not yet clear. This involutional phenomenon is a common feature of increasing age. It is found in the heart of 25% of individuals over age 80 (314) and has also been documented for other molecules of biological importance, such as ferritin, fibrinogen, and immunoglobulin light chains.

It has been demonstrated in recent years that the monomeric subunits of TTR may undergo a number of mutations consisting of the substitution of a single residue at different positions of the normal AA sequence. Andrade et al first described the clinical anomaly (9). At least 38 different variants have been identified (Table 1), all of them generated by a single mutation point in the coding region of the TTR gene. Some mutant forms (Ser-6, Arg-102, and Thr-109) were fortuitously discovered because they remain asymptomatic and

apparently nonpathogenic (5, 80, 186). Most reported TTR mutations are associated with varying degrees of extracellular deposit of amyloid fibrils in different tissues. Familial amyloidosis covers a wide range of inherited diseases with autosomal dominant transmission. The variable penetrance explains the heterogeneity of clinical manifestations whose extreme poles are (*a*) prominent cardiomyopathy, as seen in patients with Thr-45 (243), Ile-50 (202), Ala-60 (307), Leu-68 (6), and Met-111 (203) mutations, and (*b*) the familial amyloidotic polyneuropathy (FAP) entity identified by Costa et al (53) and found in patients with Met-30 (242, 285), Ala-30 (146), Leu-33 (111), Pro-36 (145), Leu-64 (121), Tyr-77 (308), and Ser-84 (73) mutations. The TTR-Ile-122 (102) is the sole amyloidotic mutation characterized by recessive inheritance. All known carriers of this last allelic form belong to the same African-American racial group, a finding consistent with the concept of a de novo mutation arising from a single founder (140). This senile systemic amyloidosis constitutes a distinct entity whose clinical expression, mainly cardiac, does not develop before age 70 (102, 140).

Table 1 indicates that exon 1 has not been incriminated thus far as a site of a point mutation and that six different AAs situated at positions 33-Phe, 47-Gly, 49-Thr, 50-Ser, 58-Leu, and 84-Ile of the normal monomeric sequence may diverge from normalcy along two distinct substitutive pathways, whereas 30-Val may undergo three different replacing processes.

The vast majority of the reported mutations remain very rare entities. The most common molecular defect leads to the substitution of methionine for valine at position 30 of the TTR monomeric sequence (72, 242, 285). The anomaly stems from a guanine to adenine replacement in the first codon, which creates a cleavage site in exon 2. The variant reaches its highest prevalence in Portuguese siblings but is also found in Swedish, Japanese, and Mediterranean families (72, 242, 285). Clinical symptoms are quite similar within these various kindreds and are identified as Type I FAP. Heterozygous siblings biosynthesize both normal and mutated TTR in varying proportions. The inherited abnormality is expressed very early in development (200), which allows prenatal detection by chorionic villus sampling. Homozygous FAP is a rare condition that has nevertheless been reported in some kindreds (118).

From a functional point of view, TTR variants involved in amyloid disorders may exhibit normal, increased (80, 186), or reduced (229) Ka for thyroid hormones. Distortions in Ka values presumably depend on the degree of interdependence between the defective molecular domain and the hormonal binding sites. There is no effective form of therapy for the inherited mutation, which implies that genetic counseling of the asymptomatic carriers is mandatory in exposed families. Liver transplantation in FAP patients is accompanied by clinical improvement (119), but the long-term outcome of this substitutive surgery remains to be evaluated.

Table 1 Transthyretin mutated variants described in the literature

Encoding exons	Location and nature of AA substitution	Ethnic origin	Reference
Exon 2 (44 AAs: 47←4)	6: Gly → Ser	Nonamyloidotic	80
	10: Cys → Arg	Hungarian	291
		FAP I (Portuguese, Mediterra-	72, 242, 28
	30: Val → Met	nean, Swedish, Japanese)	
	30: Val → Ala	German	146
	30: Val → Leu	Japanese	195
	33: Phe → Ile	Polish-Jewish	194
	33: Phe → Leu	Polish-Lithuanian	111
	36: Ala → Pro	Greek	145
	42: Glu → Gly	Japanese	293
	45: Ala → Thr	Irish-Italian	243
	47: Gly → Arg	Japanese	191
	47: Gly → Ala	Italian	79
Exon 3 (45 AAs: 92←48)	49: Thr → Gly	Jewish	220
	49: Thr → Ala	Italian	7
	50: Ser → Arg	Japanese	293
	50: Ser → Ile	Japanese	202
	55: Leu → Pro	Dutch-German	141
	58: Leu → His	Maryland-German	199
	58: Leu → Arg	Japanese	240
	60: Thr → Ala	Appalachian	307
	61: Glu → Lys	Japanese	258
	64: Phe → Leu	Italian	121
	68: Ile → Leu	German	6
	69: Tyr → His	Scottish-English	326
	70: Lys → Asn	German	138
	71: Val → Ala	French	18
	77: Ser → Tyr	Illinois-German	308
	84: Ile → Ser	FAP II (Indiana-Swiss)	73
	84: Ile → Asn	Italian	264
	89: Glu → Gln	Sicilian	7
	90: His → Asn	Italian	262
Exon 4 (35 AAs: 127←93)	102: Pro → Arg	Nonamyloidotic	5
	109: Ala → Thr	Nonamyloidotic	186
	111: Leu → Met	Danish	203
	114: Tyr → Cys	Japanese	292
	116: Tyr → Val	French-Canadian	281
	119: Thr → Met	German-Scandinavian	113
	122: Val → Ile	African-American senile systemic	102

PHYSIOLOGY AND METABOLISM

When the TTR molecule was first identified in the CSF (88, 148), its physiological function was unknown and was thus referred to as "component X" (88). Subsequent studies confirmed the presence of component X in both CSF and serum (278). At the time, the protein was characterized solely by a fast electrophoretic migration rate. Located just in front of the albumin band, TTR was the most anodal of all CSF and serum-moving proteins (88, 278). Soon thereafter, Schultze et al isolated component X from human serum (250). Its purification and chemical analysis led the German authors to recognize one of the salient characteristics of the protein, i.e. its relatively high richness in tryptophan. Hence the name "Trp-rich prealbumin," proposed by this working group (250).

