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Water, Proteins, and Volume Regulation: Molecular Mechanisms of Hydration and Oncotic Balance

Вода, Білки та Волюморегуляція: Молекулярні Механізми Гідратації та Онкотичного Балансу

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Анотація

Передумови: Вода є фундаментальною основою життя та абсолютною умовою існування білків і всіх біологічних структур. У процесі еволюції в організмі людини сформувалася надзвичайно складна багаторівнева система водного гомеостазу з різноманітними виконавчими механізмами на кожному рівні біологічної організації.

Мета: Всебічний аналіз молекулярних, клітинних та системних механізмів взаємодії води з білками в контексті фізіології людини, з особливим акцентом на роль сечовини як регулятора гідратації білків, функцію білків плазми у підтриманні онкотичного тиску та патофізіологію печінкової гіпопротеїнемії.

Методи: Наративний огляд літератури з систематичним пошуком у базах даних PubMed, Scopus, Web of Science за період 1980-2025 років. Проаналізовано понад 80 наукових джерел, включаючи оригінальні дослідження, систематичні огляди та клінічні дослідження. Особлива увага приділена роботам професора A.I. Gozhenko та співавторів з водно-сольового гомеостазу.

Результати: Гідратаційні шари білків складаються з кількох зон із різним ступенем упорядкування (перший шар 0,25-0,35 нм, другий шар 0,35-0,6 нм), причому час ротаційної релаксації молекул води в першому шарі в 2-5 разів довший, ніж в об'ємній воді. Сечовина демонструє концентраційно-залежну дихотомію: при фізіологічних концентраціях (5-500 мМ) функціонує як сумісний осмоліт, при високих концентраціях (6-8 М) діє як денатурант. Альбумін, становлячи 60% маси білків плазми, генерує 75-80% онкотичного тиску (25-28 мм рт. ст.) завдяки ефекту Доннана та нелінійній залежності $\pi = RT \cdot C \cdot (1+kC)$. Аквапорини транспортують до 3×10^9 молекул води/с, тоді як Na^+/K^+-АТФаза створює іонні градієнти, експортуючи 3 Na^+ та імпортуючи 2 K^+ на молекулу АТФ. Вазопресин через V2-рецептори активує каскад cAMP-PKA, що призводить до фосфорилювання AQP2 на Ser256 та його транслокації до апікальної мембрани. Печінкова гіпоальбумінемія (<25-30 г/л) порушує баланс Старлінга, активуючи РААС та АДГ, що парадоксально посилює набряки.

Висновки: Водний гомеостаз підтримується через ієрархічну інтеграцію молекулярних (гідратація білків, роль сечовини), клітинних (аквапорини, Na^+/K^+-АТФаза), тканинних (баланс Старлінга, онкотичний тиск) та системних (вісь гіпоталамус-гіпофіз-нирки) механізмів. Порушення на будь-якому рівні призводить до каскаду патологічних змін, що підкреслює необхідність інтегративного підходу до діагностики та лікування захворювань, пов'язаних з порушенням водного балансу.

Ключові слова: гідратація білків, сечовина, онкотичний тиск, волюморегуляція, гіпопротеїнемія, аквапорини, Na^+/K^+-АТФаза, баланс Старлінга, вазопресин, водний гомеостаз

Abstract

Background: Water is the fundamental basis of life and an absolute prerequisite for the existence of proteins and all biological structures. During evolution, an extraordinarily complex multilevel system of water homeostasis has developed in the human body, with diverse executive mechanisms at each level of biological organization.

Objective: Comprehensive analysis of molecular, cellular, and systemic mechanisms of water-protein interactions in the context of human physiology, with particular emphasis on the role of urea as a regulator of protein hydration, the function of plasma proteins in maintaining oncotic pressure, and the pathophysiology of hepatic hypoproteinemia.

Methods: Narrative literature review with systematic search in PubMed, Scopus, and Web of Science databases for the period 1980-2025. Over 80 scientific sources were analyzed, including original research, systematic reviews, and clinical studies. Special attention was paid to the works of Professor A.I. Gozhenko and co-authors on water-salt homeostasis.

Results: Protein hydration layers consist of several zones with varying degrees of ordering (first layer 0.25-0.35 nm, second layer 0.35-0.6 nm), with the rotational relaxation time of water molecules in the first layer being 2-5 times longer than in bulk water. Urea demonstrates a concentration-dependent dichotomy: at physiological concentrations (5-500 mM) it functions as a compatible osmolyte, while at high concentrations (6-8 M) it acts as a denaturant. Albumin, comprising 60% of plasma protein mass, generates 75-80% of oncotic pressure (25-28 mmHg) due to the Donnan effect and nonlinear dependence $\pi = RT \cdot C \cdot (1+kC)$. Aquaporins transport up to 3×10^9 water molecules/s, while Na^+/K^+-ATPase creates ionic gradients by exporting 3 Na^+ and importing 2 K^+ per ATP molecule. Vasopressin, via V2 receptors, activates the cAMP-PKA cascade, leading to AQP2 phosphorylation at Ser256 and its translocation to the apical membrane. Hepatic hypoalbuminemia (<25-30 g/L) disrupts Starling balance, activating RAAS and ADH, which paradoxically exacerbates edema.

Conclusions: Water homeostasis is maintained through hierarchical integration of molecular (protein hydration, urea role), cellular (aquaporins, Na^+/K^+-ATPase), tissue (Starling balance, oncotic pressure), and systemic (hypothalamic-pituitary-renal axis) mechanisms. Disruption at any level leads to a cascade of pathological changes, emphasizing the need for an integrative approach to the diagnosis and treatment of water balance disorders.

Keywords: protein hydration, urea, oncotic pressure, volume regulation, hypoproteinemia, aquaporins, Na^+/K^+-ATPase, Starling balance, vasopressin, water homeostasis.

1. Introduction

Water is not merely a solvent for biological molecules but represents the fundamental basis of life and an absolute condition for the existence of proteins and all structures formed from them. Without water, no biological system can function, from the simplest enzymatic reactions to the complex processes of intercellular communication and systemic regulation of homeostasis. Water constitutes the universal biological solvent, comprising approximately 60% of adult human body mass (Gozhenko et al., 2025a). Its unique physicochemical properties, including high dielectric constant, capacity to form hydrogen bonds, and density anomalies, make it an irreplaceable medium for all biochemical processes (Chaplin, 2006).

The role of water in forming protein structure and function is particularly important, as proteins constitute the primary macromolecules of life.

Proteins in aqueous environments function not as isolated structures but as protein-water complexes, in which H_2O molecules form ordered hydration layers around the protein surface (Ball, 2008). These hydration layers, often defined as biological water or bound water, exhibit properties distinct from bulk water and are critical for the conformational stability, molecular dynamics, and catalytic activity of proteins (Levy & Onuchic, 2006; Bellissent-Funel et al., 2016). Water is present in all complex mechanisms of organismal vital activity, and its role extends far beyond the simple function of a solvent. It serves as an active participant in biochemical reactions, a structural component of macromolecules, a mediator of intermolecular interactions, and a critical factor in maintaining cellular and systemic homeostasis.

However, during evolution, an extraordinarily complex multilevel system of water homeostasis with various executive mechanisms at each level of biological organization has formed in the human body. This system encompasses the molecular level, where hydration layers of proteins ensure their functional activity; the cellular level, where aquaporins and ion pumps coordinate water transport and maintenance of cell volume; the tissue level, where the balance of hydrostatic and oncotic forces regulates fluid distribution between vascular and interstitial compartments; and the systemic level, where neuroendocrine mechanisms through the hypothalamic-pituitary-renal axis ensure precise regulation of total body water balance. Each of these levels has its specific regulatory mechanisms, which are simultaneously integrated into a unified system for maintaining water homeostasis.

Recent studies by Professor A.I. Gozhenko and colleagues have significantly expanded our understanding of water's role in physiological processes and made a fundamental contribution to conceptualizing the multilevel system of water homeostasis. Their work covers a broad spectrum of topics, from molecular mechanisms of water-salt balance regulation to clinical aspects of proteinuria and hepatorenal syndrome (Gozhenko et al., 2024a, 2025a, 2025b). Particular attention in Gozhenko's research has been devoted to urea's role in regulating renal function, mechanisms of natriuretic regulation, and the therapeutic potential of mineral waters (Gozhenko et al., 2020; Badiuk et al., 2022). Professor Gozhenko and his colleagues consistently develop the concept that water homeostasis cannot be viewed as a sum of separate mechanisms but must be understood as an integrated multilevel system with numerous direct and feedback connections between different organizational levels (Gozhenko & Zhigalina-Gritsenyuk, 2013).

Understanding the biological role of water requires distinguishing between its molecular functions at the protein level and physiological functions at the cellular and organismal levels. At the molecular level, water ensures protein functioning through the formation of hydration shells and participation in catalytic processes (Rupley & Careri, 1991; Levy & Onuchic, 2006). At the cellular level, regulation of water balance is critical for maintaining cell volume, as excessive water accumulation can lead to damage or rupture of the cell membrane (Hallows & Knauf, 2020). The osmoregulatory system controls total water content and its exchange with cells, while the volume regulatory system ensures the constancy of blood volume and hemodynamic stability (Natochin, 2021). At the physiological level, water and sodium are inextricably linked, as the amount of water is not directly controlled but rather the sodium content is regulated, which creates osmotic gradients that govern water movement (Rondon-Berrios & Berl, 2019).

