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The role of hyperosmotic stress in inflammation and disease

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Abstract

Hyperosmotic stress is an often overlooked process that potentially contributes to a number of human diseases. Whereas renal hyperosmolarity is a well-studied phenomenon, recent research provides evidence that many non-renal tissues routinely experience hyperosmotic stress that may contribute significantly to disease initiation and progression. Moreover, a growing body of evidence implicates hyperosmotic stress as a potent inflammatory stimulus by triggering proinflammatory cytokine release and inflammation. Under physiological conditions, the urine concentrating mechanism within the inner medullary region of the mammalian kidney exposes cells to high extracellular osmolarity. As such, renal cells have developed many adaptive strategies to compensate for increased osmolarity. Hyperosmotic stress is linked to many maladies, including acute and chronic, as well as local and systemic, inflammatory disorders. Hyperosmolarity triggers cell shrinkage, oxidative stress, protein carbonylation, mitochondrial depolarization, DNA damage, and cell cycle arrest, thus rendering cells susceptible to apoptosis. However, many adaptive mechanisms exist to counter the deleterious effects of hyperosmotic stress, including cytoskeletal rearrangement and up-regulation of antioxidant enzymes, transporters, and heat shock proteins. Osmolyte synthesis is also up-regulated and many of these compounds have been shown to reduce inflammation. The cytoprotective mechanisms and associated regulatory pathways that accompany the renal response to hyperosmolarity are found in many non-renal tissues, suggesting cells are commonly confronted with hyperosmotic conditions. Osmoadaptation allows cells to survive and function under potentially cytotoxic conditions. This review covers the pathological consequences of hyperosmotic stress in relation to disease and emphasizes the importance of considering hyperosmolarity in inflammation and disease progression.

Keywords

disease; hyperosmotic stress; inflammation; osmoadaptation

Introduction

Mammalian cells and tissues have developed a number of adaptive mechanisms to compensate for increases in extracellular osmolarity. Osmolarity (Osm) is used to describe the number of solute molecules per solution volume or solution weight. The latter is also often referred to as osmolality. The total number of particles or solutes influences the

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osmotic pressure exerted by a given solution. In biological systems, semipermeable membranes facilitate the separation of two solutions with varied compositions. The ability to regulate and preserve distinct intracellular and extracellular solute microenvironments is crucial in maintaining cellular homeostasis. The osmolarity of human serum is restricted within a tightly regulated range (285–295 mOsm/kg) and, by convention, termed isotonic because this describes the extra- and intracellular osmolarity found within most tissues (1). Fluids with osmolarities above or below this range are referred to as being hypertonic or hypotonic, respectively. Some tissues, including the kidney and gastrointestinal tract, are exposed to significant fluctuations in osmolarity as a direct consequence of normal, physiological processes. An imbalance between extracellular and intracellular fluid osmolarity, and therefore osmotic pressure, is the underlying cause of osmotic stress. By definition, when extracellular fluid osmolarity is greater than that of the intracellular fluid, cells and tissues experience hyperosmotic stress. Conversely, hypoosmotic stress describes the situation where intracellular solute concentrations exceed those outside the cell. Such osmotic imbalances detrimentally affect water flux, cell volume, and cell homeostasis. When osmoadaptive responses fail to compensate for solute concentration asymmetry, negative consequences manifest and potentially contribute to inflammation and disease. The issue of hypertonicity and hyperosmotic stress response is well investigated within the kidney owing to the unique environment encountered within this specialized organ. More recently, studies have revealed that non-renal tissues commonly experience hyperosmotic stress, especially under pathological conditions. Furthermore, a number of studies have found a strong association between microenvironmental hypertonicity and inflammation. This review will focus on hyperosmotic stress-related pathologies; however, recent evidence also suggests hypoosmotic stress can act as an inflammatory stimulus and is also associated with a number of disorders, including acetaminophen toxicity and brain edema (2–4).

An increase in extracellular osmolarity has many damaging effects on cells by promoting water flux out of the cell, triggering cell shrinkage, and intracellular dehydration (5). The loss of intracellular water adversely affects protein structure and function, a consequence of which is altered enzyme activity. Cell shrinkage places a great deal of mechanical stress on the cytoskeleton as well as on the nucleus (6). DNA strand breaks trigger activation of growth arrest and DNA damage (*GADD*)-inducible genes, such as *GADD45* and *GADD153*(6). p53 expression and activation also increases during exposure to hyperosmotic stress (6). Together, up-regulation of these proteins results in cell cycle arrest. Protein translation and degradation are also significantly hindered, in addition to transcription (6). As damage accumulates, cells become primed for, and ultimately undergo, apoptosis (6). Generally speaking, the degree of damage is proportional to the degree of osmotic imbalance. However, studies have shown that certain compounds exhibit solute-specific effects and, similarly, elicit a solute-specific response (6). The responsiveness to hyperosmotic stress varies between cell types and tissues, suggesting that, although there may be a general response mechanism shared by cells, pathway activation and overall outcome differs from cell to cell.

Cells have developed several adaptive response mechanisms to counter hyperosmotic stress and restore osmotic equilibrium, including induction of genes involved in the synthesis and transport of osmolytes. Osmolytes or, more specifically, ‘compatible osmolytes’, are small, inert, organic molecules concentrated intracellularly to counter water flux out of the cell and restore osmotic balance. Many osmolytes also serve as chemical chaperones and preserve protein structure and function under non-optimal conditions (7). For example, heat shock protein expression increases, presumably to provide additional protein stability (6). Antioxidant enzymes are up-regulated as a result of increased reactive oxygen species (ROS) generation (8). Cytoskeletal remodeling also takes place to offset the increase in mechanical stresses imposed on the cell surface by excessive osmotic pressure (6). Adhesion

molecules, such as integrin β_1 and CD9, are also up-regulated (6). Although cells and tissues have developed elaborate response mechanisms to offset the damaging effects caused by hyperosmotic stress, acute or chronic hypertonicity can prime cells for apoptosis and stimulate the release of pro-inflammatory cytokines to promote tissue damage and inflammation.

Most studies investigating osmotic challenge have focused on the kidney where the renal concentrating mechanism can lead to an extracellular osmolarity in excess of 1200 mOsm/kg (7). However, more recent studies have discerned that many tissues, especially those that are metabolically active, do, in fact, encounter and respond to hyperosmotic stress. In addition to the kidney, the cornea, liver, gastrointestinal tract, intervertebral discs, and joints are exposed to hyperosmotic fluids under non-pathogenic conditions (9). The osmolarity of most physiological fluids is regulated within a relatively narrow range and slight perturbations outside this range can have profound consequences (7, 8). As a result, cells and tissues have developed sensory and signaling systems that monitor and precisely regulate fluid osmolarity. Despite this regulation, many non-renal tissues are often exposed to hyperosmolar environments. For example, both liver and lymphoid tissues are hyperosmotic when compared with serum under physiological conditions (10). In addition, these tissues and many others express nuclear factor of the activated T cells-5 (NFAT5) in response to a hyperosmotic environment. As it turns out, NFAT5 is the predominant transcription factor activated in response to cellular hyperosmotic stress (11). As previously mentioned, hyperosmolarity stimulates the release of pro-inflammatory cytokines from immune and epithelial cells. Only a small increase in extracellular osmolarity above the physiological range is necessary to elicit cytokine secretion (11). Studies have also shown that many osmolytes synthesized in response to osmotic stress can reduce inflammation (12–17). A number of disorders are associated with local and systemic elevations in extracellular fluid osmolarity, including diabetes, inflammatory bowel disease, hypernatremia, and dry eye syndrome, to name a few (11, 18–20). All of these conditions are tightly linked to inflammatory processes. Additional studies have found a positive correlation between elevated patient plasma osmolarity and obesity, aging, impaired glucose tolerance, as well as diabetes (21, 22). Over recent years, evidence has been accumulating that shows local and systemic hyperosmolarity may contribute significantly to acute and chronic inflammation (11, 23, 24). It is apparent from these studies that underlying elevated extracellular osmolarity can be a driving factor behind the initiation and progression of many human diseases.

Systemic water balance

Fluctuations in osmolarity can detrimentally affect a wide range of cellular and systemic processes. Homeostatic osmolarity is tightly regulated through the release of the antidiuretic hormone (arginine vasopressin, AVP) by the posterior pituitary. AVP release into the bloodstream is triggered by an increase in plasma osmolarity or a reduction in extracellular fluid volume, both of which induce osmosensory neuron stimulation. These neurons, found within the hypothalamus, possess mechanoreceptors activated by changes in cell volume, such as cell shrinkage (25). The kidney is a primary site of action for AVP where it regulates fluid and electrolyte balance through modulation of renal tubular water reabsorption. In addition, AVP increases arterial blood pressure by inducing constriction of arterial smooth muscle cells. Through activation of AVP receptor 2 in the renal medullary collecting duct cells, AVP stimulates urine concentration by promoting water reabsorption from the collecting duct. This process is due, in part, to aquaporin-2 (AQP2) water channel trafficking (6, 25). Through such mechanisms, the kidney plays a critical role in maintaining fluid balance in the body. Environmental insults, disease, and genetic factors can lead to

abnormal kidney function. As kidney function diminishes, so does the ability to regulate and maintain systemic fluid osmolarity.