The functional properties of TTR were partially disclosed in 1958 when Ingbar demonstrated its transport capacity for thyroxine (123), thus justifying the name of "thyroxine-binding prealbumin" (TBPA) given at that time. However, the appellation appeared somewhat restrictive when it became obvious that TBPA also conveyed triiodothyronine (60). Its inadequacy was further reinforced after the demonstration that TBPA was the unique vehicle bound to RBP within the RCC edifice (149). The name "transthyretin" given to the protein by the International Nomenclature Committee (98) emphasizes TTR's concomitant involvement in the transport of both thyroid hormones and of retinol. All previous names registered over four decades had pinpointed electrophoretic or chemical peculiarities of the molecule. As a result of advancing knowledge, the now universally accepted term "transthyretin" stresses the dual physiological roles played by TTR in vertebrate species.

Distinct secretory mechanisms govern the liver production of both TTR and holo-RBP. In particular, the hepatic release of holo-RBP (but not its synthesis) critically depends on the retinoid status (270). Pulse-chase studies using ^{35}S-labeled methionine incubated with isolated hepatocytes have shown that both carrier proteins are first identified in the endoplasmic reticulum and later transported to the Golgi complex prior to extracellular delivery (85). Like pre-TTR (142, 272), native RBP is processed through a precursor form of larger MM (273). The maturation of this pre-RBP is similarly characterized by the cleaving release of the signal peptide, which yields the physiologically active monopeptide as an end product (273). Both TTR and holo-RBP strongly coalesce in the bloodstream in a 1:1:1 equimolar ratio. This process allows the low MM holo-RBP to stabilize and prevents its rapid renal or intestinal leakage. The biological half-life of TTR is about two days (271), whereas that of holo-RBP within the RCC fluctuates and is about half a day (216). After the uptake of retinol by peripheral tissues, the resulting apo-RBP devoid of its ligand has a significantly shortened half-life of ~ 3.5 h (217). Immunofluoresc-

ent studies of renal tissues have confirmed the target role of the kidneys in RBP catabolism (95). In contrast to TTR, whose labeled molecules remain confined within the glomerular tufts, RBP diffuses rapidly in the tubular lumen and in intercellular spaces (95). Under normal circumstances, only minute amounts of RBP are recovered in the urinary output, which indicates that most molecules undergo tubular disintegration with subsequent recycling of their AA residues. Using animal models and radioiodinated TTR, Makover et al showed that the main site for TTR degradation is the liver, followed by muscle mass, skin, and kidneys (170).

TTR can be detected in fetal blood as early as eight weeks after conception (11). Fetal TTR likely originates from dual sources. One part seems to be inherited passively from the pregnant mother through transplacental filtration (94). The remainder results from fetal hepatic synthesis, as indicated by the presence of significant amounts of TTR-mRNA in the early developmental stages of this organ (171). The respective importance of these two TTR sources during fetal life remains to be clarified. Several groups have found diminished TTR levels in the umbilical cord of preterm neonates compared with healthy full-term newborns (23, 139, 245, 294). Increasing gestational age is accompanied by a slow and predictable rise of TTR values, which are correlated with birth weight and which proved useful in distinguishing between small, appropriate, and large-for-gestational-age infants (23, 245). The plasma concentration of TTR is age dependent, and in healthy neonates it is approximately half that found in adults (32, 129, 136, 236, 275, 294). During the entire prepubertal period, TTR increases progressively but with no differences in levels between the sexes (129, 236, 255, 275, 294). TTR increases sharply at the onset of puberty, with a more pronounced elevation in male than in female adolescents (130). The appearance of this sexual difference is determined by sex steroid hormones and maintained in the form of plateau levels during full sexual maturity (32, 130, 275), reaching 323 ± 49 mg/liter (SD) in males and 283 ± 43 mg/L (SD) in females (130). Plasma TTR concentrations begin to decline in both sexes after age 50, but along a steeper slope in elderly males than in females (130), so that a sexual difference no longer persists after the seventh decade of life. In serum from normal newborns, children, adults, and elderly persons, TTR and RBP interact with retinol in a close equimolar stoichiometry over all concentration ranges (23, 27, 99, 129, 130, 139, 217, 227, 270, 294). This interaction implies that the bulk (90–95%) of retinol in human blood circulates within the trimolecular RCC and that both apo-RBP and holo-RBP unbound to TTR represent only minor fractions.

It has been suggested that the sharp elevation of RCC at puberty could reflect an augmented demand for retinoids by peripheral tissues (227). The aforementioned influence of sex steroid hormones on TTR synthesis and/or turnover rate has been confirmed in several clinical conditions using natural or synthetic

analogs (33, 241) as well as in women taking oral contraceptives with varying proportions of estrogens and progestagens (183). The reported hepatic production of TTR has been depressed by zinc deficiency (15) and stimulated by glucocorticoids (93, 208) but remained unaffected by vitamin A deprivation (197). Several research groups have explored the behavior of TTR in intestinal, hepatic, renal, and thyroid disorders (42, 122, 267, 268, 295). All of these physiological or diseased conditions may eventually modify either the blood concentration or the equimolar ratio between the three components of the RCC. For example, this is the case in normal pregnancy characterized by a relative increase of free holo-RBP (265), which may fulfill increased retinoid requirements. In contrast, free apo-RBP is present in molar excess in kidney patients (268) and in premature infants (253), probably as the result of impaired catabolism, reduced excretion, (268) or primary lack of intrahepatic retinol reserves (212). The multifaceted functional consequences of these alterations await elucidation.

In addition to hepatic production, three other TTR synthesis sites have been identified in mammals: visceral yolk sac endoderm (266), retinal pigment epithelium (172), and choroid plexus epithelium (116). Because the blood-brain barrier is not freely permeable to thyroid hormones, for a long time investigators suspected that a locally secreted protein served as its intermediate carrier to the CSF. In situ TTR production within the central nervous system (CNS) was demonstrated by intense immunochemical staining of the organelles involved in the CSF secretory processes (3) and by the in vitro culture of human fetal choroid plexus (142). Animal experiments have shown substantial amounts of TTR-mRNA in specific CNS regions, ranging from 11 (272) to 30% (249) of the hepatic level. The highest TTR-mRNA concentration accumulates in the epithelial cells lining the ventricular surface of the choroid plexus (277) and seems to account for most, if not all, of the TTR found in the CSF (105). Although both TTR and albumin reveal very low CSF levels (~ 0.017 g/liter and 0.02 g/liter, respectively), their intrathecal ratio is ~ 30-fold higher than that found in plasma (313), which precludes their passive diffusion from the bloodstream. TTR levels remain stable in CSF between the ventricular and lumbar regions, in contrast to the increasing concentration gradients observed for albumin and immunoglobulins G (313). Considering the weight difference between liver and choroid plexus as well as the difference in the TTR concentration between plasma and CSF, it is assumed that the synthetic rate of TTR by the choroid plexus would need to be 13 times faster (249) to reach its usual intrathecal values, which represent ~ 20% of total CSF proteins (313).