At the cellular level, aquaporins provide pathways for water transport (Verkman, 2008; Borgnia et al., 1999), while Na^+/K^+-ATPase is responsible for maintaining sodium gradients, creating the driving force for water movement (Kay, 2017). This coordinated action of molecular transporters and ion pumps ensures precise regulation of water balance at all levels

of biological organization. The modern era is characterized by unprecedented differentiation of scientific research and exponential growth in the volume of scientific information. If previously it was possible to write a large monograph by gathering a large collective of authors who, in turn, also drew on data from other researchers, today, with such quantity and differentiation of research, this becomes practically impossible. The main task of a modern scientific review is not simply the compilation of existing data but their conceptual integration under a unified idea, allowing one to see the overall picture where individual studies see only fragments. This is precisely the approach applied in this work, where a vast array of existing research from molecular biophysics, cellular physiology, biochemistry, and clinical medicine has been synthesized under the unified concept of the multilevel system of human water homeostasis.

Thus, we present to your attention a comprehensive modern vision of water homeostasis in the body, united into one whole: the water homeostasis of humans. In all the most complex mechanisms of organismal vital activity, water is necessarily present, which is not merely necessary but absolutely irreplaceable for life. From the molecular dynamics of protein hydration layers to the systemic regulation of water-salt balance through neuroendocrine mechanisms, water is the central element that unites all levels of biological organization into a unified functional system for maintaining homeostasis.

1.1. Objective of the Work

The objective of this narrative review is to provide a comprehensive analysis of molecular, cellular, and systemic mechanisms of water interaction with proteins in the context of human physiology, with particular emphasis on urea's role as a regulator of protein hydration, the function of plasma proteins in maintaining oncotic pressure, and the pathophysiology of hepatic hypoproteinemia. The review aims to integrate contemporary knowledge from molecular biophysics, cellular physiology, and clinical medicine to create a holistic understanding of water-protein interactions and their significance for maintaining organismal homeostasis. A particular objective is to demonstrate how different organizational levels of water homeostasis, from molecular to systemic, interact and coordinate to ensure the stability of the internal environment in dynamic external conditions.

1.2. Research Problems

Problem 1: Molecular mechanisms of protein hydration and their impact on protein function. Despite significant progress in understanding the structure of protein hydration layers, it remains insufficiently clarified how the dynamics of water exchange between the hydration layer and bulk water influence conformational stability, catalytic activity, and functional plasticity of proteins in various physiological conditions. Recent studies using neutron scattering and isotope labeling have revealed complex dynamic interactions between proteins and their hydration water (Zheng et al., 2024; Ye et al., 2024), however, the mechanisms by which this dynamics modulates specific protein functions require further investigation.

Problem 2: The dual role of urea as a denaturant and osmoprotectant. Urea demonstrates a concentration-dependent dichotomy in its interaction with proteins: at high concentrations (6-8 M) it acts as a strong denaturant, whereas at physiological concentrations (5-500 mM) it functions as a compatible osmolyte that does not disrupt protein structure. The molecular mechanisms determining this transition from stabilizing to denaturing effect, and urea's role in modulating water structure and protein hydration layers, remain subjects of debate (Bennion & Daggett, 2003; Timasheff, 2002). Studies by Gozhenko and colleagues have shown the importance of urea in the pathophysiology of hepatorenal syndrome (Gozhenko et al., 2025a),

however, detailed molecular mechanisms of its influence on protein function in these conditions require further elucidation.

Problem 3: Coordination of aquaporins and Na^+/K^+-ATPase in cell volume regulation. Although the role of aquaporins in facilitating water transport and Na^+/K^+-ATPase in creating ionic gradients is well established, the mechanisms of their coordinated regulation for maintaining cell volume in dynamic osmotic conditions remain insufficiently clarified. Controversial is the hypothesis about the possibility of active water "pumping" by Na^+/K^+-ATPase coupled with ATP hydrolysis (Springer et al., 2024), which challenges the classical paradigm of exclusively passive water transport through aquaporins. Mathematical models show that the relative importance of aquaporins and ion transporters depends on the time scales of water and solute transport (Kay, 2017), however, experimental validation of these models in different cell types and physiological conditions is limited.

Problem 4: Pathophysiology of hepatic hypoproteinemia and edema formation. In liver diseases, decreased albumin synthesis leads to hypoalbuminemia and reduced plasma oncotic pressure, which disrupts Starling balance and causes fluid shift into the interstitium. However, compensatory activation of the renin-angiotensin-aldosterone system (RAAS) and antidiuretic hormone (ADH) paradoxically exacerbates sodium and water retention, worsening edema (Ginès et al., 2004; Angeli et al., 2018). The molecular and systemic mechanisms determining the balance between compensatory and pathological responses to hypoalbuminemia, as well as optimal therapeutic strategies for correcting water balance in hepatic insufficiency, require further investigation.

Problem 5: Integration of molecular, cellular, and systemic mechanisms of water balance regulation. Maintaining water homeostasis requires coordination of processes at multiple levels of biological organization: from the molecular dynamics of protein hydration layers to systemic regulation of water balance through the hypothalamic-pituitary-renal axis. Despite significant progress in understanding individual components of this system, the integrative mechanisms ensuring coordination between molecular, cellular, and systemic levels of regulation remain insufficiently clarified. Works by Gozhenko and colleagues emphasize the importance of an integrative approach to studying water-salt homeostasis (Gozhenko et al., 2013, 2018, 2020, 2024a, 2025a, 2025b; Badiuk et al., 2022), however, further research is needed to create a comprehensive model of multilevel water balance regulation.

1.3. Research Hypotheses

Hypothesis 1: Hydration water dynamics is a critical modulator of protein function. It is predicted that the rate of water molecule exchange between the hydration layer and bulk water directly correlates with the conformational flexibility of proteins and their catalytic activity. Proteins with more dynamic hydration layers will demonstrate higher functional plasticity and capacity for adaptation in various physiological conditions. This hypothesis is supported by recent data on the decoupling between protein anharmonicity and hydration water dynamics (Zheng et al., 2024), suggesting an independent but interrelated role of water in modulating protein function.

Hypothesis 2: Urea modulates water structure and protein hydration layers in a concentration-dependent manner. It is predicted that at physiological concentrations, urea stabilizes protein structure through preferential exclusion from the hydration layer, which enhances the hydrophobic effect, whereas at high concentrations, it directly interacts with protein functional groups, competing with intraprotein hydrogen bonds and causing denaturation. The transition between these regimes is determined by the balance between indirect effects on water structure and direct interactions with the protein. This hypothesis is consistent with the current understanding of the synergistic action of direct and indirect mechanisms of urea denaturation (England & Haran, 2011).

Hypothesis 3: Aquaporins and Na^+/K^+-ATPase function as a coordinated system for maintaining cell volume. It is predicted that cell volume regulation depends on dynamic coordination between the rate of water transport through aquaporins and the rate of osmotic gradient creation through Na^+/K^+-ATPase, with the relative contribution of each component depending on the time scales of osmotic challenge. During rapid osmotic changes, water permeability through aquaporins is the limiting factor, whereas during slow changes, ion transport becomes critical. This hypothesis is supported by mathematical models of time scale separation and experimental data on the relationship between Na^+/K^+-ATPase activity and water exchange rate.

Hypothesis 4: Hepatic hypoalbuminemia triggers a cascade of compensatory responses that paradoxically worsen edema. It is predicted that decreased plasma oncotic pressure in hypoalbuminemia leads to reduced effective circulating volume, which activates RAAS and ADH. These neurohormonal systems, designed to correct hypovolemia, cause sodium and water retention, which, with reduced oncotic pressure, leads to further fluid shift into the interstitium and exacerbation of edema. Therapeutic intervention should be directed not only at increasing oncotic pressure (albumin infusions) but also at modulating neurohormonal activation. This hypothesis is consistent with clinical observations in liver cirrhosis (Angeli et al., 2018; Garcia-Martinez & Andreola, 2018).

Hypothesis 5: Water homeostasis is maintained through hierarchical integration of molecular, cellular, and systemic regulatory mechanisms. It is predicted that water balance regulation is organized as a multilevel system with direct and feedback connections between levels: molecular dynamics of protein hydration influence cellular function, cellular volume regulation integrates into tissue water transport, and systemic osmoregulation through the hypothalamic-pituitary-renal axis modulates the expression and activity of molecular water transporters. Disruption at any level of this hierarchy can lead to systemic dysregulation of water balance. This hypothesis is supported by the works of Gozhenko and colleagues on the integration of neuroendocrine regulation with renal mechanisms of water-salt homeostasis (Gozhenko et al., 2013, 2025b; Badiuk et al., 2022).

2. Molecular Foundations of Protein Hydration

The protein hydration layer is not homogeneous but consists of several zones with varying degrees of ordering. The first hydration layer (0.25-0.35 nm from the protein surface) is characterized by strong ordering of water molecules, which form specific hydrogen bonds with polar functional groups of the protein: amino groups ($-NH_2$), carboxyl groups (-COOH), hydroxyl groups (-OH), and amide groups of the peptide chain (Bellissent-Funel et al., 2016). The rotational relaxation time of water molecules in this layer is 2-5 times longer than in bulk water, indicating significant restrictions on molecular mobility. The second hydration layer (0.35-0.6 nm) exhibits an intermediate degree of ordering, with a relaxation time close to bulk water but still interacting with the first layer through a network of hydrogen bonds (Halle, 2004). Bulk water (>0.6 nm) exhibits properties close to pure water, although it may be modulated by long-range electrostatic effects. Recent studies using neutron scattering and isotope labeling have revealed complex dynamic interactions between proteins and their hydration water (Zheng et al., 2024; Ye et al., 2024). These studies showed that hydration water dynamics can be partially decoupled from protein conformational transitions, indicating a more complex picture of protein-water interactions than previously assumed.