Hyperosmotic stress-induced cell damage

Elevated fluid osmolarity can negatively affect cells in a variety of ways. Osmotic imbalance initially manifests as cell shrinkage as water moves out of the cell. Intracellular water loss disrupts many homeostatic processes, including DNA synthesis and repair, transcription, protein translation and degradation, as well as mitochondrial function. As a result, cell cycle progression and cell proliferation are halted. There is a concomitant increase in oxidative stress and activation of apoptotic pathways (5, 26). Nuclear shrinkage accompanies overall cell shrinkage and the nucleus assumes a convoluted shape. As cell and nuclear volumes decrease, intracellular macromolecule concentrations increase significantly (6, 7). Nuclear alterations brought about by extracellular changes in osmolarity have profound effects on many processes, including chromatin condensation and nucleocytoplasmic transport. Hypertonicity causes DNA strand breaks and activates G_2 and G_1 cell cycle check points (27). Mitogen-activated protein kinase 14 (MAPK14, also termed p38 MAPK) mediates G_2 phase delays in response to increasing NaCl concentrations (6). The cell cycle delays associated with G_1, as well as S phase, are attributed to ataxia telangiectasia mutated (ATM)-mediated p53 phosphorylation, p21 induction, and retinoblastoma protein hypophosphorylation (27). It is also interesting to note that the underlying signaling pathways closely parallel those activated during ultraviolet radiation damage (6).

Hyperosmotic stress and apoptotic cell death are both characterized by cell shrinkage, and there are similarities between the signaling pathways found within the two processes. Increases in hypertonicity are known to trigger both autophagy and apoptosis *in vitro* and *in vivo* (6, 28). Hypertonicity-induced cell death is characterized by many classic apoptotic features, including nuclear condensation, DNA fragmentation, caspase activation, the appearance of apoptotic bodies, and extracellular phosphatidylserine exposure. Both intrinsic and extrinsic apoptotic signaling pathways appear to be activated during prolonged hyperosmotic stress (6).

Protein translation and degradation are significantly influenced by increases in extracellular osmolarity. Unlike activation of signaling pathways, the degree of translation inhibition appears to be independent of the specific solute responsible for the imbalance (6). A buildup of polyubiquitinated proteins is also observed in cells exposed to hyperosmotic stress. Mechanistically, this buildup was recently shown to be mediated by the MAPK14-dependent phosphorylation of the proteasome subunit Rpt2 (29).

Osmoprotective signaling pathways

The initial event triggering osmotic stress response signaling within mammalian cells is not completely understood. In yeast, two structurally distinct and functionally independent cell surface osmosensing receptor systems are present (10, 11). Each system directly monitors and responds to changes in extracellular osmolarity; however, the two pathways, referred to as the Sln1 branch and the Sho1 branch, differ mechanistically. The Sho1 branch takes advantage of the Rho-type small G-protein, Cdc24, to propagate downstream signaling cascades, whereas the Sln1 branch uses a multistep phosphorelay mechanism (30). The two yeast pathways converge through activation of Pbs2, a MAPKK homologous to mammalian MAP2K4 (also known as MKK4). Pbs2, in turn, activates Hog1 MAPK, the homologue of mammalian MAPK14 (31). To date, mammalian osmosensory cell surface receptor proteins analogous to those found in yeast have not been identified. There is strong evidence supporting the presence of active adaptive response pathways to hyperosmotic stress in most

mammalian cell types and tissues. T cells, B cells, macrophages, neurons, myoblasts, fibroblasts, vascular smooth muscle cells, and epithelial cells all take action against hyperosmotic stress through the use of analogous intracellular machinery and signaling pathways (11, 32–34). While the signaling pathways mediating the mammalian cellular response to hypertonicity are not completely understood, studies indicate the involvement of a large heteromeric protein complex. Proteins within the complex include Rho-type small G-proteins and protein kinases (including MAPK14), suggesting the transduction pathway may be functionally analogous to the Sho1 branch characterized in yeast (31). A kinase anchor protein 13 (AKAP13, also termed Brx) is associated with the complex and serves as a guanine exchange factor (GEF) for the Rho-type small G-proteins (35). Recent studies have revealed the requirement of this GEF for the cellular response to hyperosmotic stress through stimulation of the MAPK14 signaling cascade. In addition, AKAP13 interacts with sperm associated antigen 9 (SPAG9, also termed JIP4), a scaffolding protein known to facilitate interactions between up- and down-stream signaling factors and MAPK14 (9). The specific activation pathways used appears to be cell type specific and involves various MKKs and MKKKs. Identified upstream kinases include MAP2K3 (also termed MKK3), MAP2K6 (also termed MKK6), and MAP3K3 (also termed MEKK3) (36). A recent *in vitro* study identified that a focal adhesion protein, known as tensin-1, may play an important role in linking MAPK14-, tyrosine kinases-, and RhoGTPases-related signaling pathways within the liver (37). The presence of a focal adhesion protein within this complex hints at the possibility that the changes in cell shape caused by hyperosmolarity may act as an initiating event in pathway activation. Another study revealed elevation of mitogen-activated MAPK phosphatase (MKP-1) expression in hyperosmotically challenged rat hepatoma cells with the up-regulation being preceded by activation of the MAP kinases Erk-1, Erk-2, and JNK-2 (6). Insulin is known to induce robust up-regulation of MKP-1. Interestingly, hyperosmolarity delays MKP-1 accumulation and impairs synthesis after insulin treatment when compared with insulin-treated cells under isosmotic conditions (38). These data suggest that the systemic hyperosmolarity experienced by diabetes patients could significantly contribute to insulin resistance by altering downstream signaling pathways.

A major function of signaling through the above pathways is activation of NFAT5 by phosphorylation. NFAT5, previously known as tonicity-responsive element binding protein (TonEBP) or as osmotic response element binding protein (OREBP), is considered the principal transcription factor activated in response to osmotic stress. An increase in intracellular ionic strength caused by extracellular hyper-tonicity appears to directly influence a transactivation domain (TAD) found within the C-terminus of NFAT5 (6). *In vitro* studies suggest that this TAD is regulated osmotically through a tonicity-dependent phosphorylation event. Dimerization and nuclear translocation are also required for transactivation of NFAT5 target genes. A nuclear localization signal, nuclear export signal, and auxiliary export domain were identified within the amino terminus of NFAT5 (39). In addition, ATM kinase activity was shown to significantly contribute to nuclear translocation of NFAT5 (40). However, it remains unclear whether translocation is a direct consequence of ATM kinase-mediated phosphorylation of NFAT5 or indirectly by association with another factor in the signaling pathway (41). In either case, NFAT5 activation results in nuclear translocation and subsequent regulation of target genes, which include those associated with osmolyte transport and synthesis, antioxidant defense, as well as many molecular chaperones.

NFAT5 is a member of the NF-κB/Rel family of transcription factors. This family comprises well-known regulators of many genes intimately involved in immune and inflammatory responses. NFAT5 expression has been identified in many tissues, including the kidney, brain, and thymus (42). Environmental stimuli activate the MAPK14 pathway, which, among other effects, results in increased expression of NFAT5. As noted,

extracellular hypertonicity also causes NFAT5 phosphorylation. Proteins implicated in NFAT5 phosphorylation include casein kinase 1, cyclin-dependent kinase 5 (CDK5), ATM kinase, c-Abl kinase, phosphatidylinositol 3-kinase class IA, Fyn kinase, MAPK14, and protein kinase A (6, 43–45). Upon phosphorylation, NFAT5 translocates from the cytoplasm into the nucleus and controls target gene expression (6). NFAT5 regulates gene transcription by binding to a highly conserved sequence known as a tonicity-responsive enhancer (TonE, also referred to as an osmotic response element, ORE) within the target gene's promoter region. The mammalian TonE consensus sequence is 5′-NGGAAAWDHMC(N)-3′ and the element is often repeated multiple times within a target gene's promoter region (6). NFAT5 knockout mice are embryonic lethal, underscoring the protein's physiological importance (10). Interestingly, embryonic lethality was observed in both mixed 129/sv-C57BL/6 and isogenic C57BL/6 backgrounds; however, survival rates increased significantly when bred into a pure 129/sv background, suggesting genetic background is an important factor influencing NFAT-null lethality (46). The surviving adult 129/sv NFAT knockout mice exhibit marked hypernatremia, or elevated plasma sodium levels (leading to hypertonicity), and severe immunodeficiency (46). Results from the same study indicated that systemic hypernatremia was a major factor contributing to immunodeficiency in NFAT knockouts. It was recently reported that miRNAs play a role during hypertonicity-induced, NFAT5-mediated signal transduction (47). Researchers have found that the elevation of two miRNAs, miR-200b and miR700, result in a significant decrease in *NFAT5* gene transcription. NFAT5 mRNA and protein levels also dropped, underscoring the complexity of the signaling systems driving osmotic response. Most studies have focused on the role of NFAT5 within the renal medulla. However, NFAT5 expression is noted in many tissues, implying a fundamental, more global requirement for hyperosmotic stress response pathways (10). NFAT5 is responsible for the up-regulation of a number of genes that influence an even wider spectrum of biological processes in a variety of tissues and cell types (Table 1). It is important to note, however, that recent studies have indicated hypertonicity-independent regulation of NFAT5 by cytokines, growth factors, and ROS, implying NFAT5-mediated gene regulation may represent a more universal response mechanism to various cellular signals and stressors (48).