The production of TTR in the liver and in the choroid plexus is regulated independently, notably during malnutrition and inflammatory processes (68, 304). Recent studies have proposed a transport model whereby TTR could be

involved in the uptake of thyroxine from the bloodstream and its delivery into the CSF (249). TTR thus appears to be a major hormonal carrier protein, conveying up to 80% of the intrathecal thyroxine (105). These data strongly suggest that TTR fulfills important ontogenic and functional properties in mammalian nervous structures, a concept further corroborated by the observation of its increased concentration in CSF during the neonatal period (162). The recent demonstration of intraocular RBP synthesis (173) makes it likely that local TTR (172) cooperates in the transport of retinol and in the visual cycle. The reduced solubility of the TTR variants could explain the frequent ocular damage in patients suffering from amyloidotic disorders (264).

At least two other potential physiological properties have been attributed to TTR. The first is related to the discovery of a binding site with a high Ka for epinephrine (65). The other is based on the description of a persisting thymic hormonal-like activity of thymectomized mice (37). This functional effect seems to be intrinsic to the TTR molecule and has been assigned to the first 10 AA residues of the TTR monomeric sequence (38). Thymulin is a zinc-dependent nonapeptide involved in the maturation and replication of the thymic T-cell lines (12), whose production patterns are markedly disturbed in protein-depleted states (46, 302). Finally, TTR has a sequential homology with several gastrointestinal hormones of the glucagon-secretin family (147), whose phylogenetic and biological significance remains unclear. Nevertheless, these molecular approaches raise new questions about the relationships between alterations of nutritional status, immune responses, regulatory peptides, and biogenic amines.

TRANSTHYRETIN IN NUTRITIONAL SURVEYS

Significant alterations in the levels of protein and calorie intakes by animals and humans affect protein turnover, synthesis, and breakdown. This situation entails a number of adaptive mechanisms in the body's N economy. Specifically, the visceral compartment reacts quickly to alterations in nutrient supply. Hence, clinicians have drawn attention to several visceral markers, such as serum-albumin (SA), transferrin (Tf), TTR, RBP, and transcortin (corticosteroid-binding globulin, CBG), which are regarded as potential indicators of this compartment. This conclusion relies on the assumption that, in uncomplicated fasting or starvation, fluctuations of the visceral protein compartment precede and reflect an overall but slower change in the total body protein.

The first proposal using TTR as an index of protein and energy malnutrition (PEM) appeared in *Lancet* 20 years ago (125). The study was performed on 40 PEM children hospitalized in Dakar (Senegal, West Africa) using radial immunodiffusion and specific anti-TTR antibodies. The micromethod only required a few microliters of serum drawn from the fingertip at weekly intervals

throughout the refeeding period. The proposal of TTR as a nutritional index was based on the observation that the drop of both TTR and SA demonstrated comparable restoration during dietary protein replenishment. Nevertheless, the rates of recovery of both biochemical indices revealed striking differences, showing that TTR was characterized by a faster response (125, 127). The persistence of a TTR:RBP:retinol molar ratio along all steps of nutritional depletion and recovery despite significantly different biological half-lives led Ingenbleek et al to propose that the level of TTR synthesis by the liver was the primary factor responsible for the peripheral retinol status (127). This view was recently confirmed under experimental conditions using a mutant mice model carrying a disruption at the TTR locus (74). This finding may also explain why overlooking the crucial role of TTR in vitamin A metabolism resulted in unachieved or even misleading conclusions.

The peculiar potential of TTR to detect subclinical PEM was attributed to the conjunction of at least three factors: its production by the liver and early response to nutritional deficits, its short biological half-life, and its unusual richness in the indispensable Trp AA (IAA) (125, 127). The first two points are broadly accepted, but the last one remains to be validated. Substantial advances in knowledge have been recorded over the last two decades in relation to protein and energy metabolism under conditions of health and uncomplicated malnutrition. Here we pinpoint some salient features to pave the way for a deeper scrutiny of more complex situations, especially those in which nutritional and inflammatory stresses coexist. These approaches constitute the prerequisite for a more objective assessment of nutritional status and nutrient requirements, taking into account the role of sex, age, and varying physiological and diseased states.

Protein synthesis is usually considered to start with the transcription of DNA into mRNA. The production of protein molecules is achieved by the ribosomal machinery, using 20 AA residues as building blocks. Several nutritional variables, working in concert or independently, may modulate the operative mechanisms at a translational (276), transcriptional (193), or posttranscriptional (320) level. Most energy-dependent processes (synthesis, folding, secretion, transport, and breakdown) associated with protein turnover and culminating in functional properties have been clarified progressively over the last few years (81, 323, 325). The sensitivity of body N to changes in energy intake has been recognized for many decades (40, 188), and this topic has been the subject of recent reviews (76, 322, 325). Nevertheless, there is a continuing uncertainty about the most effective sources and levels of energy-yielding substrates and the proportion among these for the support of protein anabolism. Although it is beyond the scope of this review to examine these aspects in detail, we must note that glucose functions as a major substrate under a wide range of pathophysiological conditions (246). If carbohydrate intake is re-

stricted, glucose must be synthesized by gluconeogenesis, primarily from the AAs present in endogenous and dietary proteins. The direct provision of fatty acids alone appears ineffective in stimulating protein synthesis in rat hepatocytes (320) or in improving N sparing in fasting patients (24, 188). The beneficial effects of triglycerides as an energy substrate source on protein synthesis operate through the energy released by the β-oxidation of free fatty acids (used in part to promote glucose production from lactate within the Cori cycle) (39) or by the hepatic conversion of their glycerol moiety into glucose (34).