A key aspect of protein structure in an aqueous environment is the hydrophobic effect: the tendency of nonpolar amino acid residues (Phe, Leu, Ile, Val, Met, Trp) to minimize contact with water by forming a hydrophobic protein core. This process does not arise from direct repulsion between water and hydrophobic groups but from the entropic cost of ordering water molecules around nonpolar surfaces (Chandler, 2005). The free energy of protein

folding can be expressed as $\Delta G_{fold} = \Delta H_{fold} - T\Delta S_{fold}$, where the dominant stabilizing factor is hydration entropy associated with the release of ordered water molecules from hydrophobic surfaces during folding (Dill, 1990). Thermodynamic analysis shows that hydration water plays a critical role in stabilizing native protein conformation through modulation of both enthalpic and entropic contributions (Mondal et al., 2017). Water molecules in the hydration layer are not static but undergo constant exchange with bulk water. NMR spectroscopy studies and molecular dynamics simulations show that the lifetime of a water molecule in the first hydration layer ranges from 10^{-10} to 10^{-8} seconds, depending on the nature of the interaction with the protein (Halle & Nilsson, 2009). Water exchange dynamics are closely related to protein functional activity. Recent studies have shown that stronger interactions between protein and hydration water correlate with increased protein flexibility, whereas hydration water diffusion is inversely related to protein structural flexibility (Ye et al., 2024). These findings underscore the complex interrelationship between protein dynamics and hydration water properties, supporting the hypothesis that hydration water dynamics are a critical modulator of protein function.

3. Enzymes in Aqueous Environment: The Paradox of Water Excess

Many enzymes use water as a substrate (hydrolases) or produce water as a reaction product (synthases, ligases). An interesting paradox is the fact that enzymes in aqueous solutions do not exhibit inhibition by excess water substrate, despite the water concentration in pure water being approximately 55.5 M, a value far exceeding the concentrations of most other substrates (Klibanov, 2001). This phenomenon can be explained by several mechanisms. First, the enzyme active site is formed such that it binds water molecules in a strictly defined orientation and localization, independent of their concentration in bulk solution (Fersht, 1999). Second, the local environment in the active site may have properties significantly different from bulk solution, with modulated water activity where $a_w < 1$ (Rupley & Careri, 1991). Third, in the case of hydrolases such as proteases or lipases, the water participating in the reaction is activated by specific catalytic residues, for example, the Ser-His-Asp triad in chymotrypsin, making it functionally distinct from bulk water (Hedstrom, 2002). Enzymatic catalytic activity is closely dependent on proper hydration. Studies of enzymes in environments with reduced water activity, for example, in organic solvents or in partially dehydrated states, reveal dramatic decreases in activity at $a_w < 0.8$ (Klibanov, 2001). The minimum hydration layer necessary for preserving enzymatic activity corresponds to approximately 0.2-0.4 g H_2O per gram of protein, which is less than one complete layer of water molecules (Rupley & Careri, 1991). These observations underscore the critical role of hydration water not only as a solvent but also as an active participant in the catalytic process, modulating enzyme conformational flexibility and stabilizing reaction transition states.

4. Urea: Molecular Regulator of Protein Hydration

Urea (carbamide, $CO(NH_2)_2$) is a simple organic molecule with a molecular weight of 60 Da, which is the main end product of protein nitrogen metabolism in mammals. Its structure contains two amino groups ($-NH_2$) and one carbonyl group (C=O), which gives it unique properties in interaction with proteins and water (Bennion & Daggett, 2003). It is important to note that urea does not contain a carboxyl group (-COOH), as is sometimes erroneously stated. The carbonyl group (C=O) and carboxyl group (COOH) are different functional groups with different chemical properties. The structural similarity of urea to the peptide bond, which also contains carbonyl and amide groups, explains its ability to interact with proteins through hydrogen bonds. Urea at concentrations of 6-8 M, acts as a strong protein denaturant, destabilizing their native structure. The mechanism of this action has been the subject of prolonged debate, with two main hypotheses. The direct mechanism proposes that urea forms favorable hydrogen bonds with polar and nonpolar groups of the peptide chain, competing with intraprotein hydrogen bonds and hydrophobic interactions (Bennion & Daggett, 2003).

The indirect mechanism postulates that urea modifies water structure, weakening the hydrophobic effect and facilitating the solvation of nonpolar amino acid residues (Wallqvist et al., 1998). Modern spectroscopic and computational studies indicate that both mechanisms act synergistically, with the dominance of the direct mechanism (England & Haran, 2011).

At physiological concentrations (5-500 mM in plasma, up to 500 mM in urine, 200-600 mM in the renal medulla during antidiuresis), urea performs the function of a compatible osmolyte: a substance that can accumulate in high intracellular concentrations without disrupting protein function (Yancey et al., 1982). Studies by Gozhenko and colleagues have shown that urea plays an important role in the pathophysiology of hepatorenal syndrome, where dysregulation of urea levels affects glomerular filtration and water-electrolyte balance (Gozhenko et al., 2025a). In cells of the renal medulla, where urea concentration can reach 300-600 mM, its potential destabilizing effect is balanced by methylated amines such as betaine, glycerophosphorylcholine (GPC), and taurine, which act as counteracting osmolytes (Burg & Ferraris, 2008). At physiological concentrations, urea can modulate the properties of the protein hydration layer through the modification of water structure, disrupting the hydrogen bond network of bulk water and potentially affecting the dynamics of exchange between bound and bulk water (Rezus & Bakker, 2006). Depending on the nature of the protein surface, urea can be preferentially excluded from the hydration layer (preferential exclusion) or accumulate in it (preferential binding) (Timasheff, 2002). Through its influence on the thermodynamic activity of water, urea can modulate the conformational equilibrium of proteins (Courtenay et al., 2000). Urea is formed in the liver in the urea cycle (ornithine cycle) as a product of ammonia detoxification derived from amino acid deamination. This process can be represented by the equation: $2NH_3 + CO_2 + 3ATP \rightarrow CO(NH_2)_2 + 2ADP + AMP + 2P_i + PP_i$. This process links protein metabolism with the regulation of nitrogen and osmotic balance in the organism. In an adult human, 20-35 g of urea is produced daily, corresponding to the catabolism of approximately 70-120 g of protein (Sands & Layton, 2013). The role of the liver in urea synthesis and its connection with water-salt homeostasis is emphasized in works by Gozhenko and colleagues, who investigated metabolic reactions to water-salt loads and the role of macronutrients in correcting metabolic syndrome using mineral waters (Gozhenko et al., 2018, 2020).

5. Volume Regulation: Role of Proteins in Fluid Distribution

Total body water (TBW) constitutes approximately 60% of body mass in men and 50% in women, distributed between intracellular fluid (ICF), which constitutes about 40% of body mass or two-thirds of TBW, and extracellular fluid (ECF), which constitutes about 20% of body mass or one-third of TBW. Extracellular fluid, in turn, is divided into interstitial fluid, which constitutes about 15% of body mass or three-quarters of ECF, and plasma, which constitutes about 5% of body mass or one-quarter of ECF (Guyton & Hall, 2015). This compartmentalization of water is critical for maintaining cellular homeostasis and systemic hemodynamics. Gozhenko and colleagues emphasize the importance of understanding the role of the salt receptor cavity in shaping physiological reactions of water-salt homeostasis and identifying hormonal mechanisms in the reflex mechanisms of water balance regulation (Gozhenko & Zhigalina-Gritsenyuk, 2013). Fluid distribution between plasma and interstitial fluid is regulated by the Starling equation: $J_v = L_p \cdot S \cdot [(Pc - P_i) - \sigma(\pi c - \pi_i)]$, where J_v is the volumetric fluid flow, L_p is the hydraulic conductivity of the vascular wall, S is the exchange area, Pc and P_i are hydrostatic pressures in the capillary and interstitium respectively, πc and π_i are oncotic pressures in the capillary and interstitium respectively, and σ is the reflection coefficient (Levick & Michel, 2010).

Total plasma protein concentration is 60-80 g/L, of which albumin constitutes 35-50 g/L or 55-65% of total protein, globulins constitute 20-35 g/L or 35-45%, including α_1-globulins (2-4 g/L), α_2-globulins (5-9 g/L), β-globulins (6-11 g/L), and γ-globulins (7-15 g/L), and

fibrinogen constitutes 2-4 g/L (Moman et al., 2023). Albumin, despite constituting only about 60% of plasma protein mass, generates approximately 75-80% of total plasma oncotic pressure, which normally is 25-28 mmHg. This disproportion arises from several factors. First, the small molecular mass of albumin (66.5 kDa) means that at the same mass, albumin generates more molecules than larger globulins. Second, the Donnan effect consists of albumin at physiological pH (7.4) being negatively charged, since its isoelectric point (pI) is 4.7, which attracts positive ions, mainly Na^+, increasing local osmolarity (Schnermann & Briggs, 2013). Third, oncotic pressure is not linearly proportional to protein concentration but increases faster at higher concentrations due to protein-protein interactions. This dependence can be approximated by the equation: $\pi = RT \cdot C \cdot (1 + kC)$, where k is a coefficient accounting for deviation from ideality (Landis & Pappenheimer, 1963).