Hyperosmotic stress and adaptive response

Under physiological conditions, the osmolarity of extracellular fluid in mammals remains extremely stable despite large fluctuations in water and solute intake and excretion. As extracellular fluid osmolarity increases, osmosensory receptors become activated and trigger downstream signaling pathways. A number of compensatory and adaptive mechanisms exist to maintain and restore a cell's original volume and to counter the damaging consequences caused by osmotic imbalance (Figure 1). In response to an increase in extracellular tonicity, cells initially activate transporters to increase intracellular ion concentrations. Non-selective cation channels become activated and promote an influx of Na^+ ions (6). The resultant increase in intracellular ions counteracts cell shrinkage through osmosis. However, such a compensatory ion movement severely disrupts intracellular ion homeostasis. As a secondary response, inorganic ions are continuously replaced by small organic compounds known as 'compatible' osmolytes. In such a response, solute carrier family proteins (including SLC2A4, SLC5A3, SLC6A8, SLC9A1) are activated and result in increased transport of a wide variety of compounds, such as choline, creatine, myoinositol, and glucose (49). These compounds either directly act as osmolytes or serve as precursors for osmolyte synthesis. Hyperosmotic stress-mediated ERK activation induces *AQP1* and *AQP5* gene expression to facilitate water movement (50, 51). AQP1 expression also has been shown to involve p38 kinase and JNK kinase in renal medullary cells grown *in vitro* (6). Additional studies identified NFAT-mediated up-regulation of both AQP1 and AQP2 (52–54). In astrocytes, hyperosmolarity stimulates p38 MAPK-dependent up-regulation of AQP4 and AQP9,

suggesting AQP isoform induction may be cell type and/or tissue specific (55). Channel and transporter activation is accompanied by increased expression of channel and transporter proteins as well as enzymes involved in osmolyte synthesis. Together, coordinated water, ion, and osmolyte transport responses restore the osmotic equilibrium between the intra- and extracellular environments. Nuclear transport proteins are also up-regulated in response to hypertonic stress. For example, inner medullary collecting duct 3 cells exposed to acute osmotic stress exhibited up-regulation of nucleoporin 88 (56). Up-regulation of this nuclear pore protein facilitates retention of NFAT5 within the nucleus.

As noted previously, hyperosmotic stress initially influences cell shape through the loss of intracellular water. Mechanical stress caused by cell shrinkage play an important role in the cellular response to changes in osmolarity. It remains unclear whether mechanical stress directly activates signaling pathways associated with hyperosmotic stress response as no extracellular receptors have been identified. However, some proteins (including transmembrane channels) are directly influenced by mechanical stimuli, an example of which is the transient receptor potential vanilloid (TRPV) family of receptors. The Ca^{2+}-permeable, transient receptor potential vanilloid (TRPV1) ion channel can be osmotically activated through mechanical processes associated with water loss and subsequent alterations in cell shape (57). Recently, the magnitude of TRPV1 channel activation was found to be proportional to actin filament density within the cell (58). TRPV4 was also recently found to play an important role in regulating liver tissue osmolarity (57). It is also highly expressed in chondrocytes (59). The observation that TRPV4 knockout mice exhibit osteoarthritic joint degradation and increased bone density is suggestive of an important role for this osmo-sensory protein (and potentially an osmotic stress response) in maintaining joint and skeletal health (60). TRPV4 was also recently found to play an important role in regulating liver tissue osmolarity and is highly expressed in hepatic sensory neurons (57). Interestingly, Ca^{2+} flux is required for NFAT5 activation and subsequent nuclear translocation in T cells. This controls T-cell activation and proliferation as well as cytokine gene expression, and further supports a possible connection between inflammation and osmotic stress (61).

Cells adapt to increases in hypertonicity through the up-regulation of osmoprotective genes (26). NFAT5-mediated transactivation regulates the majority of osmoresponsive genes, including those involved in osmolyte transport and synthesis, cytoskeletal remodeling, antioxidant response, and unfolded-protein response. Intracellular accumulation of organic osmolytes, including betaine, sorbitol, myoinositol, taurine, as well as a number of other compounds, is a key factor in protecting cells from hyperosmotic stress. NFAT5-regulated genes related to osmolyte accumulation include aldose reductase (*AR*) and patatin-like phospholipase domain-containing esterase (*PNPLA6*, also known as *NTE*), which participate in the synthesis of the osmolytes sorbitol and α-glycerophosphocholine (α-GPC), respectively. Expression of betaine and taurine transporters, as well as solute carrier family 5 (SLC5) transporters (inositol transporters, also known as SMITs), increases in response to hyperosmotic stress. The importance of osmolytes will be discussed in more detail in subsequent sections.

Cyclooxygenase 2 (COX2) is up-regulated in the liver, lung, and kidney cells *in vitro* in response to hyperosmotic stress (62–64). COX2 expression also increases during oxidative stress (62). This enzyme, normally expressed at low levels in most tissues, plays a key role in the biosynthesis of prostaglandins, especially during inflammation. In the kidney, COX2 is thought to play a major role in the synthesis of prostaglandin E_2 (PGE_2). PGE_2, like other prostaglandins, acts as a vasodilator and dilates the afferent arteriole, increasing glomerular filtration rates within the kidney (25). Studies have also shown that PGE_2 exerts antiapoptotic effects on tubular epithelial cells both *in vitro* and *in vivo* (65, 66). COX2 up-

regulation involves transactivation of the epidermal growth factor receptor (EGFR) (6). NF-κB, MAPK family members (including ERK, JNK2, and MAPK14), and Src kinases are also associated with hypertonicity-mediated COX2 gene regulation (6, 67).

Disruptions in water homeostasis directly contribute to an increase in ROS generation and oxidative stress (8). In response, antioxidant enzymes are also up-regulated. Several studies have shown heme oxygenase 1 (HMOX1) expression is elevated in response to hyperosmotic stress (68). HMOX1 expression also increases in response to other stresses associated with oxidative stress, such as heavy metal toxicity and cytokine secretion. There is currently no direct evidence showing NFAT5 regulation of HMOX1. Nevertheless, hypertonicity-induced HMOX1 expression is observed under conditions in which NFAT5 is known to be activated (68). NFAT5 is a potent regulator of cytochrome P450 3A (CYP3A) family members (69). CYP proteins are heme-containing mono-oxygenases that are principally responsible for metabolizing xenobiotics and toxins. CYP3A enzymes may play a role in osmolyte synthesis and/or aid in the removal of toxic compounds produced as a result of hypertonicity-induced oxidative stress; however, neither of these roles has been shown experimentally. Ironically, elevated CYP activity is associated with ROS production and thereby contributes to further cell damage (70). Hyperosmotic conditions increase expression of a number of other proteins with known antioxidant functions, including peroxiredoxin-2 (PRDX2), PRDX6, α-enolase, glyceraldehyde-3-phosphate dehydrogenase, and lactate dehydrogenase (71).

Water efflux out of the cell through osmosis also has profound effects on the structure of macromolecules. As water leaves the cell, intermolecular crowding occurs, subjecting macromolecules to mechanical stress. To counteract these structural changes, the cell up-regulates protein chaperones associated with the unfolded-protein response, such as heat shock 27 kDa protein 1 (HSPB1, also known as HSP27), HSP70, HSP90, HSP110, α(B)-crystallin (CRYAB), and heat shock protein 4-like (HSPA4L, also known as OSP94) (6, 71). In addition, HSP70 and HSP90 phosphorylation increases during hyperosmotic stress (72). Protein chaperones help stabilize protein structure and function. A number of studies have also shown that HSP expression prevents apoptosis, potentially giving the cell more time to adapt to the changing extracellular environment (73, 74).