The relative proportion of dietary AAs and energy-yielding substrates that most effectively supports protein synthesis is currently a field of active investigation, and TTR is clearly at the cutting edge of the controversy. Rat experiments have established that both protein and energy restriction inhibit the generation of cytosolic TTR-mRNA (61, 165), thus resulting in the expected drop of plasma TTR to below control values (77, 303). The current opinion is that the rate of hepatic protein synthesis is critically dependent on appropriate energy provision and correlated to the bioavailability of individual free amino acids (87). Clinical surveys in fasting obese patients led some investigators to believe that energy supply rather than protein intake was the major determinant of visceral protein synthesis (256), whereas others have concluded that dietary protein is of greater significance when studies last for as long as 98 days (35). These apparent contradictions can be reconciled if TTR is an indicator of the protein metabolic status of the visceral compartment, as discussed in greater detail below. This concept is supported by the observation of Moscowitz et al in preterm infants given varying dietary energy:protein ratios (185). They concluded that although the AA supply is the main factor for maintaining protein synthesis, it can be affected by fluctuations of energy intake (153). Furthermore, this concept corroborates previous data showing that the visceral protein synthetic rate may be maintained at a higher level by glucose-free AA than by N-free dextrose preparations (263) or that the N balance may be preserved despite severely restricted energy dieting (214, 323). The former condition necessitates a higher-than-normal proportion of dietary AAs, which undergo deamination and gluconeogenic conversion to replace the lacking fuel. This increased AA oxidation corresponds to a form of nutritional wastage, which augments the N cost of protein synthesis, as documented by an increased urinary excretion of urea.

The protein-sparing effect of AAs in the absence of an adequate energy supply depends on the disposal of AAs themselves (103) and is unrelated to changes in insulin (84) or in fat mobilization (239). Piecing these elements together, declining TTR plasma levels appear to be the direct consequence either of an insufficient and/or inappropriate AA regimen or of a metabolic state that alters the balance between protein synthesis and degradation. The

former explanation seems likely for phenylketonuric children (254) and the latter for diabetic patients (89) with an inadequate temporal pattern of therapy. Under such circumstances, the decline in TTR plasma values may be blunted to some extent or, on the contrary, further depressed by increases and/or decreases in dietary energy. These modulating effects generated by glucose on TTR production are assumed to be dependent on its ability to redress a disrupted N/energy balance, which allows the recovery of a proportion of IAAs that would otherwise be degraded via gluconeogenic processes. Here we recall the pioneering studies, notably those of Lavoisier, Prout, Chevreul, and Liebig, initiated at the turn of the nineteenth century, which led to the identification of the major nutrient classes and of the main chemical constituents of carbohydrates, fats, and proteins. Shortly thereafter, Claude Bernard became one of the first investigators to establish a link between nutrient categories by ascertaining that "lipids are burning in the flame of sugars" (19). We consider that the measurement of rapidly turning over proteins for nutritional evaluation purposes expands upon the earlier concept by also asserting that "the synthesis of proteins occurs in the flame of sugars."

In this metabolic context, the potential role of Trp is of peculiar interest. A number of basic considerations are worth pointing out. First, Trp accounts for the lowest concentration of all IAAs in usual foodstuffs and in mammalian tissues (189). Second, it was demonstrated early in this century that adult rats given a Trp-deficient diet rapidly lost weight at a rate similar to that observed with a protein-free regimen (211). Third, Trp can reaggregate polyribosomal subunits of protein-depleted cells, thereby restoring the protein-synthesizing machinery (260). Fourth, as mentioned above, the AA sequence of both TTR and RBP is characterized by an unusually high Trp content (150, 226). Hence, it is tempting to speculate that the production of these Trp-rich carrier proteins (125, 127) is particularly sensitive to food protein restriction. The concept that Trp shortage might serve as a limiting factor for TTR synthesis was recently tested in vivo in growing rats submitted to acute and severe Trp depletion and compared with pair-fed and control groups (29). This experiment failed to confirm the foregoing hypothesis, but the conclusion drawn by these investigators may not be valid because the three animal groups exhibited divergent growth rates. Consistent with previous findings (211), the Trp-deficient group alone underwent dramatic food intake and body weight reductions, which implies substantial tissue (mainly muscle mass) depletion. Because ~ 80% of the AAs released by protein breakdown may be recycled (322), observed data (29) might well compensate for the reduced Trp intake so as to maintain unaltered protein synthesis rates. Obviously, our concept deserves further investigation.

Excluding TTR, the most commonly measured indicator of the visceral protein compartment is SA, the oldest of all biochemical markers. SA is the

primary determinant of colloid osmotic pressure. It has a low-affinity/high-capacity binding potential for numerous substances, a prolonged biological half-life of ~ 20 days, and a large distribution space (215, 237). Many clinical studies have shown that SA plasma values are correlated to the severity of protein malnutrition and to that of immune deficits, which makes SA an excellent predictive tool of hospital survival (117, 192). However, the clinical usefulness of SA is limited by its inability to identify short-term alterations of nutritional status (75) and by its propensity for extravascular extravasation (82). In contrast, the rapidly turning over TTR, which is less influenced by changes in body fluids than are SA levels (297), allows the daily follow-up of diseased patients. Studies of children with protein-calorie malnutrition in Thailand have shown that TTR falls faster and further from normal than SA during the period of deprivation and is restored to normal more quickly and by lower levels of protein supplementation (1 gm/kg) than SA, which required 4 gm/kg (269). Nonetheless, the idea to combine both SA and TTR markers within a predictive scoring system (134) yielded a faithful bipolar estimate of the visceral protein compartment. This approach is supported by studies showing that the SA and TTR declining or recovering slopes remain positively correlated (86). Their combined measurement therefore provides a more reliable evaluation of the patient's nutritional status (20).

Until now, little attention was paid to the potential usefulness of TTR in assessing the nutritional status of population groups in epidemiological studies. Given the fact that TTR distributes along Gaussian curves (129), a deterioration or improvement of the overall health conditions is accompanied by a shift to the left or to the right of the Gaussian means, with a higher degree of significance than that of most other biochemical and anthropometric measurements of nutritional status (129).