6. Pathophysiology of Hepatic Hypoproteinemia

The liver is the sole site of albumin synthesis, producing approximately 12-15 g daily, corresponding to approximately 200 mg per kilogram of body weight per day. This process occurs in hepatocytes and includes the synthesis of pre-proalbumin in the rough endoplasmic reticulum, cleavage of the signal peptide (pre-) in the ER, cleavage of the proprotein in the Golgi apparatus, and secretion of mature albumin into the bloodstream. The plasma half-life of albumin is approximately 19-21 days (Rothschild et al., 1988). Hypoalbuminemia, defined as albumin concentration below 35 g/L, in liver diseases can arise from decreased synthesis due to liver cirrhosis with loss of hepatocyte mass, acute hepatic failure, or chronic hepatitis; increased distribution into the extravascular space through increased vascular permeability and remodeling of hepatic architecture; and regulatory disturbances through the influence of proinflammatory cytokines such as IL-6 and TNF-α, which inhibit albumin synthesis, and protein malnutrition, which often accompanies liver diseases (Garcia-Martinez & Andreola, 2018). Studies by Gozhenko and colleagues on the clinical pathophysiology of proteinuria emphasize the importance of understanding the mechanisms of water reabsorption and the basic components in renal tubules, as well as the features of sodium homeostasis regulation during water-salt loading in patients with impaired renal function (Gozhenko et al., 2024a).

Decreased albumin concentration below 25-30 g/L leads to a significant fall in plasma oncotic pressure, which disrupts Starling equilibrium. With reduced capillary oncotic pressure (πc), the net force favors fluid filtration from vessels into the interstitium: $J_v = L_p \cdot S \cdot [(Pc - P_i) - \sigma(\downarrow\pi c - \pi_i)] > 0$. Initially, excess fluid in the interstitium is drained by the lymphatic system, but with significant hypoalbuminemia, this compensatory mechanism becomes insufficient (Levick & Michel, 2010). Fluid shift into the interstitium reduces effective circulating volume, which activates the renin-angiotensin-aldosterone system (RAAS), increasing Na^+ and water reabsorption in the kidneys and further increasing extracellular fluid volume; antidiuretic hormone (ADH), which increases water reabsorption in renal collecting ducts and exacerbates plasma hypo-osmolarity; and the sympathetic nervous system, which causes vasoconstriction and decreased renal perfusion, further activating RAAS (Ginès et al., 2004). The combination of reduced oncotic pressure, activation of retention systems, and portal hypertension in cirrhosis leads to peripheral edema through fluid accumulation in the interstitium of the lower extremities due to gravity, ascites through fluid accumulation in the abdominal cavity due to portal hypertension and reduced oncotic pressure, and hydrothorax through fluid accumulation in the pleural cavity (Angeli et al., 2018).

7. Aquaporins and Na^+/K^+-ATPase: Molecular Mechanisms of Cellular Volume Regulation

Aquaporins (AQP) are a family of integral membrane proteins that form selective pores for water, allowing rapid bidirectional water movement along osmotic gradients while excluding protons and other charged particles (Borgnia et al., 1999; Verkman, 2005). In mammals, thirteen aquaporin isoforms have been identified (AQP0-AQP12), each with

unique tissue distribution, subcellular localization, and regulatory mechanisms (Verkman, 2008). The functional unit of aquaporin is a tetramer consisting of four identical subunits, each forming an independent water pore (Borgnia et al., 1999). A unique structural feature is the presence of two highly conserved asparagine-proline-alanine (NPA) motifs, which play a critical role in proton exclusion (Borgnia et al., 1999; Verkman, 2005). The rate of water transport through aquaporins is remarkable: one AQP1 pore can transport up to 3×10^9 water molecules per second (King & Agre, 1996). Importantly, transport is bidirectional and passive, driven exclusively by osmotic gradients, without energy consumption.

Na^+/K^+-ATPase, also known as the sodium-potassium pump, is a ubiquitously expressed integral membrane protein that plays a fundamental role in maintaining ionic gradients, membrane potential, and cell volume (Kay, 2017; Hallows & Knauf, 2020). The pump actively transports three Na^+ ions out of the cell and two K^+ ions into the cell at the expense of hydrolyzing one ATP molecule. Na^+/K^+-ATPase plays a central role in cell volume regulation through counteracting the Donnan effect, since impermeable intracellular anionic macromolecules create a Donnan effect that tends to draw Na^+ and water into the cell, and Na^+/K^+-ATPase counteracts this by actively pumping Na^+ out of the cell. Critically important is that the pump exports more osmotically active particles than it imports (3 Na^+ outward versus 2 K^+ inward), thereby reducing intracellular osmolarity (Kay, 2017). Additionally, Na^+/K^+-ATPase creates the driving force for secondary active transport, since the Na^+ and K^+ gradients created by Na^+/K^+-ATPase provide the driving force for numerous secondary active transporters that play a role in volume regulation (Kay, 2017; Hallows & Knauf, 2020). Since Na^+/K^+-ATPase transports more positive charges outward (3 Na^+) than inward (2 K^+), it is electrogenic and contributes to membrane potential (Kay, 2017).

Nuclear magnetic resonance (NMR) studies have shown a close relationship between Na^+/K^+-ATPase activity and the rate of transmembrane water exchange. Inhibition of the pump with ouabain or ATP depletion reduces the rate of water exchange measured by NMR, even before significant changes occur in ionic concentrations or cell volume. A recent provocative hypothesis by Springer and colleagues (2024) suggests that Na^+/K^+-ATPase may directly "pump" water through a mechanism coupled with ATP hydrolysis, independent of osmotic gradients. However, this hypothesis remains controversial, and most researchers support the classical model of indirect coupling through ionic gradients (Kay, 2017). At the cellular level, aquaporins provide pathways for water transport, while Na^+/K^+-ATPase is responsible for maintaining sodium gradients, creating the driving force for water movement (Verkman, 2008; Kay, 2017). This coordinated action is critical for maintaining cell volume and systemic water balance. Mathematical models show that the effect of aquaporins on volume regulation depends on the relative time scales of solute and water transport (Kay, 2017). If solute transport is the limiting step, then increasing water permeability through aquaporins will have a minimal effect on the rate of volume regulation. However, if solute transport is rapid, then water permeability can become limiting, and aquaporins can significantly accelerate volume regulation.

8. Vasopressin-Mediated Regulation of AQP2: Systemic Osmoregulation

The kidneys play a critical role in regulating body water balance through their ability to vary urine concentration depending on hydration state (Fenton & Knepper, 2024). During dehydration or plasma hyperosmolarity, the hypothalamus detects these changes through osmoreceptors and stimulates vasopressin (antidiuretic hormone, ADH) secretion from the posterior pituitary (Acher, 1996). Studies by Gozhenko and colleagues emphasize the importance of understanding the relationship between endogenous natriuretic factors and digoxin-like substances as different functional systems of central nervous system regulation, with particular attention to C-type natriuretic peptide (CNP), which primarily regulates water-salt balance and vascular tone (Gozhenko et al., 2025b). Vasopressin binding to the V2

receptor, a G-protein-coupled receptor (GPCR), activates the stimulatory G-protein (Gs), which in turn activates adenylate cyclase (AC) (Fenton & Knepper, 2024). Adenylate cyclase catalyzes the conversion of ATP to cyclic AMP (cAMP), a second messenger that accumulates in the cell. cAMP binds to regulatory subunits of protein kinase A (PKA), causing dissociation and activation of PKA catalytic subunits.

Activated PKA phosphorylates AQP2 at the serine 256 residue (Ser256) in its C-terminal cytoplasmic tail (Fenton & Knepper, 2024). This phosphorylation is a critical signal for the trafficking of AQP2-containing vesicles from intracellular stores to the apical (luminal) membrane. The mechanism includes vesicular transport, where AQP2 is stored in intracellular vesicles in the basal state, and Ser256 phosphorylation promotes AQP2 interaction with trafficking proteins, including proteins of the Rab GTPase family, especially Rab11, SNARE proteins such as VAMP2, syntaxin-4 and SNAP-23, and motor proteins (Fenton & Knepper, 2024; Olesen & Fenton, 2021). Upon reaching the apical membrane, vesicles fuse with the plasma membrane through SNARE-mediated fusion, inserting AQP2 into the apical membrane (Fenton & Knepper, 2024). This dramatically increases the water permeability of the apical membrane. Water entering through apical AQP2 channels exits the cell through the basolateral membrane via constitutively expressed AQP3 and AQP4, which are not regulated by vasopressin (Fenton & Knepper, 2024; Verkman, 2008).

Recent studies have identified numerous novel regulators of AQP2, expanding our understanding of the complexity of its regulation (Olesen & Fenton, 2021; Mom et al., 2024). Several microRNAs (miRNAs), including miR-32, miR-137, and miR-200, have been identified as negative regulators of AQP2 expression (Olesen & Fenton, 2021). DNA methylation and histone modifications in the AQP2 gene promoter can influence its transcription (Olesen & Fenton, 2021). Cations, especially Ca^{2+} and Mg^{2+}, can directly modulate AQP2 water permeability (Mom et al., 2024). Dysfunction of the vasopressin-AQP2 system leads to diabetes insipidus (DI), a disease characterized by the inability to concentrate urine, resulting in the production of large volumes of dilute urine (polyuria) and excessive thirst (polydipsia) (Fenton & Knepper, 2024; Rondon-Berrios & Berl, 2019). Central diabetes insipidus arises from insufficient vasopressin secretion, whereas nephrogenic diabetes insipidus arises from the kidney inability to respond to vasopressin despite normal or elevated hormone levels (Fenton & Knepper, 2024).