Cytoskeletal rearrangement is a key adaptation in response to increased extracellular osmolarity. This is intended to maintain normal cell volume and to structurally reinforce cellular integrity. AMP-activated protein kinase (AMPK) is also associated with signaling pathways that control the actin rearrangements, a mechanism by which it regulates cell cycle, cell polarity, and cell migration (75). AMPK activation triggers F-actin predominance. Hypertonicity causes an increase in F-actin expression and cofilin phosphorylation (76). Cofilin is a member of the ADF/cofilin family of proteins, which are responsible for actin disassembly. Phosphorylation inhibits cofilin, encouraging F-actin polymerization and subsequent hypertonicity-induced cytoskeletal rearrangements. In addition to F-actin, β-actin, and α-actinin 4 (ACTN4) expression increases under hyperosmotic conditions (71). ACTN4 is a cytoplasmic protein found localized within adherens-type junctions and microfilament bundles where it helps bind actin to the plasma membrane. Hypertonicity has also been shown to elevate expression of several additional tight junction-related proteins, including multi-PDZ protein-1, zonula occludens 1, and afadin 6 within inner medullary cells (77).

NFAT5 regulates several genes, including vascular endothelial growth factor C (*VEGFC*), tumor necrosis factor α (*TNF*), and S100 calcium binding protein A4 (*S100A4*). The diverse functions of these molecules (angiogenesis, immune response, and tumor metastasis, respectively) provide a tantalizing insight into the vast array of processes that may be

influenced during osmoadaptation (78–80). Even relatively minor increases in extracellular hypertonicity can damage cells, thereby activating response pathways and subsequent NFAT5 target gene transactivation (6).

Osmolytes and inflammation

Organic osmolytes are characterized as inert compounds that can accumulate to high concentrations without perturbing cellular homeostasis. As such, they are often referred to as ‘compatible osmolytes’. Osmolyte accumulation within the cell equalizes intracellular osmotic pressure with that of the extracellular environment. It is also interesting to note that experiments have shown supplementation with a variety of osmolytes reduces inflammatory cytokine release and inflammation (12–17). Intracellular accumulation of these compounds has little effect on cellular homeostasis because most are neutral at physiological pH, i.e., either lack charge or are zwitterionic. Osmolyte accumulation prevents water flux out of the cell, thereby preserving cell volume. A key feature of the cellular response to hyperosmotic stress involves increasing the intracellular osmolyte concentration by (i) increasing osmolyte transport and/or (ii) increasing osmolyte synthesis. Osmolyte accumulation not only plays an important role in maintaining cell volume but also preserves and protects cellular homeostasis. Osmolytes act as chemical chaperones by stabilizing protein structure and thereby preserving enzyme function. These compounds are thought to promote folding of unstructured or denatured proteins through osmophobic interactions created between an unfolded or misfolded peptide backbone and the osmolyte (81). In addition, osmolytes help promote protein-protein interactions and protein-DNA interactions (82). Several classes of osmolytes have been identified, including polyols (e.g., sorbitol), methylamines (e.g., betaine glycine), and certain amino acids (e.g., taurine). While the common belief is that most osmolytes are interchangeable, recent studies suggest some compounds may be more advantageous under certain conditions (7).

The carbohydrate osmolytes can be structurally divided into two groups: polyols and cyclitols. Examples of polyol osmolytes include sorbitol, xylitol, mannitol, glycerol, and adonitol. Myoinositol and trehalose are examples of cyclitol osmolytes (Figure 2). However, the term polyol is often used to describe both groups. In mammals, the predominant carbohydrate compounds acting as osmoprotectants include sorbitol and myoinositol (7). Unlike many of the methylamine osmolytes, most carbohydrate compounds do not exert stabilizing effects on protein structure under physiological conditions but do so as the pH decreases (83). Sorbitol is formed from glucose by AR, an enzyme up-regulated during hyperosmotic stress by NFAT5. Ironically, elevated AR activity can be pro-inflammatory during oxidative stress as the result of AR-mediated metabolism of aldehydes to corresponding alcohols, which then mediate inflammatory signals (84). Studies have also shown that AR inhibitors are a promising therapy for inflammatory disorders (84). In the kidney, myoinositol is not synthesized but rather transported into cells through the solute carrier family 5, member 3 (SLC5A3) transporter. Similar to the *AR* gene, the *SLC5A3* gene is regulated by binding of NFAT5 (85). Gastrointestinal epithelial cells co-cultured with *Helicobater pylori*, a pathogen associated with gastric and duodenal inflammation, produced less interleukin (IL) 8 when grown in the presence of mannitol (12). Xylitol inhibited lipopolysaccharide (LPS)-induced production of the inflammatory cytokines IL1β and TNF by mouse macrophages (13). Similarly, trehalose, a natural α-linked disaccharide, suppressed IL1β and TNF production by murine peritoneal macrophages (14). Trehalose also reduced IL6 production by bone marrow cells grown in culture and prevented osteoclastogeneisis in both ovariectomized and LPS-stimulated osteoclast induction models of osteoporosis in mice (86).

The predominant methylamine osmolytes are betaine, trimethylamine N-oxide (TMAO), and α-GPC (Figure 3). These compounds are chemically characterized by the presence of methyl-ammonium functional groups. Betaine (also known as glycine betaine) is formed predominantly in the liver and kidney through the irreversible $NADP^+$-dependent oxidation of betaine aldehyde by aldehyde dehydrogenases (ALDH). The two predominant betaine aldehyde-metabolizing enzymes in the liver are believed to be ALDH7A1 and ALDH9A1 (87, 88). Betaine aldehyde is derived from the oxidation of choline by choline oxidase. Choline predominantly originates from dietary sources. In addition to acting as an osmolyte, betaine is used to re-methylate homocysteine to *S*-adenosyl methionine in the methionine cycle. Competition for betaine during hyperosmotic stress could conceivably have negative sequelae on cellular methylation status. Methylamine osmolytes can act as excellent molecular chaperones preserving the structure and function of DNA, RNA, and proteins (82). The ability of these compounds to stabilize is dependent on the surrounding solvent conditions as well as the specific molecule being stabilized. For example, despite exerting very strong protein-stabilizing effects at neutral pH, both betaine and TMAO lose these beneficial actions when the pH falls below 5 (89). Interestingly, studies looking at the stabilizing effect of osmolytes on DNA found that betaine prevented double helix formation in a concentration-dependent manner (90). α-GPC is formed from phosphatidylcholine (PC) under the action of phospholipase. PNPLA6 has been shown to metabolize PC to α-GPC. PNPLA6 is classified as a B family phospholipase and, like other osmolyte-associated genes, is up-regulated by NFAT5 during hyperosmotic stress. Betaine has shown to be hepatoprotective against a wide range of insults, including ethanol toxicity (91). Betaine has also been suggested for use as a supplemental therapy against cholestasis in patients with chronic hepatitis B or C viral infections undergoing interferon therapy (92). Betaine supplementation reduced TNF production and the size of atherosclerotic lesions in apolipoprotein E-deficient mice, suggesting it exerts anti-inflammatory actions (93). In addition, high dietary betaine in humans is associated with a significant reduction in inflammatory biomarkers, including TNF, IL6, and C-reactive protein (15, 16). Renal medullary cells cultured under hypertonic conditions and supplemented with betaine exhibit reduced up-regulation of NFAT5 target genes, indicating attenuation of the hyperosmotic response pathways (51). In rat hepatoma cells, hyperosmotic conditions elicit down-regulation of betaine-homocysteine *S*-methyltransferase (BHMT), an enzyme that catalyzes the conversion of betaine and homocysteine to dimethylglycine and methionine (94). Such down-regulation of BHMT may represent a cellular homeostatic response to preserve pools of betaine that would serve to counter the extracellular osmotic pressure.

There are a number of amino acids and amino acid derivatives that act as osmoprotectants, including glycine, proline, isoleucine, leucine, phenylalanine, valine, β-alanine, taurine, and hypotaurine (Figure 4). Taurine, a sulfur-containing β-amino acid, represents the predominant amino acid osmolyte serving as an osmoprotectant in mammals (7). It is derived from cysteine and formed principally by the liver and then released into the systemic circulation. At least within the kidney, increased taurine transport (rather than synthesis) appears to drive intracellular accumulation during hypertonicity. The solute carrier family 6, member 6 (SLC6A6), previously known as taurine transporter (TauT) protein, is responsible for taurine transport across the plasma membrane and, like many other genes, is regulated by NFAT5 during hyperosmotic stress. As the synthesis of both taurine and glutathione (GSH) requires cysteine, it is feasible that, during increased osmotic stress where oxidative stress is significantly elevated, these metabolic pathways may compete for the intracellular cysteine pool. Similar to the observation noted for betaine, proline was shown to destabilize the double helix structure of DNA in a concentration-dependent fashion (95). Studies have revealed that taurine also influences a number of processes unrelated to its role in osmotic stress. Taurine has been implicated in regulating cardiac rhythm, cardiac contractile function, and blood pressure (6). Moreover, pretreatment with taurine significantly reduced

the levels of TNF and IL1β in a rat model of severe acute pancreatitis. In this model, taurine also inhibited MAPK14 expression and its activation through phosphorylation (17).