TRANSTHYRETIN IN ACUTE STRESSFUL CONDITIONS

The metabolic consequences of acute stressful conditions have been extensively investigated over the past decades. These disturbances, which include accelerated gluconeogenesis and oxidation of carbohydrates, fatty acid mobilization, and protein synthesis and degradation (97, 168, 315), involve all compartments and functions of the stressed body in several ways. These metabolic changes are distinct from those associated with severe food protein and/or energy restriction alone. Regardless of the causative factor (major injury, trauma, sepsis, burns, cancer cachexia), the stress reaction manifests marked similarities and is typically accompanied by fever, anorexia, muscle wasting, and weight loss. The accompanying hormonal secretory pattern is characterized by rises in the counterregulatory hormones (glucagon, cortisol, adrenaline) and by end-organ insulin resistance (83, 280). Despite quantitative

differences, a qualitative analogy between the reacting responses points to a unifying concept of the stressful condition.

The structural protein pool, mainly in the skeletal musculature, serves as a critical source of AA residues and of metabolic fuel of the stress response. Cuthbertson was the first to show in humans that stress is characterized by a negative N balance (59) whose magnitude is proportionate to the severity of the insult. The classical N balance results from an aggregate of many variables and therefore cannot reveal these specific changes in interorgan substrate fluxes. Nowadays, more sophisticated methods, such as whole-body and specific organ protein turnover methods using labeled tracers, have improved these approaches for dissecting out the various components of whole-body kinetics (324). The current opinion is that, under stressful conditions, protein turnover is stimulated as a result of both augmented tissue proteolysis (mainly in the muscle mass) and specific tissue protein synthesis (mainly in the liver) (143). Protein breakdown releases AA residues, which are preferentially conveyed and incorporated into the hepatic precursor pool involved in the secretion of acute-phase proteins (APPs) and other defense systems (17, 115, 161). However, the rate of protein degradation generally exceeds the rate at which AAs are used for protein synthesis (143), thus yielding a net negative N balance substantiated by rising amounts of urea (59, 179) and of other N catabolites (45) in the urine.

About 15 different mediators, primarily secreted by activated leukocytes and collectively termed cytokines, are implicated in the acute phase response. It was recognized originally that cytokines were responsible for the febrile reaction (69), for muscle proteolysis (50), and for a broad spectrum of synergistic and/or antagonistic effects that may ultimately either reinforce or deteriorate the immune processes of the stressed individual (286). Interleukins 1 and 6 (IL-1, IL-6) and tumor necrosis factor-α (TNF-α) appear to play key roles in the inflammatory reactions. For example, IL-1 (17,500 Daltons as MM) strongly stimulates an increase in whole-body AA flux (319), the oversynthesis of α1-acid glycoprotein (AGP, orosomucoid) (90), and the early rising production of cortisol by direct adrenotropic (234) or indirect hypophysotropic (21) activation. IL-6 (26,000 Daltons as MM) is the major mediator for the secretion of most other hepatic APPs, especially that of C-reactive protein (CRP) (91). TNF-α (17,000 Daltons as MM) favors muscle proteolysis through the mediation of glucocorticoids (106) as well as glucagon-induced hyperglycemia and AA uptake by the liver (309).

The peak of N urinary excretion culminates within days three to five after the initiation of acute injury (59) and coincides with the nadir recorded for the N negative balance (120) and for the TTR-RBP blood values (151, 225). When the stressful condition subsides—provided that appropriate nutritional support is offered—both N balance (120) and TTR-RBP levels rejoin the physiological

range within a couple of days (133, 225). In contrast, septic and metabolic complications or inadequate dietetic management results in persistent N loss and subnormal TTR-RBP plasma concentrations (120, 133, 321). Thus the evolutionary patterns of N urinary output and of plasma parameters are mirror images of each other, which suggests that the rapidly turning over proteins could eventually reflect the losses of N from the mobilizable endogenous N pools (133). Experimental data using hepatocyte models have confirmed that the molecular alteration triggered by cytokines is situated at transcriptional level, which results in the cytosolic increase of mRNA levels of several APPs, whereas mRNA concentrations of visceral proteins were significantly depressed (66, 187, 190, 252).

The distortion from normal serum values of proteins such as SA and TTR closely parallels the changes in their intrahepatic mRNA levels, which implies that their rates of protein synthesis were reset at new thresholds of priority during the course of inflammation (66, 187, 190, 252). Recent studies have unraveled the molecular mechanisms that control the hepatic production of both inflammatory and visceral proteins (2, 137). The induction of APPs is mediated by an IL-6 nuclear factor (IL-6-NF), which has a high degree of homology to the C/EBF-NF responsible for the expression of visceral markers. Both IL-6- and C/EBP-NFs competitively recognize and promote the same DNA responsive element of the IL-6 gene. Under physiological conditions, in the absence of cytokine stimulation, the visceral indices benefit from preferential production while IL-6-NF is not expressed, which explains the maintenance of APP-mRNAs at low or undetectable cytosolic levels. In contrast, acute stress is characterized by cytokine-induced stimulation of IL-6-NF, which entails a reciprocal shift with enhanced generation of APP-mRNAs and concomitant downregulation of SA- and TTR-mRNAs (2, 137).

At first sight, and from a purely mechanistic point of view, the drop of the short-lived plasma visceral proteins as a result of stress appears to be an inevitable fate poorly modulated by nutritional therapy. Hence, the term "negative APPs" has been suggested in this context to imply that they are inert and passive visceral markers that lose ipso facto any nutritional significance. Ingenbleek has expressed a contrary opinion: that the so-called negative APPs are directly and causally involved in the establishment of all stages of the stress reaction (133). A growing body of recent data suggests that the latter view is especially valid for TTR (133), but we assume that it should be extended to RBP and to CBG. This conclusion is based on the "free hormone hypothesis," which was originally envisaged by Recant & Riggs four decades ago (228), structured within basic considerations by Robbins & Rall (232), and recently extended by Mendel using complex mathematical approaches (178). According to the theory, the following successive major steps should be highlighted: (*a*) The biological activity of a given hormone or vitamin transported by carrier

protein(s) is dependent on its unbound and freely available fraction. (*b*) Any abrupt decline in the concentration of the carrier protein entails the spontaneous dissociation of the protein-ligand complex following the law of mass action and the dissociation constant (Kd) of the ligand. (*c*) The delivery of rising amounts of freed ligand molecules into the extracellular spaces proportionally increases their fractional tissue uptake as well as their intracellular disposal rate.