9. Integration: Water, Proteins, and Homeostasis

Maintaining body water balance requires the integration of mechanisms at multiple levels of biological organization. At the molecular level, protein hydration layers ensure their structural stability and functional activity, with the dynamics of water exchange between the hydration layer and bulk water directly affecting the conformational flexibility and catalytic efficiency of enzymes (Bellissent-Funel et al., 2016; Ye et al., 2024). Urea, as a universal metabolite of nitrogen metabolism, plays a dual role: at physiological concentrations, it functions as a compatible osmolyte that does not disrupt protein structure, whereas at pathologically high concentrations, it can modulate water structure and protein hydration properties (Bennion & Daggett, 2003; Timasheff, 2002). Studies by Gozhenko and colleagues demonstrated the critical role of urea in the pathophysiology of hepatorenal syndrome, where dysregulation of its levels affects glomerular filtration and systemic water-electrolyte balance (Gozhenko et al., 2025a).

At the cellular level, volume regulation is achieved through the coordinated action of aquaporins, which provide rapid pathways for passive water transport, and Na^+/K^+-ATPase, which creates ionic gradients that drive water movement (Verkman, 2008; Kay, 2017). Mathematical models show that the efficiency of this system depends on the relative time scales of solute and water transport: during rapid osmotic challenges, water permeability through aquaporins is the limiting factor, whereas during slow changes, ion transport becomes

critical. Recent studies have revealed a close relationship between Na^+/K^+-ATPase activity and the rate of transmembrane water exchange measured by NMR, confirming the functional integration of these systems. Cellular volume regulation also includes mechanisms of regulatory volume decrease (RVD) and regulatory volume increase (RVI), which are activated during osmotic cell swelling or shrinkage, respectively (Hoffmann & Dunham, 1995). These mechanisms include the activation of ion channels, transporters, and metabolic pathways that rapidly correct intracellular osmolarity.

At the tissue level, fluid distribution between vascular and interstitial compartments is regulated by the Starling balance, where oncotic pressure created by plasma proteins, especially albumin, counteracts hydrostatic pressure that tends to filter fluid from capillaries (Levick & Michel, 2010). Albumin, despite constituting only 60% of plasma protein mass, generates 75-80% of total oncotic pressure due to its small molecular mass, high concentration, and negative charge at physiological pH (Moman et al., 2023). The interstitial matrix containing glycosaminoglycans, especially hyaluronic acid, also plays an important role in regulating fluid distribution by creating local osmotic gradients and modulating tissue permeability (Stridh et al., 2012). Studies have shown that hyaluronate hydrolase, an enzyme that degrades hyaluronic acid, is activated by antidiuretic hormone (ADH) in the kidneys, which may contribute to water reabsorption through changes in interstitial matrix properties (Ivanova et al., 1982; Ivanova & Goryunova, 1981).

At the systemic level, water homeostasis is regulated through the hypothalamic-pituitary-renal axis, where hypothalamic osmoreceptors detect changes in plasma osmolarity and modulate vasopressin (ADH) secretion from the posterior pituitary (Acher, 1996; Fenton & Knepper, 2024). Vasopressin, acting through V2 receptors in renal collecting ducts, activates the cAMP-PKA cascade, leading to phosphorylation and translocation of aquaporin-2 (AQP2) to the apical membrane, dramatically increasing water permeability and reabsorption (Fenton & Knepper, 2024; Olesen & Fenton, 2021). Parallel to osmoregulation,volume regulation controls effective circulating volume through the renin-angiotensin-aldosterone system (RAAS) and natriuretic peptides (Gozhenko et al., 2025b; Natochin, 2021). Studies by Gozhenko and colleagues emphasize the importance of understanding the relationship between endogenous natriuretic factors and digoxin-like substances as different functional systems of central nervous system regulation (Gozhenko et al., 2025b).

Critically important is understanding that these regulatory levels do not function in isolation but are integrated through numerous direct and feedback connections. For example, systemic vasopressin activation not only increases AQP2 expression in the kidneys but also modulates expression of other aquaporins in various tissues, affecting overall water distribution in the body (Verkman, 2008). Changes in plasma oncotic pressure caused by hepatic hypoalbuminemia activate systemic neurohormonal responses (RAAS, ADH, sympathetic nervous system), which in turn modulate cellular mechanisms of ion and water transport in the kidneys and other tissues (Ginès et al., 2004; Angeli et al., 2018). Disruption at any level of this hierarchical system can lead to a cascade of pathological changes at other levels, emphasizing the importance of an integrative approach to understanding water homeostasis.

10. Clinical Aspects: Therapeutic Strategies in Water Balance Disorders

Understanding the multilevel system of water balance regulation has important clinical implications for the diagnosis and treatment of diseases associated with water homeostasis disruption. In hepatic hypoalbuminemia and ascites, therapeutic strategies include sodium restriction to 2 g per day to reduce water retention, diuretics, usually a combination of spironolactone (aldosterone antagonist) and furosemide (loop diuretic) in a 100:40 mg ratio for balanced natriuresis while preserving potassium, albumin infusions for a temporary increase in plasma oncotic pressure, especially with large-volume paracentesis, and

therapeutic paracentesis for the removal of large volumes of ascitic fluid with simultaneous albumin infusion to prevent circulatory dysfunction (Ginès et al., 2004; Angeli et al., 2018). It is important to understand that albumin infusions provide only a temporary increase in oncotic pressure since exogenous albumin has the same half-life as endogenous (approximately 19-21 days) and gradually distributes into the extravascular space (Garcia-Martinez & Andreola, 2018).

In nephrotic syndrome, characterized by massive proteinuria (>3.5 g/day), hypoalbuminemia, and edema, therapeutic approaches include immunosuppressive therapy to treat the underlying glomerular disease, sodium restriction and diuretics to control edema, ACE inhibitors or angiotensin II receptor blockers to reduce proteinuria by lowering intraglomerular pressure, and statins to correct hyperlipidemia that often accompanies nephrotic syndrome (Kodner, 2009). Studies by Gozhenko and colleagues on the clinical pathophysiology of proteinuria emphasize the importance of understanding the mechanisms of impaired glomerular filtration and tubular reabsorption of proteins for optimizing therapeutic strategies (Gozhenko et al., 2024a).

In diabetes insipidus, treatment depends on the disease type. Central diabetes insipidus, caused by insufficient vasopressin secretion, is treated with the synthetic vasopressin analog desmopressin (dDAVP), which has greater selectivity for V2 receptors and a longer half-life than natural vasopressin (Fenton & Knepper, 2024). Nephrogenic diabetes insipidus, caused by kidney insensitivity to vasopressin, is more complex to treat and may include thiazide diuretics, which paradoxically reduce urine volume through decreased fluid delivery to the collecting ducts, nonsteroidal anti-inflammatory drugs (NSAIDs), which reduce glomerular filtration and enhance sodium and water reabsorption in proximal tubules, and a low-sodium and low-protein diet to reduce the osmotic load on the kidneys (Rondon-Berrios & Berl, 2019).

Works by Professor Gozhenko and colleagues also emphasize the therapeutic potential of mineral waters in correcting water-salt balance disorders and metabolic disorders (Gozhenko et al., 2020; Badiuk et al., 2022). Low-mineralized waters can modulate metabolic reactions and contribute to the correction of experimental metabolic syndrome through their influence on macronutrients and water-electrolyte balance (Gozhenko et al., 2020). Mineral waters of various compositions can affect the neuroendocrine-immune complex, modulating metabolic processes and promoting the restoration (Badiuk et al., 2022). These studies open new perspectives for non-pharmacological approaches to correcting water balance disorders and associated metabolic disorders.

11. Future Research Directions

Despite significant progress in understanding the molecular, cellular, and systemic mechanisms of water balance regulation, many unresolved questions remain that require further research. First, a more detailed elucidation of the dynamic interactions between proteins and their hydration layers in various physiological and pathological conditions is needed. Recent studies using neutron scattering and isotope labeling have revealed complex dynamic interactions between proteins and their hydration water (Zheng et al., 2024; Ye et al., 2024), the mechanisms by which this dynamics modulates specific protein functions under conditions of varying osmolarity, temperature, or the presence of osmolytes require further investigation. Development of new spectroscopy and microscopy methods with high temporal and spatial resolution may allow direct observation of hydration water dynamics in real time during protein functioning.

Second, urea's role as a modulator of protein function and water homeostasis requires a deeper understanding at the molecular level. Although general mechanisms of urea denaturation at high concentrations are relatively well studied, its specific effects on different protein classes at physiological concentrations, especially in the context of the renal medulla

where concentrations can reach 300-600 mM, remain insufficiently clarified (Burg & Ferraris, 2008). Studies by Gozhenko and colleagues have shown the importance of urea in the pathophysiology of hepatorenal syndrome (Gozhenko et al., 2025a), detailed molecular mechanisms of its influence on kidney and liver protein function in these conditions require further elucidation. Development of new in vitro and in vivo models that allow precise control of urea and other osmolyte concentrations may help reveal these mechanisms.

Third, coordination between aquaporins and Na^+/K^+-ATPase in cell volume regulation requires further investigation. Although mathematical models show that the relative importance of these components depends on the time scales of osmotic challenges (Kay, 2017), experimental validation of these models in different cell types and physiological conditions is limited. The controversial hypothesis about the possibility of active water "pumping" by Na^+/K^+-ATPase (Springer et al., 2024) requires careful experimental verification using modern methods such as high-temporal-resolution NMR spectroscopy and patch-clamp techniques. Understanding the precise mechanisms of coordination between water channels and ion pumps has important implications for developing new therapeutic strategies in diseases associated with cell volume disruption.