It is also important to note that, under certain conditions and concentrations, some osmolytes can actually destabilize protein structure and function, as well as hinder intramolecular interactions. The pH dependence of stabilization noted for many osmolytes suggests that the synthesis and accumulation of a certain osmolyte within a cell may be very dependent on the specific insult and conditions generated as a result. For example, as mentioned above, many methylamine compounds can have a negative impact on protein stability at a pH < 5.0, whereas many of the polyol compounds only exert a stabilizing force at lower pH values. This suggests that a cell may generate osmolytes in an insult-specific fashion. This may also help explain why certain tissues and cell types show a preference for one osmolyte over another. It is reasonable to propose that intracellular osmolyte concentrations have the potential to significantly influence protein homeostasis through their stabilizing (and destabilizing) effects on protein structure and folding.

Extracellular hypertonicity as an inflammatory stimulus

Changes in extracellular osmolarity can contribute to the initiation and development of both local and systemic disorders. Several common disorders are known (or thought) to be caused by perturbations in fluid osmolarity. Tissue burns, dehydration, heat stroke, diabetes mellitus, diabetes insipidus, hypernatremia, and uremia are all associated with elevated extracellular osmolarity (11, 96–99). Extracellular fluid hyperosmolarity is also believed to play an inflammatory role in asthma and cystic fibrosis (11). Furthermore, NFAT5 was found to regulate both synovial proliferation and angiogenesis in chronic, inflammatory arthritis (23). In many cases, mild to moderate elevations in extracellular osmolarity appear to be sufficient for pathological sequelae. Interestingly, all of these disorders are also strongly associated with inflammation and cytokine secretion. The pro-inflammatory cytokines linked to hyperosmotic stress-related pathologies include TNF, IL1β, IL6, IL8, and IL18 (11, 24, 100, 101). *In vitro* studies have implicated an important role for hypertonicity in the inflammatory response. Hyperosmotic stress was found to directly cause TNF, IL1β, IL6, and IL8 secretion from a variety of cell types (24). Interestingly, many of these cytokines, including IL1, IL6, and IL18, contain NFAT5 binding sites within their promoter regions, suggesting the potential for their direct up-regulation in response to hypertonicity (11). Insults that disrupt osmoregulation and lead to increased osmolarity or osmotic load are often characterized by a strong inflammatory response. For instance, damage to the cerebral ventricles may lead to a buildup of cerebrospinal fluid and intracranial pressure, known as hydrocephalus (102). This condition is accompanied by up-regulation of inflammatory cytokines (103).

Eye disease

Maintaining osmolarity is a key feature of ocular tissues. The eye is a fluid-filled organ, and osmolality plays an important role in eye health. The intraocular space separating the cornea and lens is filled with a fluid, aqueous humor. Vitreous humor is a gel-like fluid that fills the space between the lens and retina. The cornea is constantly exposed to the external environment and corneal hydration by means of tear secretion is required for its proper function. Two features of dry eye disease include an increase in tear osmolarity and ocular surface inflammation. Indeed, tear hyperosmolarity serves as a key diagnostic for this disorder (104). As noted previously, hyperosmolarity represents a potent inflammatory stimulus. A strong correlation exists between tear hyperosmolarity and the severity of dry eye syndrome. In patients exhibiting the most severe pathological grade of this condition, tear osmolarity increased to 344 mOsm/L on average. Studies using *in vitro* dry eye models

have revealed tear hyperosmolarity activates many of the same downstream pathways noted in other tissues, which involve MAPK14, JNK MAP kinase, and NF-κB. Exposure to hypertonic solutions elicits the secretion of the pro-inflammatory cytokines IL6 and IL8 from corneal epithelial cells *in vitro* (20). Further, production of IL1β, TNF, and IL8 are up-regulated after treatment with hyperosmolar media in primary human limbal epithelial cultures (105). EGFR transactivation and the MAPK14 kinase pathway appear to also be involved in the corneal epithelial cell response to hypertonicity. Topical administration of hypertonic saline solution to the eyes of mice resulted in elevated levels of IL1β, TNF, and matrix metallopeptidase 9 levels on their corneal surface (11). Under these same conditions, JNK, ERK, and MAPK14 signaling pathways were also activated in the corneas of the animals. These results, in combination with the presence of hyperosmotic tears in chronic dry eye patients, are consistent with hyperosmotic stress being a very important contributory factor in this condition.

Patients with diabetes often develop diabetic retinopathy, a low-grade chronic inflammatory disease that results in retinal damage and eventual blindness. More than 80% of these patients who have had the disease for > 10 years have experienced some degree of retinopathy (106). Osmotic stress caused by sorbitol accumulation is believed to play a role in microvascular complications associated with diabetic retinopathy, as is high AR activity (107). As previously mentioned, hyperosmotic stress and NFAT5 activation are potent inducers of AR expression and AR is responsible for the metabolism of glucose to sorbitol. *In vitro* studies have revealed that retinal pigment epithelial cells exposed to hypertonic conditions exhibit a > 11-fold increase in AR mRNA expression (108, 109). A large number of studies have linked the activity of AR to diabetic retinopathy, and AR inhibitors are currently being used therapeutically in the disorder (110). The observation that hyperosmolarity induces significant AR expression supports a possible link between hypertonicity and diabetic retinopathy (109). The exact roles played by AR and hyperosmotic stress in the development and progression of diabetic retinopathy still remains to be determined.

Insulin resistance and diabetes

Diabetes has many clinical manifestations including neuropathy, retinopathy, nephropathy, and hypertension. Significant increases in blood glucose contribute to plasma hyperosmolarity. A cross-sectional study revealed that elevated plasma osmolarity positively correlated with impaired glucose tolerance and diabetes (22). Patients exhibiting serum hyperglycemia (elevated blood glucose) and electrolyte hypertonicity (elevated blood sodium and potassium) were found to be more than four times more likely to develop diabetes than patients with hyperglycemia only (111). This observation suggests that elevated plasma tonicity is an important contributing factor to disease progression. As noted above, vasopressin is released in response to high plasma osmolarity (112). Patients with diabetes insipidus exhibit decreased release of vasopressin and/or decreased renal sensitivity to vasopressin, leading to an inability to respond to systemic hyperosmotic stress (113). Hyperglycemia is also linked with negative outcomes in clinical conditions including myocardial infarction and postoperative complications, such as increased risk of infection (114). Interestingly, hyperosmotic stress has been shown to inhibit interferon-γ (IFN-γ) expression in blood lymphocytes (115). Given the critical role of IFN in mounting an immune response to both viral and intracellular bacterial infections, it is possible that hyperosmotic stress may contribute to the increased susceptibility to infection observed in patients with hyperglycemia. *In vitro* studies suggest that elevated glucose levels enhance inflammation during infection by means of hyperosmotic stress-induced cytokine release (116). Other studies indicate that elevated oxidative stress caused by glucose metabolism through the polyol pathway, rather than osmotic stress, is the underlying cause of many

diabetic complications (117). Studies using *in vivo* mouse models of diabetic cataract formation identified both oxidative stress (as a result of glucose metabolism) and osmotic stress as contributing to the pathology (118, 119). It has also been suggested that chronic oxidative stress associated with glucose metabolism impairs osmoregulatory pathways, thereby hindering a tissue's ability to respond to osmotic stress (119).

Hyperosmotic stress may also be associated with insulin resistance (120, 121). In skeletal myocytes and adipocytes, insulin regulates blood glucose levels by stimulating translocation of SLC2A4 glucose transporters (also known as Glut4) to the plasma membrane, leading to increased glucose transport into the cell (122). Increases in extracellular non-glucose solute concentrations also cause a corresponding increase in glucose uptake by adipocytes and myocytes (123, 124). Insulin receptors belong to a subfamily of the tyrosine kinase receptor protein family. The binding of insulin results in activation of receptor kinase activity and phosphorylation of cellular proteins. Insulin receptor-mediated tyrosine phosphorylation of insulin receptor substrate-1 (IRS1) promotes the binding and activation of phosphoinositide 3 (PI3)-kinase. PI3 kinase then activates protein kinase B (PKB), which mediates SLC2A4 translocation. Insulin resistance develops through diminished PKB and IRS1-associated PI3-kinase activity, as well as by IRS1 degradation (121, 122). This causes down-regulation of the action of insulin, thereby diminishing the effects of physiological insulin concentrations. Short-term hyperosmotic challenge of adipocytes was shown to inhibit the action of IRS1 through serine phosphorylation (121). Furthermore, long-term osmotic challenge resulted in IRS1 degradation. Together, these results shed light on the mechanisms underlying hyperosmolarity-induced insulin resistance and substantiate a physiological role during the initiation and progression of diabetes.