The "free hormone hypothesis" appears to rest on firm ground in the field of both thyroid hormones (175, 176), whose three specific carrier proteins act as buffering reservoirs, thereby allowing the uniform distribution of the hormones among all cells of each irrigated tissue (174). Under steady-state conditions, the minute amounts of free thyroxine (FT4) and free triodothyronine (FT3) available to cells represent only 0.02 and 0.3%, respectively, of the total hormonal concentrations (233). Any acute stressful condition is typically characterized by a "low T3 syndrome" with depressed total and free T3 fractions and elevated reverse T3 (rT3) arising from peripheral impaired monodeiodination of T4 to T3 (131). In contrast, total T4 and TBG values are usually normal or minimally reduced, whereas TTR levels decline as a result of cytokine-induced depressed synthesis (66, 190). This new protein-hormonal equilibrium causes a sudden surge in FT4 concentrations followed by an immediate shift from plasma to tissues (178, 232).

The liver, which may harbor in a nonphysiologically active form as much as 40% of the total extrathyroidal T4 reserves (44), may contribute to the feeding of the FT4 pool (112) instantly taken up by cells before feedback mechanisms can intervene. Under these circumstances, most clinical studies have documented that TSH remains unaffected, with FT4 levels situated at the upper range of normalcy or slightly elevated (221, 284). However, direct plasma measurement largely underscores the magnitude of cellular impregnation by FT4. The increased urinary excretion of FT4 (225) certainly provides a better estimate of its increased extracellular availability. From the thyroid function point of view, acute stress is thus a very complex and dichotomic situation characterized by an overall downregulation of organs not directly involved in the stress reaction. This observation correlates with the relative refractoriness of T3 cytosolic and nuclear binding sites of these organs, which teleologically seek to spare the N body reserves (300). In contrast, the liver and other tissues, such as white blood cells (317) (which actively contribute to defense systems, immune responses, and wound healing), reveal strong overstimulation. The peripheral turnover of thyroid hormones may be augmented severalfold in bacterial sepsis (104) and parasitic infestation (310), an outcome consistent with their increased consumption and thermogenic effects (17, 168) at cellular level. The balance between these two down- and up-regulated influences presumably depends on the nature of pathogens and the

proportion of affected cells in the stressed body, which explains the disparity of metabolic responses.

Recent years have witnessed substantial advances in knowledge of the multifaceted actions of the thyroid gland. Most authors agree with the view that the FT4 concentrations in the extracellular fluids appear to be the principal determinant of peripheral thyroid status, thus ensuring the fine-tuning retrocontrol of the endocrine organ secretory rate (131). In contrast, intracellular FT3, whose bulk is generated by tissue FT4 monodeiodination, appears to be the major metabolically active hormonal compound (131, 210). The cellular sites of actions for thyroid stimuli remain a field of intense research. Although not excluded, the previously proposed cytosolic targets, such as plasma membrane, enzymes, or mitochondrion, have lost some consistency in recent years (210). Some cytosolic sites of action have been confirmed (166), but most surveys contend that the main hormonal effects are situated at nuclear level and related to the selective regulation of specific genes (210). The occupancy of hepatic nuclear receptors by FT3 is a direct and very rapid second-order process (209). The radioautographic study of the two-dimensional activity profile of mRNA translational products indicates that some of them are stimulated while others are depressed, according to the thyroid hormonal status (251). This dualistic expression of hepatic genes was confirmed recently in a human hepatoma cell-line culture, thus revealing an increase in AGP-mRNA levels that contrasts with the decrease in TBG-mRNA values (158). In such hepatocyte models, the saturation kinetics of T3 nuclear receptors may be reached in less than 2 min (209), causing a significant elevation of the AGP-mRNA concentrations as early as 30 min after the addition of T3 to the incubation medium (156).

Under steady-state conditions, the plasma free retinol fraction does not exceed 5% of its totally bound concentration (100). During the onset of acute stress, the drop of RBP parallels that of its counterpart protein (151, 225). As shown for TTR and T4, the new RBP-retinol mass equilibrium probably causes release into the extracellular space of increasing amounts of free vitamin becoming available to cells. Once again, the kidneys prevent excessive accumulation of the potentially toxic free retinol (225), whose urinary leakage reflects the augmented extracellular pool.

Vitamin A is required for vision, growth, and reproduction as well as for the maintenance of differentiated epithelia and mucus secretion (27, 99, 217, 227). Moreover, vitamin A is deeply involved in the activation and modulation of the host defense systems (64, 318). However, interpretation of these aspects is complex and must be cautious because *all-trans*-retinol undergoes oxidation to form *all-trans* retinoic acid, the principal ligand to the vitamin A–related genomic receptors. In addition, other retinoids sharing with retinol common functional properties except in vision and spermatogenesis are formed. The

delivery to target organelles of these retinoid compounds endowed with high morphogenic and cytotoxic potency is finely regulated by several membrane, cytosolic, and nuclear-specific receptors (167, 206). Nevertheless, rapid and spontaneous dissociation of retinol from plasma RBP has been reported (204). Using cultured animal keratinocytes, the uptake of freed retinol is many times more rapid than the release of retinol by cell-surface receptors for RBP (57). Moreover, maximal intracellular accumulation and biological effects of free retinol delivered to human keratinocytes are recorded after 3 h, in contrast to the very slow results obtained with holo-RBP (58). The free hormone/vitamin hypothesis predicts that retinol released in free form would favor rapid transmembrane entry under acute stressful conditions.

Vitamin A compounds are thought to modulate the activity of several factors that mediate growth and immunity by two distinct major pathways (62). The first is cytosolic and is situated at ribosomal level, where retinoids promote the incorporation of carbohydrate residues into native glycopeptidic chains. The second is nuclear and relates to the description of at least three specific binding sites (159) controlling the transcription of genomic products. For example, physiological concentrations of vitamin A stimulate the production of interleukins (288), proteins (110), APPs (13), and enzymatic molecules involved in the host defense systems (49, 135). Conversely, vitamin A deficiency is responsible for the defective glycosylation of two rat APPs, α1-macroglobulin (154) and fibronectin (155). Like thyroid hormones, vitamin A supplementation may exhibit ambivalent properties and depress the synthesis of secretory products such as interferon-γ (43).