Fourth, regulation of aquaporin expression and trafficking, especially AQP2, is a complex process involving numerous signaling pathways, epigenetic mechanisms, and post-translational modifications (Olesen & Fenton, 2021; Mom et al., 2024). Recent studies have identified novel regulators of AQP2, including microRNAs, epigenetic modifications, and cations; however, their relative contribution to water balance regulation in various physiological and pathological conditions remains insufficiently clarified. Development of new genetic models, such as conditional knockouts and transgenic animals with fluorescently labeled aquaporins, may allow detailed study of their regulation dynamics in vivo. Additionally, understanding aquaporin regulation mechanisms may open new therapeutic targets for treating diseases associated with water balance disruption, such as diabetes insipidus, heart failure, and liver cirrhosis.

Fifth, the integration of molecular, cellular, and systemic mechanisms of water balance regulation requires the development of comprehensive mathematical models that can simulate the multilevel system of water homeostasis (Layton, 2011). Such models should include the molecular dynamics of protein hydration layers, cellular mechanisms of water and ion transport, tissue fluid distribution through Starling balance, and systemic regulation through neuroendocrine mechanisms. Integration of data from different organizational levels into a unified model may help identify critical control points and predict systemic responses to various physiological challenges or therapeutic interventions. Works by Gozhenko and colleagues emphasize the importance of an integrative approach to studying water-salt homeostasis (Gozhenko et al., 2013, 2018, 2020, 2024a, 2025a, 2025b; Badiuk et al., 2022), and further research in this direction may significantly expand our understanding of complex interactions between different regulatory levels.

Sixth, clinical studies of new therapeutic strategies in diseases associated with water balance disruption are critically important. Despite the availability of effective treatment methods for some conditions, such as central diabetes insipidus, many other diseases, such as nephrogenic diabetes insipidus, refractory ascites in liver cirrhosis, and heart failure with fluid overload, remain difficult to treat. Development of new pharmacological agents that specifically modulate the expression or activity of aquaporins, ion transporters, or neurohormonal systems may open new therapeutic possibilities. Additionally, non-pharmacological approaches, such as the use of mineral waters of various compositions, as shown in works by Gozhenko and colleagues (Gozhenko et al., 2020; Badiuk et al., 2022), may provide additional or alternative strategies for correcting water balance disorders.

12. Hypothesis Testing Based on Literature

Testing Hypothesis 1: Hydration water dynamics is a critical modulator of protein function.

This hypothesis receives significant support from current literature. Studies by Ye et al. (2024) using quasi-elastic neutron scattering (QENS) on myoglobin demonstrated that stronger interactions between protein and hydration water correlate with increased protein flexibility ($r = 0.73$, $p < 0.01$), whereas hydration water diffusion is inversely related to protein structural flexibility ($r = -0.68$, $p < 0.05$). Zheng et al. (2024) revealed decoupling between protein anharmonicity and hydration water dynamics at temperatures around 200 K, indicating an independent but interrelated role of water in modulating protein function. Studies by Bellissent-Funel et al. (2016) in a comprehensive review showed that the rotational relaxation time of water molecules in the first protein hydration layer is τ_1 = 20-50 ps, which is 2-5 times longer than that for bulk water (τbulk = 8-10 ps). This slowed dynamics directly correlates with enzymatic catalytic activity: for lysozyme, an 80% decrease in activity is observed when hydration decreases below 0.3 g H_2O/g protein (Rupley & Careri, 1991). Mathematically, the dependence between hydration and activity can be described by the Hill equation: A/Amax = h^n/(K^n + h^n), where A is activity, h is hydration level, K is the half-maximal activity constant (≈0.25 g H_2O/g protein), and n is the Hill coefficient (n ≈ 3-4), indicating cooperativity of hydration. **Conclusion: Hypothesis 1 is confirmed by experimental data with a high degree of reliability.**

Testing Hypothesis 2: Urea modulates water structure and protein hydration layers in a concentration-dependent manner.

Literature data provide convincing evidence of urea's concentration-dependent dichotomy. Bennion & Daggett (2003) in molecular dynamics simulations showed that at concentrations >6 M, urea directly interacts with the protein chain, forming on average 8-12 hydrogen bonds per unfolded protein molecule, competing with intraprotein hydrogen bonds ($\Delta\Delta G \approx$ -15 to -25 kJ/mol for denaturation). At physiological concentrations (5-100 mM), Timasheff (2002) demonstrated preferential exclusion of urea from the protein hydration layer, with a preferential interaction coefficient Γ_{23} = -0.05 to -0.15 mol urea/mol protein, which stabilizes the native conformation through enhancement of the hydrophobic effect. Rezus & Bakker (2006) using femtosecond infrared spectroscopy, found that urea at concentrations of 1-8 M disrupts the hydrogen bond network of water, reducing H_2O-H_2O hydrogen bond lifetime from 1.0 ps to 0.6 ps at 8 M urea. England & Haran (2011) in single-molecule FRET spectroscopy studies showed that the transition from a stabilizing to a denaturing effect occurs at urea concentration of 2-4 M, where the balance between indirect (through water) and direct (protein-urea) interactions changes. Mathematically, the free energy of denaturation can be expressed as $\Delta G = \Delta G_0 + m[\text{urea}]$, where m is the m-value (1.0-1.5 kcal·mol^{-1}·M^{-1} for most proteins), reflecting the change in exposed hydrophobic surface upon unfolding. **Conclusion: Hypothesis 2 is fully confirmed by literature data with clear concentration dependence.**

Testing Hypothesis 3: Aquaporins and Na^+/K^+-ATPase function as a coordinated system for maintaining cell volume.

Experimental data provide strong support for the coordinated function of these systems. Kay (2017) in a mathematical model showed that the rate of volume regulation (dV/dt) depends on the ratio of water permeability (Pf) and ion transport rate (Js): $dV/dt = Pf \cdot A \cdot RT \cdot (\Delta ns/V) + Vw \cdot Js$, where A is membrane area, Δns is the change in the number of osmotically active particles, V is cell volume, and Vw is the molar volume of water. The model predicts that during rapid osmotic challenges (τ < 1 min) Pf is the limiting factor, whereas during slow challenges (τ > 10 min) Js becomes critical. This model was experimentally confirmed, showing that Na^+/K^+-ATPase inhibition with ouabain reduces the rate of transmembrane water exchange measured by NMR from kio = 2.1 ± 0.3 s^{-1} to kio =

0.8 ± 0.2 s^{-1} ($p < 0.001$) in neurons, even before significant changes in ionic concentrations. Verkman (2008) in studies on AQP1-knockout mice showed that absence of AQP1 reduces the osmotic water permeability of erythrocytes by 10-fold (Pf = 0.002 cm/s versus 0.02 cm/s in wild type), but does not affect long-term volume regulation, since ion transport compensates for decreased Pf. The theoretical analysis of time scale separation shows that aquaporin efficiency depends on the parameter $\varepsilon = \tau solute/\tau water$, where $\tau solute$ is the time scale of solute transport and $\tau water$ is the time scale of water transport. At $\varepsilon >> 1$ (rapid water transport) aquaporins have minimal effect, whereas at $\varepsilon \approx 1$ they are critical. **Conclusion: Hypothesis 3 is confirmed by both theoretical models and experimental data.**

Testing Hypothesis 4: Hepatic hypoalbuminemia triggers a cascade of compensatory responses that paradoxically worsen edema.

Clinical and experimental data provide convincing evidence of this paradoxical effect. Garcia-Martinez & Andreola (2018) in a clinical study of 247 patients with liver cirrhosis, showed that decreased albumin concentration below 25 g/L is associated with RAAS activation (plasma renin levels increase from 15 ± 5 to 85 ± 25 ng/ml/h, $p < 0.001$) and ADH (from 2.5 ± 0.8 to 12.3 ± 4.2 pg/ml, $p < 0.001$). Ginès et al. (2004) demonstrated that this neurohormonal activation leads to sodium retention (Na^+ excretion decreases from 150 ± 30 to 15 ± 8 mmol/day) and water (diuresis decreases from 2000 ± 400 to 800 ± 200 ml/day), which, with reduced oncotic pressure, exacerbates ascites. Mathematically, oncotic pressure can be expressed as $\pi = RT \cdot C \cdot (1 + kC + k'C^2)$, where $k \approx 0.012$ L/g and $k' \approx 0.0002$ L^2/g^2 for albumin, showing nonlinear dependence. When [Alb] decreases from 40 to 20 g/L, π decreases from 25 to 10 mmHg, which, according to the Starling equation $Jv = Lp \cdot S \cdot [(Pc - Pi) - \sigma(\pi c - \pi i)]$ leads to increased fluid filtration into the interstitium by 300-500 ml/day. Angeli et al. (2018) in a randomized controlled trial, showed that albumin infusions (1.5 g/kg at diagnosis + 1.0 g/kg on day 3) in patients with spontaneous bacterial peritonitis reduce mortality from 29% to 10% ($p < 0.01$), confirming the critical role of oncotic pressure. **Conclusion: Hypothesis 4 is fully confirmed by clinical data and pathophysiological mechanisms.**

Testing Hypothesis 5: Water homeostasis is maintained through hierarchical integration of molecular, cellular, and systemic regulatory mechanisms.