Diabetic nephropathy is the predominant cause of kidney failure in the United States. Hyperosmotic stress is believed to enhance cellular susceptibility to renal tubular fibrosis (125). This is particularly relevant given that tubular fibrosis significantly contributes to the pathogenesis of diabetic nephropathy, which in turn is responsible for > 40% of cases that progress to end stage renal disease. Increases in NFAT5 DNA binding activity has been observed in mesangial cells of diabetic patients with diabetic nephropathy when compared with diabetic control and non-diabetic groups (11). NFAT5 activity was also elevated in the peripheral blood mononuclear cells of these patients, implying that NFAT5 activation extends beyond cells of the kidney. Glucose-associated increases in plasma solute concentrations are thought to increase hyperosmotic stress within the renal medulla. Renal distal tubule cells exposed to this environment exhibit down-regulation of Smad7 (125). This promotes Smad pathway activation, one effect of which is increased TGFβ receptor stability. The resultant increase in TGFβ signaling is thought to enhance susceptibility of renal tubule cells to extracellular matrix synthesis and fibrosis (125). The mechanisms underlying injury in diabetic nephropathy parallel those driving diabetic retinopathy, including microvasculature damage associated with hyperosmotic stress (126). As with other diabetic-related pathologies, it appears that both hyperglycemia-induced osmotic stress and glucose metabolism-associated oxidative stress contribute to the pathogenesis of this condition (125, 127).

Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a term used to describe a number of inflammatory diseases that affect the gastrointestinal tract. It is characterized by chronic inflammation of one or more regions of the gastrointestinal tract with reoccurring flare-ups (128, 129). Crohn's disease and ulcerative colitis comprise the most common forms of IBD. These conditions share many clinical features but differ in location and the nature of the inflammatory changes. Crohn's disease manifests as transmural lesions found anywhere

along the gastrointestinal tract and involves multiple cell layers and types. Ulcerative colitis is limited to the epithelial lining of the colon and rectum. A commonly used mouse model of IBD uses oral administration of dextran sulfate sodium (DSS) (128). A recent study revealed that DSS feeding causes hyperosmolarity and hyperosmotic stress within the colon, which in turn triggers inflammation (11). Other studies in rats using alanine, mannitol, or NaCl as hyperosmotic stimuli produced similar results, suggesting any compound elevating colonic hypertonicity may have pathological consequences (130). These results corroborate observations in human patients with IBD, including neonatal necrotizing enterocolitis, Crohn's disease, and ulcerative colitis, where elevated osmolarity in fecal fluid within the colon is noted (18, 19, 24). Furthermore, the fecal osmolarity elevation in Crohn's disease patients shows a close correlation with disease severity (18, 19, 129). Under physiological conditions, both pro- and anti-inflammatory cytokines are present within the gastrointestinal tract, with the pro-inflammatory cytokines being kept in check by the anti-inflammatory cytokines. Dysregulation between the pro- and anti-inflammatory signals is believed to be a major factor contributing to the pathogenesis of IBD. Epithelial cells and infiltrating immune cells are believed to be the predominant cytokine-secreting cells in IBD (128, 131). Cultured intestinal epithelial cells exposed to hyperosmotic media exhibit elevated IL1β and IL8 production (132). Studies in human intestinal cells also have revealed NFAT5 activation after treatment with hyperosmotic media (132, 133). These data are supported by clinical studies documenting IBD patients to have elevated levels of pro-inflammatory cytokines, including TNF, IL1, IL6, and IL18 (131). The overall significance of hyperosmotic stress and epithelial cell cytokine release on IBDs has yet to be determined but represents a potentially important therapeutic target for the treatment of these disorders.

Cardiovascular disease

Cardiovascular diseases are the leading cause of premature deaths worldwide, being responsible for > 17 million deaths in 2008 alone (134). Hypertension is a major risk factor for coronary heart disease (135). Osmoreceptor activation in the central nervous system can elevate blood pressure through a mechanism involving increased sympathetic nerve activity, a process thought to contribute to salt-induced hypertension (136). In rats, a high salt diet activates NFAT5 in macrophages and results in VEGFC secretion (78). VEGFC then diminishes interstitial hypertonic volume retention by inducing hyperplasia of the lymph capillaries and expression of endothelial nitric oxide synthase. In isolated vascular smooth muscle cells, NFAT5 was shown to be activated by hypertonicity, the vasoconstrictor angiotensin II, and the mitogen platelet-derived growth factor-BB. These effects were selective in that NFAT5 was unaffected by other vasoconstrictors, mitogens, or a variety of cytokines, including IL1β, IL8, IL10, TNF, or IFN (34). NFAT5 is also significantly up-regulated in models of vascular injury, such as atherosclerotic lesions and neointimal hyperplasia. It was proposed that NFAT5 was involved in the regulation of the vascular smooth muscle cell phenotype (48). NFAT5 also appears to have a role in the heart. In cardiac myocytes, NFAT5 degradation was found to be a key event mediating doxorubicin cytotoxicity (137). Exposure of the myocytes to hypertonic media causes NF-κB and caspase activation through a mechanism involving ROS (138). This would suggest that the hyperosmotic stress triggers oxidative stress and ROS production within the cells. Hyperosmotic stress also triggers apoptosis in cardiac myocytes through a p53-dependent manner. In this respect, it appears to be a more potent stimulus than other factors known to induce cardiac myocyte death, including doxorubicin or angiotensin II (6). NFAT5 mRNA and protein is up-regulated in cardiac myocytes exposed to hyperosmotic media *in vitro* (139). After hyperosmotic challenge with sorbitol, cardiomyocytes also exhibited an increase in AR expression that was accompanied by AR-mediated activation of apoptotic signaling pathways (140).

The osmolyte taurine is the most abundant free amino acid in cardiac tissues and in skeletal muscle. In response to hyperosmotic stress, cells take up taurine to counter the elevations in extracellular osmolarity. One mechanism responsible for the intracellular taurine accumulation is the NFAT5-mediated up-regulation of the taurine transporter SLC6A6 (6). Consistent with this observation is the finding that SLC6A6 has a NFAT5 binding site within its promoter (6). Several studies have shown that taurine deficiency leads to cardiomyopathy, suggesting an important role for this osmolyte in maintaining heart health (141). Due to the multifaceted functions of taurine (including antioxidant and osmoprotectant properties), the precise mechanism(s) mediating protection of cardiac tissues remains to be determined (142). Using SLC6A6 knockout mice, current evidence suggests that the cytoprotective actions of taurine in cardiac and skeletal muscle are dependent on its ability to act as an osmolyte. These mice exhibited decreased cell volume, a condition often associated with the cell's inability to effectively counter extracellular osmolarity (141). In addition, SLC6A6 knockout animals exhibited a significant up-regulation of Hsp70, ATA2, and S100A4 within cardiac and skeletal muscle tissues, all of which are induced in response to osmotic stress (141).

Liver disease

There is accumulating evidence supporting an important role for osmotic stress in the pathogenesis of a number of liver disorders. Hepatic hydration heavily influences protein turnover within the liver (143). A number of factors can cause shrinkage of hepatocytes, including high urea concentrations, amino acid starvation, elevated sodium levels in the blood (hypernatremia), and oxidative stress (144). Cell shrinkage caused by hepatocellular dehydration leads to an overall increase in proteolytic activity (144). On the other hand, hepatic protein synthesis is, in turn, reduced under hyperosmotic conditions (145). The influence of cell shrinkage on protein synthesis has also been observed in mammary tissue (146). Cellular dehydration is believed to be a driving force behind the severe protein wasting observed within the liver and skeletal muscles of extremely ill patients (143). The osmolarity of mouse liver is approximately 35 mOsm/kg greater than serum, suggesting this organ is physiologically functioning under slightly hypertonic conditions (10). In addition, the osmolarity of portal vein blood increases after feeding or ingestion of fluids with a high solute concentration, which subsequently influences hepatic hydration, further underscoring the need for a general response mechanism to hyperosmolar conditions within this tissue (147, 148). Hydration state greatly influences many hepatic processes. Bile acid uptake and canalicular secretion are both heavily dependent on osmoregulation through transporter integration into the membrane (149). Hydrophobic bile acids are known to cause hepatocyte shrinkage and it has been speculated that hyperosmotic stress contributes to cholestatic liver injury (150). Hyperosmotic stress also sensitizes hepatocytes to apoptosis, suggesting that elevated hepatic osmolarity could influence drug-induced liver injury. The hepatic $Na^+/K^+/2Cl^-$ co-transporter SLC12A2 (also known as NKCC1) and betaine transporter SLC6A12 (also known as BGT-1) are up-regulated in response to hyperosmotic stress (151, 152). Both of these proteins are also up-regulated during the transition of hepatic stellate cells to a myofibroblast-like phenotype, suggesting that an intracellular osmolyte imbalance may accompany stellate cell transformation during liver fibrosis (151, 152). CYP2E1 is up-regulated in hepatocytes exposed to hyperosmotic stress through a NFAT5-dependent mechanism (11). Such expression increases free radical generation and oxidative stress, which contributes to liver inflammation and fibrosis (153). CYP2E1 has been linked to a number of liver diseases, including alcoholic liver disease, non-alcoholic steatohepatitis, and cirrhosis (154). As mentioned above, the amino acid taurine is a physiologically important osmolyte. Hyperosmotic-challenged hepatocytes exhibit up-regulation of the taurine transporter SLC6A6 (155). Knockout mice lacking SLC6A6 protein exhibit severe taurine deficiency and develop chronic liver disease characterized by moderate hepatitis and liver

fibrosis (156). These mice are also more sensitive to ultraviolet B irradiation-induced immunosuppression, suggesting taurine uptake may serve as a protective response under these conditions (157).