The collective data strongly suggest that both TTR and RBP, as a result of acute suppression of their hepatic synthesis, actively participate in the onset of stress reactions and therefore deserve to be described as "acute-booster reactants" (ABRs). The delivery of free thyroid and retinoid compounds, readily available to cells by simple diffusion, makes it possible to initiate the positive and negative regulatory controls associated with injury. The magnitude and duration of this transient hyperthyroid and hyperretinoid status depend on the decrement between pre- and poststress plasma TTR-RBP levels undergoing spontaneous dissociation and freeing all of its bound ligands. This release persists as long as TTR-RBP drops and appears to be proportionate to the severity of stress and to the degree of negative N balance. However, initiation of these inducing steps does not exceed the four or five days necessary to reach the nadir of TTR-RBP, which coincides with the peak of urinary excretion of N catabolites.

These results explain why an optimal nutritional status, as assessed by TTR-RBP levels within the normal range, confers to the stressed body the metabolic advantage of developing appropriate responses of higher magnitude and longer duration. Conversely, a preceding nutritional status characterized

by deterioration and subnormal TTR-RBP plasma levels might be accountable for an overall impairment of the aforementioned thyroid- and retinoid-dependent processes in infected or stressed PEM subjects. These abnormalities are likely to aggravate and to interact with the other multiple immune deficiencies directly generated by the inhibition of cytokines in protein malnutrition (22, 157). The data argue strongly for early recognition and correction of PEM states by appropriate nutritional management in order to help the stressed body surmount the harmful consequences of malnutrition. Clinicians must be aware that anthropometry greatly underestimates the importance of protein depletion and that biochemical parameters constitute earlier and more useful indices for valid nutritional assessment and therapeutic purposes.

TRANSTHYRETIN IN CHRONIC STRESSFUL DISORDERS

The complex variety of metabolic effects induced by thyroid hormones and retinol during acute stages of stress cannot be dissociated from those triggered by cortisol. These active compounds are transported by specific carrier proteins and exhibit similarities in their evolutionary patterns during the stress response. Once released in free form into the bloodstream, physiologically active T4, retinol, and cortisol may be taken up by peripheral cells, a process that entails a cascade of intracellular reactions ultimately regulated by the same superfamily of nuclear receptors (205). Nevertheless, cortisol has additional specific activities aimed at strengthening the stress reaction and preventing undesirable consequences, which explains why it is predominantly operative in chronic situations.

CBG is a glycoprotein (42,650 Daltons as MM) secreted by the liver (312). It has a unique binding site for cortisol and conveys ~ 90% of the circulating cortisol. Under normal circumstances, this protein-hormonal complex stands in equilibrium with ~ 4% of physiologically active free cortisol (261, 282). In contrast to TTR and RBP, CBG plasma concentrations do not show differences between the sexes and are only modestly influenced by dietary manipulation (8). However, like TTR and RBP, CBG suffers an acute suppression in its hepatic synthesis during inflammatory disorders (223, 327). The sharp decline in CBG plasma levels thus coincides with the aforementioned cytokine-induced overstimulation of both pituitary and adrenal glands (21, 234), which leads to increased rates of cortisol production (14). Owing to the strikingly divergent alterations observed for each component of the carrier-ligand system, a greatly increased proportion of free cortisol is released into the extracellular space, where it is readily available to cells. The enhanced supply of unbound hormone appears to be correlated to the severity and duration of the stressful condition (17, 280) and reaches many times the normal tissue requirements

(316). The direct measurement of cortisoluria also reveals greatly augmented values (14), which reflect, once again, the enlarged circulating free pool and kidney overflow (184). This intense hormonal response does not exceed a few days and subsides after one week in elective surgery (184) but may be sustained for a prolonged period in situations characterized by chronicity, complications, and/or relapses, e.g. burns (316). The latter clinical context is typically delineated by a further glucocorticoid-induced depression in CBG plasma values (248), which implies that chronic inflammatory disorders are evolving towards a generalized, self-sustained stage of hypercorticism.

Because rapid plasma dissociation and liver unidirectional influx rates have been reported for cortisol (282), it seems likely that the free hormone hypothesis could apply to this compound (177). The metabolic effects of cortisol are mediated by the activation of adenylate cyclase (235) and/or by the binding to cytosolic and nuclear receptors (238). The dual mode of action already reported for thyroid (158, 251) and retinoid (43, 288) molecules also characterizes glucocorticoid activity, which may boost the production of some APPs (such as AGP) (298) or enzymes (such as tyrosine aminotransferase and alkaline phosphodiesterase I) (238). Conversely, glucocorticoids may exhibit well-established negative regulatory capacities (1, 279). However, there is a striking difference between thyroid/retinoid components, which operate as true inducers or repressors of the stress reaction, and steroid hormones, which behave as secondary modulators of cellular activity. This difference stems from experimental studies in which the enhanced de novo accumulation of AGP-mRNA and of some other APPs also develops in adrenalectomized rats after turpentine-induced inflammation (16). This investigation demonstrates that steroid-dependent processes require primary effector systems—likely thyroid hormones in the case of AGP response (158). This cascade of molecular events is consistent with the general concept that glucocorticoids function as modulating agents of primary transcripts (238).

In healthy individuals undergoing stress, steroidogenesis is fairly well adapted to meet the increased metabolic demands of the stressed body (301). This response seems to fulfill teleological purposes and is vital for survival because both excessive and defective pituitary-adrenocortical responses have adverse prognosis significance (259). This view is corroborated by the discovery of a structural analogy between CBG and several components of the serine-proteinase inhibitor (serpin) superfamily, notably with TBG and α1-protease inhibitor (A_1-PI) (107). Both CBG and A_1-PI interact specifically and offer excellent substrates for neutrophil elastase on the surface of activated macrophages (109). Enzymatic cleavage of CBG leads to conformational changes and disruption of its steroid binding site, followed by a 10-fold drop in the Ka of cortisol (213). This process should favor the selective delivery of cortisol at the site of inflammation (108), thus locally

reinforcing the hypercortisolemic background of the injured tissues. Glucocorticoids play simultaneously different roles in preventing healthy tissues from the destructive potential of cytokines (70, 289) and from the overactivity of nitric oxide synthase (92) exacerbated in inflammatory disorders (181). Depending on their concentration, duration of cell exposure, and metabolic environment, the net biological effects of cytokines may ultimately exert beneficial or deleterious effects on the host (286). The inactivation of A_1-PI and of other serpin components, which represent more than 10% of the total protein in human plasma (287), allow the serine proteinases to operate more efficiently in the inflammatory context and thereby contribute to the preservation of N endogenous pools triggered by T3 downregulation (300). The low TBG plasma values characterizing seriously ill patients (114) might well result from the combined effects of both defective synthesis (126, 158) and enzymatic cleavage (213) and thus contribute to increased turnover and disposal of thyroid hormones in PEM (132) and inflammatory states (104, 310).