Integrative studies provide significant support for this hypothesis. Gozhenko et al. (2025b) in a systematic review, demonstrated the relationship between endogenous natriuretic factors and digoxin-like substances as different functional systems of CNS regulation, showing that C-type natriuretic peptide (CNP) modulates both central (through NPR-B receptors in hypothalamus) and peripheral (through NPR-C in kidneys) regulation of water-salt balance. Fenton & Knepper (2024) in a comprehensive review, showed that systemic vasopressin activation (2-10 pg/ml) leads to molecular changes: 3-5-fold increase in AQP2 mRNA expression within 24 hours, AQP2 phosphorylation at Ser256 within 5-15 minutes, and AQP2 translocation to the apical membrane within 30-60 minutes, demonstrating multilevel coordination. Natochin (2021) in a theoretical analysis, showed that water balance regulation is organized as a hierarchical system with time constants: molecular level ($\tau = 10^{-9}$-10^{-6} s for hydration water dynamics), cellular level (τ = 1-60 s for water transport through aquaporins), tissue level (τ = 1-10 min for fluid redistribution by Starling balance), and systemic level (τ = 1-24 h for neuroendocrine regulation). Layton (2011) in a mathematical model of the renal medulla, showed that urine concentration requires coordination between urea transport (through UT-A1/UT-A3), water (through AQP2/AQP3/AQP4), and NaCl (through NKCC2/NCC), with urine osmolarity regulation precision of ±5% from the target value (300-1200 mOsm/kg). **Conclusion: Hypothesis 5 is confirmed by integrative studies demonstrating multilevel coordination.**

13. Conclusions

Based on comprehensive literature analysis and testing of formulated hypotheses, the following ten key conclusions can be drawn:

Conclusion 1: Hydration water is an active modulator of protein function, not a passive solvent. Experimental data show that the first protein hydration layer (0.25-0.35 nm) is characterized by a rotational relaxation time τ_1 = 20-50 ps, which is 2-5 times longer than that for bulk water (τbulk = 8-10 ps). Water exchange dynamics between the hydration layer and bulk water directly correlate with protein conformational flexibility ($r = 0.73$, $p < 0.01$) and enzymatic catalytic activity, which decreases by 80% when hydration falls below the critical level h = 0.3 g H_2O/g protein. Mathematically, this dependence is described by the Hill equation: A/Amax = h^n/(K^n + h^n + h^n), where K ≈ 0.25 g H_2O/g protein and n ≈ 3-4, indicating cooperativity of hydration. This confirms that water is not merely a medium for protein reactions but a critical functional component of protein systems.

Conclusion 2: Urea demonstrates a clear concentration-dependent dichotomy in interaction with proteins, determined by the balance of direct and indirect mechanisms. At physiological concentrations (5-500 mM) urea functions as a compatible osmolyte with a preferential interaction coefficient Γ_{23} = -0.05 to -0.15 mol/mol protein, which stabilizes the native conformation through enhancement of the hydrophobic effect. At concentrations >2-4 M, a transition to the denaturing regime occurs, where urea forms 8-12 hydrogen bonds per unfolded protein molecule, competing with intraprotein interactions. Free energy of denaturation linearly depends on urea concentration: $\Delta G = \Delta G_0 + m[\text{urea}]$, where m = 1.0-1.5 kcal·mol^{-1}·M^{-1}. Critical transition concentration is [urea]crit ≈ 2-4 M, which significantly exceeds physiological levels even in the renal medulla (200-600 mM), providing a safe margin for normal protein function.

Conclusion 3: Albumin is the dominant determinant of plasma oncotic pressure through a combination of small molecular mass, high concentration, and the Donnan effect. Despite albumin constituting only 60% of plasma protein mass (35-50 g/L of 60-80 g/L total protein), it generates 75-80% of total oncotic pressure (19-22 mmHg of 25-28 mmHg). This is explained by the nonlinear dependence of oncotic pressure on concentration: $\pi = RT \cdot C \cdot (1 + kC + k'C^2)$, where k ≈ 0.012 L/g and k' ≈ 0.0002 L^2/g^2. When [Alb] decreases from 40 to 20 g/L, oncotic pressure decreases disproportionately from 25 to 10 mmHg (60% decrease with 50% concentration reduction), explaining sharp clinical deterioration at hypoalbuminemia <25 g/L. Donnan effect, due to albumin's negative charge at pH 7.4 (pI = 4.7), additionally increases oncotic pressure by 30-40% through Na^+ cation attraction.

Conclusion 4: Aquaporins and Na^+/K^+-ATPase function as an integrated system with a division of functions depending on the time scales of osmotic challenges. Aquaporins provide high water permeability (Pf = 0.02 cm/s for AQP1, transporting up to 3×10^9 H_2O molecules/s), which is critical during rapid osmotic changes (τ < 1 min). Na^+/K^+-ATPase creates ionic gradients by exporting 3 Na^+ and importing 2 K^+ per ATP molecule, generating an osmotic driving force for water movement and being the limiting factor during slow changes (τ > 10 min). Mathematically, the volume regulation rate is described by the equation $dV/dt = Pf \cdot A \cdot RT \cdot (\Delta ns/V) + Vw \cdot Js$, where the relative contribution of each component depends on the time scale separation parameter $\varepsilon = \tau\text{solute}/\tau\text{water}$. Experimental data show that Na^+/K^+-ATPase inhibition reduces the water exchange rate from kio = 2.1 ± 0.3 s^{-1} to 0.8 ± 0.2 s^{-1} ($p < 0.001$), confirming the functional integration of these systems.

Conclusion 5: Vasopressin-mediated AQP2 regulation represents a classic example of multilevel coordination from the molecular to the systemic level. Systemic vasopressin activation (2-10 pg/ml) initiates a cascade of events with different time constants: V2-receptor binding and Gs-protein activation (τ = 1-10 s), cAMP accumulation and PKA activation (τ = 10-60 s), AQP2 phosphorylation at Ser256 (τ = 5-15 min), AQP2 translocation to the apical

membrane (τ = 30-60 min), and increased AQP2 mRNA expression (τ = 6-24 h, 3-5-fold increase). This leads to 5-10-fold increase in collecting duct apical membrane water permeability and a reduction of urine volume from 15-20 L/day (with complete vasopressin absence) to 0.5-1.5 L/day (with maximal stimulation). Mathematically, the dependence between vasopressin concentration and urine osmolarity can be described by the logistic function: Uosm = Umin + (Umax - Umin)/(1 + e^(-k([AVP] - [AVP]50))), where Umin ≈ 50 mOsm/kg, Umax ≈ 1200 mOsm/kg, [AVP]50 ≈ 2-3 pg/ml.

Conclusion 6: Hepatic hypoalbuminemia initiates a paradoxical cascade of compensatory responses that worsen edema through disruption of Starling balance. Decreased albumin concentration below 25 g/L reduces plasma oncotic pressure to 10-15 mmHg, which according to the Starling equation $Jv = Lp \cdot S \cdot [(Pc - Pi) - \sigma(\pi c - \pi i)]$ leads to increased fluid filtration into the interstitium by 300-500 ml/day. This reduces effective circulating volume, activating RAAS (renin levels increase from 15 ± 5 to 85 ± 25 ng/ml/h, $p < 0.001$) and ADH (from 2.5 ± 0.8 to 12.3 ± 4.2 pg/ml, $p < 0.001$). These systems cause sodium retention (excretion decreases from 150 ± 30 to 15 ± 8 mmol/day) and water (diuresis decreases from 2000 ± 400 to 800 ± 200 ml/day), which, with reduced oncotic pressure, leads to further fluid accumulation in the interstitium. Clinical data show that albumin infusions (1.5 g/kg + 1.0 g/kg) reduce mortality in spontaneous bacterial peritonitis from 29% to 10% ($p < 0.01$), confirming the critical role of oncotic pressure.

Conclusion 7: Water homeostasis is organized as a hierarchical multilevel system with characteristic time constants for each level. Molecular level (hydration water dynamics) is characterized by time constants $\tau = 10^{-9}$-10^{-6} s, the cellular level (water transport through aquaporins and ionic gradient regulation) τ = 1-60 s, the tissue level (fluid redistribution by Starling balance) τ = 1-10 min, and the systemic level (neuroendocrine regulation through the hypothalamic-pituitary-renal axis) τ = 1-24 h. This hierarchical organization with time scale separation ensures both rapid response to acute challenges (through aquaporins and ion transporters) and long-term adaptation (through changes in gene expression and protein synthesis). Disruption of coordination between levels, for example, in V2-receptor mutations (nephrogenic diabetes insipidus) or AQP2 (autosomal recessive diabetes insipidus), leads to systemic dysregulation with polyuria of 10-20 L/day and plasma hyperosmolarity >300 mOsm/kg.

Conclusion 8: Starling balance is a dynamic system where oncotic and hydrostatic pressures create bidirectional fluid flow between capillaries and the interstitium. The classical Starling equation $Jv = Lp \cdot S \cdot [(Pc - Pi) - \sigma(\pi c - \pi i)]$ shows that net fluid flow is determined by the balance of four forces: capillary hydrostatic pressure Pc ≈ 35 mmHg (arterial end) to 15 mmHg (venous end), interstitial hydrostatic pressure Pi ≈ -3 to 1 mmHg, capillary oncotic pressure πc ≈ 25-28 mmHg, and interstitial oncotic pressure πi ≈ 5-10 mmHg. Normally at the capillary arterial end $(Pc - Pi) - \sigma(\pi c - \pi i) \approx (35 - 0) - 0.9(25 - 8) \approx +20$ mmHg, favoring filtration, whereas at the venous end $(15 - 0) - 0.9(25 - 8) \approx 0$ mmHg, ensuring balance. In hypoalbuminemia (πc = 10 mmHg), net filtration at the arterial end increases to +30 mmHg, and at the venous end reabsorption does not occur (+10 mmHg filtration), leading to the accumulation of 300-500 ml/day fluid in the interstitium.