Expert opinion

Conventional wisdom holds that osmotic stress is not routinely experienced by most cells and tissues, with the exception of the kidney. Although this is true in some cases, recent evidence suggests that many cell types and tissues commonly experience osmotic stress due to microenvironmental osmotic imbalances. A major challenge associated with examining this possibility is the difficulty associated with accurate measurement of the osmolarity of physiological fluids within these microenvironments. Nevertheless, compelling evidence supports the involvement of osmotic imbalance in a wide range of human diseases. The area of hyperosmotic stress-induced inflammation is an emerging one. Consequently, the contribution of this process to disease pathogenesis requires further investigation. Osmotic stresses (or changes) in physiology have historically been focused at the level of the organism, usually in the context an adaptive response of the body to maintain fluid balance. The studies described in this review suggest a wider array of cellular and organismal adaptive responses to osmotic stress. The strong association between hyperosmotic stress, inflammation, and many disease states underscores the need for a comprehensive understanding of the cytoprotective mechanisms underlying the hyperosmotic stress response. Such knowledge may reveal a new series of therapeutic targets. Given that chronic infection and inflammation contribute to > 25% of cancer, a greater understanding of the basic mechanisms driving inflammatory process, such as hyperosmotic stress, will greatly enhance our ability to treat and, more importantly, prevent disease (158).

Outlook

The negative microenvironmental effects of systemic hyperosmolarity are still not well understood. Nor is the contribution of osmotic stress to the development and progression of chronic inflammatory diseases, such as arthritis and inflammatory bowel disease. Recent research supports a significant role for hyperosmotic stress in the release of pro-inflammatory cytokines and inflammation. Understanding the molecular mechanisms driving osmotic stress-induced inflammatory response and the subsequent contribution to chronic inflammation is further underscored by studies showing that chronic inflammation plays a major role in carcinogenesis. On-going studies over the next decade may reveal that osmotic imbalances play a significant role not only in inflammatory diseases but also in cancer, and that therapeutics targeting osmoadaptation could represent a novel class of drugs for the treatment of many disorders.

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Highlights

In vitro and *in vivo* studies suggest that hyperosmotic stress acts as pro-inflammatory stimuli.

- Most cell types and tissues demonstrate the ability to adaptively respond to osmotic stress.
- Osmolyte synthesis increases during osmotic stress, and many of these cytoprotective compounds have anti-inflammatory actions.
- A number of human diseases exhibit elevation of physiological fluid osmolarity, particularly disorders associated with chronic inflammation.
- Therapeutics targeting pathways associated with osmoadaptation may represent a promising new class of anti-inflammatory and/or chemotherapeutic drugs.

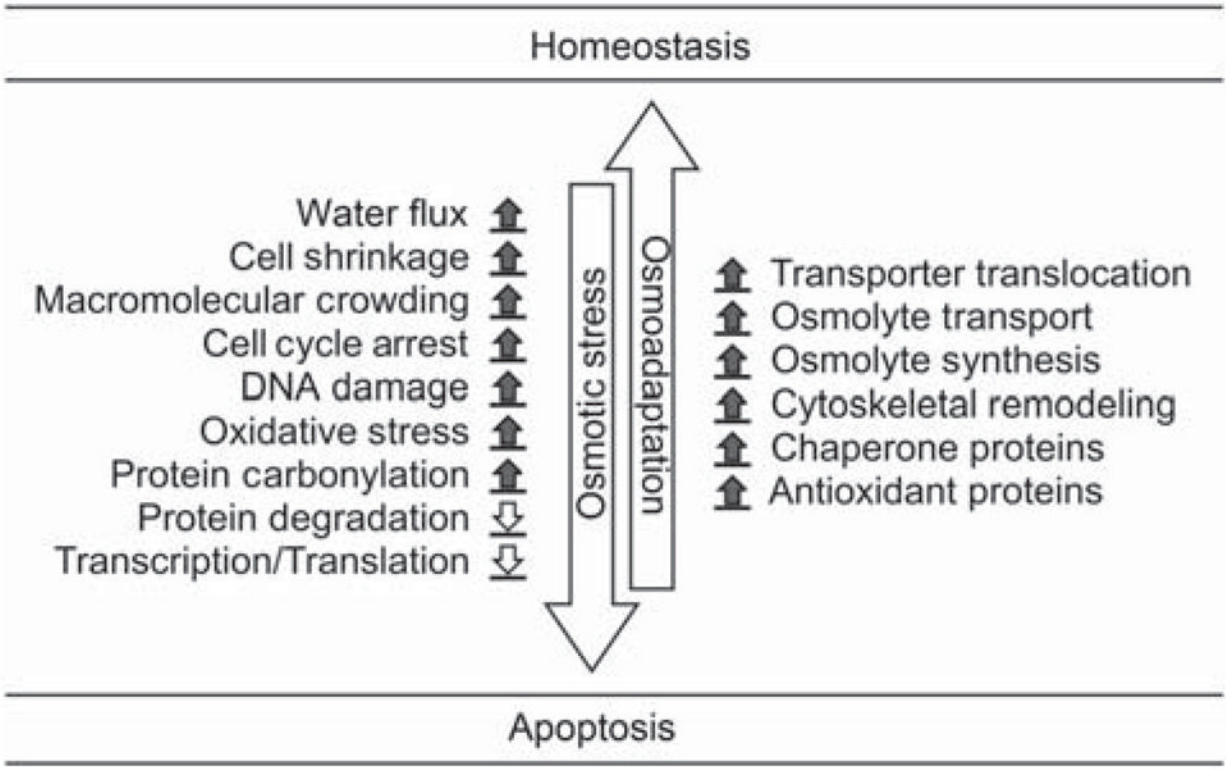

Figure 1.
Hyperosmotic stress and osmoadaptation.
Hyperosmotic stress negatively affects many cellular processes. If left unchecked, the cell is primed for, and eventually undergoes, apoptosis. Osmoadaptive mechanisms are in place to counter osmotic stress, and restore water balance and cell homeostasis.

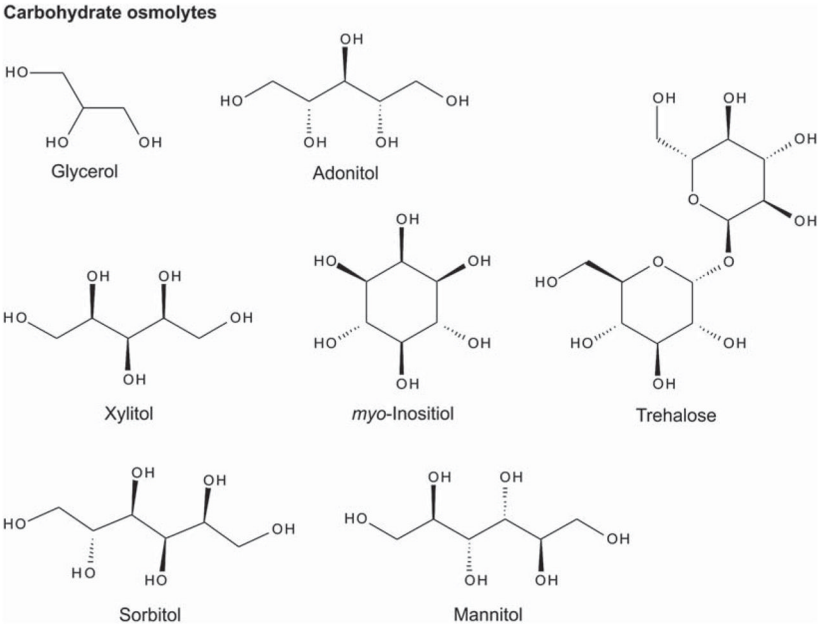

Figure 2.

Carbohydrate osmolyte structures.

The carbohydrate osmolytes include polyols, such as glycerol, adonitol, xylitol, sorbitol, and mannitol, and cyclitols, such as myoinositol and trehalose.