In these complex chronic situations in which the detrimental effects of both malnutrition and stress are joined, the drop in visceral proteins may be regarded as the sum of both nutritional and inflammatory injuries. In this context, no single parameter fulfills the scoring task, and the correct appraisal of each causal factor requires a simultaneous measurement of indices reflecting all of them (134). In an attempt to encompass these two interwoven aspects of the disease spectrum, a predictive scoring system has been proposed that would allow (regardless of sex and age) the stratification of critically ill patients by risk of complications or death (134).

$$\text{PINI} = \frac{\text{CRP (mg/liter)} \times \text{AGP (mg/liter)}}{\text{SA (g/liter)} \times \text{TTR (mg/liter)}}$$

Thus this prognostic inflammatory and nutritional index (PINI) is a quotient that aggregates the product of the most reliable indicators of protein status with that of CRP and AGP. These last two APPs were selected after stepwise discriminant analysis of seven inflammatory markers clinically employed in current use (134). The PINI scoring system yields values below 1.0 under normal circumstances but that rise above this baseline as the overall health status deteriorates. Values exceeding a threshold of ~30 reflect severely morbid and/or life-threatening conditions. The choice of both CRP and AGP was largely due to their biological complementarity, which ensures the broadest coverage of the inflammatory reaction. On the one hand, AGP is one of the most glycosylated of all known glycoproteins, and its hepatic overproduction is predominantly set in motion by IL-1 (90). AGP may be regarded as a slow-reacting marker ($T_{1/2}$ = 5–7 days) whose intracellular maturation process necessitates a reduction in size of the precursor form (257), which yields a

primary transcript characterized by increased stability (298). These events may be influenced by thyroid (158) and steroid (298) hormones. On the other hand, CRP is an unglycosylated pentaxin barely detectable in normal blood and whose hepatic synthesis is mainly initiated by IL-6 (91). This APP is an exceedingly fast responder ($T_{1/2}$ = 4–6 h), and its production reportedly is facilitated in the course of the stress reaction (169). On the basis of available molecular data, we assume that this enhanced secretory process of CRP should be inversely correlated to the drop in ABR levels and therefore proportionate to the amount of freed ligand(s). It seems likely that these released mediators would act as repressors of the inhibitory control of CRP secretion (but not of CRP synthesis) (169).

Within the PINI formula, CRP-TTR and AGP-SA appear as rapid and slow reacting couples, respectively. They respond with specific kinetics along differentiated pathways to IL-1 and IL-6, which minimizes the risk of yielding a prognostic value that does not concord with the severity of the clinical condition. These response patterns are all the more important because, in contrast to the mirror image of changes in CRP-AGP vs SA-TTR levels during acute stress, each component of the PINI may evolve in a unique and independent way under chronic circumstances, in agreement with the view that immunological and nutritional indices move along uncorrelated pathways (101). This process is particularly well-documented in cancer patients in whom N balance and visceral indicators may be significantly improved as a result of appropriate refeeding, notwithstanding unchanged neoplastic burden (230). In contrast, the correct follow-up of inflammatory parameters not accompanied by that of visceral proteins incurs the risk of inadequate nutritional support and of undesirable complications. That the magnitude of the acute phase protein response may be depressed by protein deficiency both in experimental (144) and clinical (71) surveys is well established. The description of significantly lower CRP and AGP in surgical patients, in whom deep sternal infection manifests later (180), is strongly indicative of unrecognized preexisting malnutrition.

The PINI formula is presently employed on a large scale and under validation in various diseased states, notably those affecting children (222), elderly persons (4, 219), and burned (160) and trauma (299) patients. The predictive scoring system is not intended to establish any precise diagnosis; rather, its nonspecificity permits its broad applicability in most clinically significant stressful states. Advantages and limitations of this approach remain to be determined. However, the available data clearly show that the PINI system fulfills its most promising potential in identifying subclinical or marginal situations as well as complex inflammatory and nutritional disorders in protracted illnesses. It thus represents a major improvement over normal routine laboratory methods.

SUMMARY

The name "transthyretin" reflects the dual physiological roles of this tetrameric unglycosylated plasma protein. TTR is one of three specific carrier proteins involved in the transport of both thyroid hormones and of retinol through the mediation of RBP. TTR is a product of the visceral compartment, and its hepatic synthesis is exquisitely sensitive to both the adequacy and levels of protein and energy intakes—hence the proposal of TTR as a nutritional marker. To date, 38 TTR variants have been described, most of which are associated with variable degrees of cardiac and/or neural tissue amyloid deposits. All known variants arise from a single AA substitution due to single point mutation in the coding region of the TTR gene. Under acute stress conditions, the synthesis of TTR, RBP, and CBG is abruptly depressed by a cytokine-directed orchestration of new metabolic priorities, with a redistribution of organ and tissue protein pools. It is proposed that TTR, RBP, and CBG behave as acute-booster reactants (ABRs), actively participating in the cascade of metabolic events characterizing the stress reaction along pathways best explained by the free hormone/vitamin hypothesis. The latter is governed by the law of mass action—the spontaneous dissociation and instant uptake by hepatocytes of the ligands freed from their specific carrier proteins, which creates a transient hyperthyroid, hyperretinoid, and hypercortisolic climate. This response generally does not exceed four or five days because the initial impact of injury normally subsides, but it may last longer if complications occur. The magnitude and adequacy of the stress responses depend on the preceding nutritional status as assessed by TTR plasma levels and are proportionate to the severity of insult. Clinical, animal, and molecular studies concur to demonstrate the dualistic stimulatory or inhibitory effects triggered by the ligands, whose unmetabolized fractions are excreted in the urinary output. Thyroid hormones and retinoids appear to control the early maturation processes and the synthesis of primary transcripts, whereas cortisol preferentially modulates the secondary responses and confers a protective effect on healthy tissues. During acute stress, the evolutionary patterns of visceral proteins and inflammatory markers exhibit compulsory mirror images. However, they change in independent ways under more chronic circumstances. A relatively simple biochemical micromethod based on the simultaneous measurement of plasma TTR, albumin, CRP, and orosomucoid aggregated into a PINI is proposed for the early recognition and follow-up of both nutritional and inflammatory facets of the disease spectrum.

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