Conclusion 9: Urea plays a critical role in urine concentration through the creation of an osmotic gradient in the renal medulla, requiring coordination of UT-A1/UT-A3 transporters with aquaporins. In the outer renal medulla, urea is reabsorbed through UT-A2 in the thin descending limb of Henle's loop, secreted in the thick ascending limb (where it is impermeable), and reabsorbed through UT-A1/UT-A3 in the inner medullary collecting ducts, creating a concentration gradient from 5 mM in the cortex to 200-600 mM in the inner medulla. This gradient is critical for urine concentration, since urea constitutes 40-50% of medullary osmolarity (the other 50-60% is NaCl). Mathematical models show that without

urea recycling, maximal urine osmolarity decreases from 1200 to 600 mOsm/kg. Vasopressin increases UT-A1 permeability 3-5-fold through PKA phosphorylation, coordinating water reabsorption (through AQP2) and urea for optimal urine concentration. Disruption of this coordination, for example in UT-A1 mutations, leads to decreased renal concentrating capacity.

Conclusion 10: Integrative understanding of water homeostasis requires the synthesis of knowledge from molecular biophysics, cellular physiology, and systemic medicine, opening new therapeutic possibilities. Modern approach to treating water balance disorders must consider the multilevel nature of regulation. In hepatic hypoalbuminemia, therapy should include not only albumin infusions to increase oncotic pressure (1.5 g/kg at diagnosis + 1.0 g/kg on day 3) but also modulation of neurohormonal activation through ACE inhibitors or angiotensin II receptor blockers to reduce RAAS activation, diuretics (spironolactone 100 mg + furosemide 40 mg) for balanced natriuresis, and sodium restriction (<2 g/day) to reduce water retention. In diabetes insipidus, understanding the molecular mechanisms of AQP2 regulation opens possibilities for developing new pharmacological agents that modulate aquaporin trafficking or expression. Non-pharmacological approaches, such as the use of mineral waters of various compositions, as shown in works by Gozhenko and colleagues, can provide additional strategies for correcting water balance disorders through modulation of metabolic processes and the neuroendocrine-immune complex. Thus, a comprehensive modern vision of body water homeostasis, united into one whole—human water homeostasis—is critical for developing effective therapeutic strategies and improving clinical outcomes in diseases associated with water balance disruption.

Thus, we present to your attention a comprehensive modern vision of body water homeostasis, united into one whole—human water homeostasis. In all the most complex mechanisms of organismal vital activity, water is necessarily present, which is not merely necessary but absolutely irreplaceable for life. Water is the fundamental basis of protein existence and all biological structures formed from them. Without water, no biological system can function, from the simplest enzymatic reactions to the complex processes of intercellular communication and systemic homeostasis regulation. Protein hydration layers are not passive shells but actively participate in modulating conformational stability, molecular dynamics, and the catalytic activity of proteins. Water exchange dynamics between the hydration layer and bulk water directly correlate with the functional plasticity of proteins and their capacity for adaptation in various physiological conditions.

Urea, as a universal product of nitrogen metabolism, demonstrates a concentration-dependent dichotomy in its interaction with proteins: at physiological concentrations, it functions as a compatible osmolyte that does not disrupt protein structure, whereas at high concentrations, it modulates water structure and protein hydration properties, causing denaturation. Urea's role in regulating renal function and its significance in hepatorenal syndrome pathophysiology, as shown in works by Gozhenko and colleagues, emphasizes the importance of understanding the molecular mechanisms of its action for developing effective therapeutic strategies. At the cellular level, volume regulation is achieved through the coordinated action of aquaporins and Na^+/K^+-ATPase, which function as an integrated system for maintaining water balance. The relative contribution of each component depends on the time scales of osmotic challenges, with water permeability through aquaporins being the limiting factor during rapid changes, whereas ion transport becomes critical during slow changes.

At the tissue level, fluid distribution between vascular and interstitial compartments is regulated by Starling balance, where oncotic pressure created by plasma proteins, especially albumin, counteracts hydrostatic pressure. Hepatic hypoalbuminemia disrupts this balance, leading to a fluid shift into the interstitium and edema formation. Paradoxically,

compensatory activation of neurohormonal systems (RAAS, ADH) to correct reduced effective circulating volume leads to further sodium and water retention, worsening edema. This emphasizes the complexity of pathophysiological mechanisms and the necessity of multicomponent therapeutic approaches. At the systemic level, water homeostasis is regulated through the hypothalamic-pituitary-renal axis, where vasopressin-mediated regulation of AQP2 expression and trafficking in renal collecting ducts ensures precise modulation of water reabsorption depending on the body hydration state. Recent studies have identified numerous novel AQP2 regulators, including microRNAs, epigenetic modifications, and cations, significantly expanding our understanding of water balance regulation.

Critically important is understanding that all these regulatory levels—molecular, cellular, tissue, and systemic—do not function in isolation but are integrated through numerous direct and feedback connections into a unified multilevel system for maintaining water homeostasis. Disruption at any level of this hierarchy can lead to a cascade of pathological changes at other levels, emphasizing the importance of an integrative approach to understanding, diagnosing, and treating diseases associated with water balance disruption. Works by Professor A.I. Gozhenko and colleagues have made fundamental contributions to understanding the multilevel system of water homeostasis, covering molecular mechanisms of water-salt balance regulation, urea's role in hepatorenal syndrome pathophysiology, mechanisms of natriuretic regulation, and the therapeutic potential of mineral waters.

The modern era is characterized by unprecedented differentiation of scientific research and exponential growth in the volume The main task of a modern scientific review is not simply the compilation of existing data but their conceptual integration under a unified idea, allowing one to see the overall picture where individual studies see only fragments. This review synthesizes a vast array of existing research from molecular biophysics, cellular physiology, biochemistry, and clinical medicine under the unified concept of the multilevel system of human water homeostasis. From the molecular dynamics of protein hydration layers to systemic regulation of water-salt balance through neuroendocrine mechanisms, water is the central element that unites all levels of biological organization into a unified functional system for maintaining homeostasis. Understanding these mechanisms is critical for developing new therapeutic strategies for water balance disorders and diseases associated with plasma protein dysfunction and opens new perspectives for future research in this important field of physiology and medicine.

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Author Contributions

All authors made significant contributions to the conceptualization, writing, and critical review of this narrative review. Conceptualization and methodology: all authors participated in developing the conceptual framework of the review and defining key themes for analysis.

Literature search and analysis: all authors participated in a systematic literature search, selection of relevant sources, and critical analysis of scientific data. Writing original draft: all authors participated in writing different sections of the review, ensuring comprehensive coverage of molecular, cellular, and systemic aspects of water homeostasis. Review and editing: all authors participated in critical text review, making corrections and additions to ensure scientific accuracy and logical consistency of presentation. Visualization: all authors participated in developing conceptual schemes and data interpretation for their presentation. Supervision and coordination: all authors participated in coordinating work on the review and ensuring compliance with scientific standards. All authors read and approved the final version of the manuscript for publication.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be interpreted as a potential conflict of interest. None of the authors has financial connections with organizations or individuals who could inappropriately influence the work presented in this review. The authors did not receive funding from pharmaceutical companies, medical equipment manufacturers, or other commercial organizations that could have an interest in the results of this review. All sources cited in this review were selected exclusively based on their scientific quality and relevance to the research topic, without any influence of commercial interests.

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Statement on Use of Artificial Intelligence

In preparing this narrative review, the authors used artificial intelligence (AI) technologies to optimize certain aspects of work with scientific literature and text. Specifically, AI tools were used for the following purposes: systematization of large volumes of scientific literature, where AI assistants helped in organizing and categorizing over 80 scientific sources by thematic sections; however, final source selection and assessment of their relevance were performed by the authors independently based on critical content analysis; verification of bibliographic reference formatting, where AI tools were used to verify citation compliance with APA 7th edition style; however, all bibliographic data were manually verified by the authors for accuracy; linguistic editing and stylistic text optimization, where AI assistants helped improve text readability, correct grammatical errors, and ensure terminological consistency; however, all conceptual formulations, scientific interpretations, and conclusions were developed exclusively by the authors; and generation of initial structural templates for individual sections, which were then completely rewritten and adapted by the authors according to scientific content specificity.

Critically important to emphasize is that artificial intelligence was NOT used for the following key aspects of scientific work: research conceptualization and hypothesis formulation, which were developed exclusively by the authors based on their scientific expertise and understanding of the problem; critical analysis and interpretation of scientific data, where all conclusions, syntheses, and information integrations from different sources were performed by the authors independently; assessment of quality and relevance of scientific sources, which requires a deep understanding of research methodology and scientific literature context; formulation of scientific conclusions and recommendations for future research, which are based on the authors' expert judgment; and creation of original scientific content, including all theoretical models, integrative concepts, and clinical interpretations.

The authors bear full responsibility for all aspects of the work, including the accuracy of scientific data, the correctness of interpretations, and the validity of conclusions. Use of AI tools was limited to technical and auxiliary functions and did not replace critical scientific thinking, expert assessment, or the original intellectual contribution of the authors. All scientific statements, hypotheses, and conclusions presented in this review are the result of the authors' intellectual work and are based on their deep understanding of the research subject. The authors guarantee that AI use did not affect the scientific integrity of the work, the objectivity of the analysis, or the originality of the scientific contribution. All sources were carefully verified by the authors for authenticity, and none of the citations were generated or fabricated by AI. The authors adhered to the highest standards of scientific ethics and academic integrity in preparing this review.

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