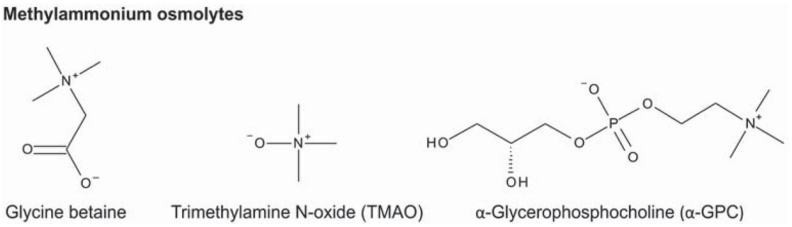

Figure 3.
Methylamine osmolyte structures.
The methyamine osmolytes include glycine betaine, triethylamine, and α-glycerophosphocholine. Under certain conditions, methylamines act as excellent molecular chaperones, stabilizing the structure of DNA, RNA, and protein.

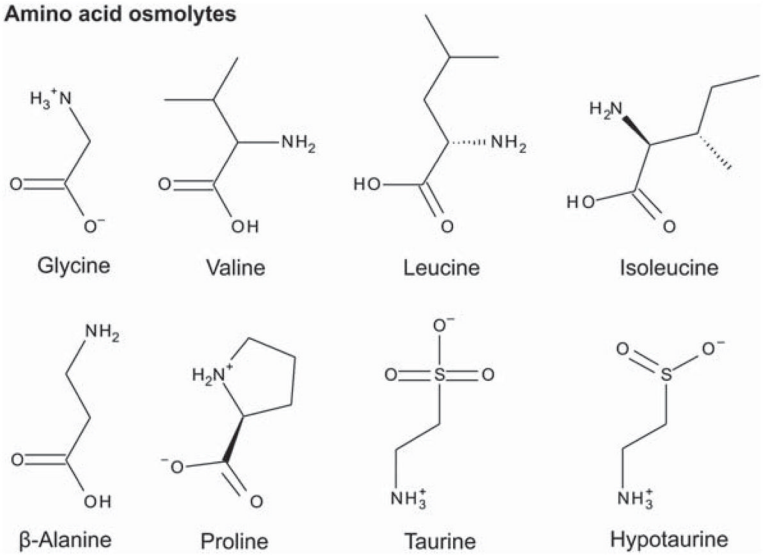

Figure 4.

Amino acid osmolyte structures.

A number of amino acids can function as osmolytes, including glycine, valine, leucine, isoleucine, β-alanine, and proline. The sulfur-containing compounds, taurine and hypotaurine, are also considered amino acid osmolytes.

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Table 1

Target genes up-regulated by NFAT5 activation.

NFAT5 target gene	Function	Tissue/cell line[a]	References
Aggrecan (*ACAN*)	Cartilagenous tissue extracellular matrix protein; chondrogenic marker	HACs	(159)
Aldo-keto reductase family 1, member B1 (*AKR1B1*)	Catalyzes the reduction of a number of aldehydes (also known as *AR*)	MDCK, CHO, MEF, PBMCs, HMCs	(11, 42, 160)
Aquaporin-1 (*AQP1*)	Molecular water channel protein	IMCD3	(52)
Aquaporin-2 (*AQP2*)	Molecular water channel protein	mpkCCDcl4, MDCK	(53, 54)
Asporin (*ASPN*)	Cartilage extracellular protein; may regulate chondrogenesis	MEF	(42)
CD24 molecule (*CD24*)	Encodes a sialoglycoprotein expressed on surface of immune cells	T cells	(46)
Chemokine (C-C motif) ligand 2 (*CCL2*)	C-C chemokine that stimulates monocytes/macrophages and CD8-positive T cells (also known as *MCP1*)	NRK52E	(161)
Collagen, type II, α1 (*COL2A1*)	Fibrillar collagen found in cartilage; chondrogenic marker (also known as *COL2*)	HACs	(159)
Crystallin, αB (*CRYAB*)	Members of the small heat shock protein family; protein chaperone	MEF	(42)
Cyclooxygenase-2 (*COX2*)	Key enzyme in prostaglandin biosynthesis	MDCK	(160)
Cysteine-rich, angiogenic inducer, 61 (*CYR61*)	Associated with cell adhesion, migration, chemotaxis, and differentiation in fibroblasts and endothelial cells	Primary myoblasts	(11)
Cytochrome P450 2E1 (*CYP2E1*)	Monooxygenase; catalyzes oxidation of a wide range of substrates	Human primary hepatocytes	(11)
Cytochrome P450 3A4 (*CYP3A4*)	Monooxygenase; catalyzes oxidation of a wide range of substrates	C_2bbe1, LS180, HepG2, human primary colonic cells	(133)
Cytochrome P450 3A5 (*CYP3A5*)	Monooxygenase; catalyzes oxidation of a wide range of substrates	C_2bbe1, LS180, HepG2, human primary colonic cells	(133)
Cytochrome P450 3A7 (*CYP3A7*)	Monooxygenase; catalyzes oxidation of a wide range of substrates	C_2bbe1, LS180, HepG2, human primary colonic cells	(133)
Ectonucleotide pyrophosphatase/ phosphodiesterase 2 (*ENPP2*)	Functions as both a phosphodiesterase and phospholipase; involved in cell proliferation and chemotaxis	MEF	(42)
Heat shock 70kDa protein 1B (*HSPA1B*)	Protein chaperone (also known as *HSP70-2*)	IMCD3, MDCK, HBE16	(160, 162)
Heat shock 70kDa protein 4-like (*HSPA4L*)	Heat shock protein identified as human hypertensive heart biomarker (also known as *OSP94*)	IMCD3	(6)
Insulin-like growth factor binding protein 5 (*IGFBP5*)	Regulates cellular proliferation by modulating insulin action	MEF	(42)
Insulin-like growth factor binding protein 7 (*IGFBP7*)	Regulates cellular proliferation by modulating insulin action	MEF	(42)
Interleukin-1, β (*IL1B*)	Important mediator of the inflammatory response	HLENs	(163)
Lymphotoxin, β (*LTB*)	TNF family membrane protein	T cells	(80)
Mucin 5AC (*MUC5AC*)	Heavily glycosylated proteins that form gel-like secretions	HBE16	(162)
Natriuretic peptide receptor 1 (*NPR1*)	Membrane-bound guanylate cyclase	MEF	(42)

NFAT5 target gene	Function	Tissue/cell line[a]	References
Protein tyrosine phosphatase, receptor-type, Z (*PTPRZ1*)	Member of the receptor protein tyrosine phosphatase family	MDA-MB-231	(79)
S100 calcium binding protein A4 (*S100A4*)	Involved in many processes including cellular processes, cell cycle progression, and differentiation	HACs, Clone A cells, IMCD3	(159, 164, 165)
Serum- and glucocorticoid-inducible kinase (*SGK1*)	Serine/threonine protein kinase associated with cellular stress responses	IMCD	(166)
NFAT5 target gene	Function	Tissue/cell line[a]	References
Solute carrier family 2, member 4 (*SLC2A4*)	Glucose transporter (also known as *GLUT4*)	C2C12	(49)
Solute carrier family 5, member 3 (*SLC5A3*)	Sodium/myoinositol cotransporter (also known as *SMIT*)	HeLa, MEF	(42, 167)
Solute carrier family 6, member 6 (*SLC6A6*)	Taurine transporter (also known as *TauT*)	HepG2	(155)
Solute carrier family 6, member 12 (*SLC6A12*)	Betaine/γ-aminobutyric acid (GABA) transporter (also known as *BGT1*)	HACs, MDCK, MEF	(6, 42, 159)
Solute carrier family 14, member 2 (*SLC14A2*)	Urea transporter (also known as *UTA*)	IMCD3, MDCK	(6)
Solute carrier family 38, member 2 (*SLC38A2*)	Amino acid transporter (also known as *ATA2*)	T cells	(32)
SRY (sex determining region Y)-box 9 (*SOX9*)	Involved in chondrocyte differentiation; chondrogenic marker	HACs	(159)
Tumor necrosis factor (*TNF*)	TNF family pro-inflammatory cytokine (also known as *TNF*α)	T cells	(80)
Vascular endothelial growth factor C (VEGFC)	Involved in angiogenesis and endothelial cell growth	MPS	(78)

[a]Cell line abbreviations: C_2bbe1, human intestinal cells; C2C12, mouse myoblasts; CHO, Chinese hamster ovary cells; Clone A cells, colon cancer cells; HACs, human articular chondrocytes; HBE16, human bronchial epithelial cells; HLECs, human limbal epithelial cells; HMCs, human mononuclear cells; IMCD3, mouse inner medullary collecting duct cells; MDCK, Madin-Darby canine kidney cells; MDA-MB-231, MDA-MB-231 breast carcinoma cells; mpkCCDcl4, immortalized mouse collecting duct principal cells; MPS, mononuclear phagocyte system cells; NRK52E, normal rat kidney cells; PBMCs, peripheral blood mononuclear cells.