Effect of Cell Hydration on Metabolism

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Abstract
Prerequisites for cell survival include avoidance of excessive cell volume alterations. Cell membranes are highly permeable to water, which follows osmotic gradients. Thus, cell volume constancy requires osmotic equilibrium across cell membranes. Cells accumulate osmotically active organic substances and compensate their osmolarity by lowering cytosolic Cl\(^{-}\) concentrations. Following cell shrinkage, regulatory cell volume increase is accomplished by ion uptake (activation of Na\(^{+}\), K\(^{+}\), 2Cl\(^{-}\) cotransport, Na\(^{+}\)/H\(^{+}\) exchange in parallel to Cl\(^{-}\)/HCO\(_{3}\)\(^{-}\) exchange and Na\(^{+}\) channels), by cellular accumulation of organic osmolytes (e.g. myoinositol, betaine, phosphorylcholine, taurine) as well as by proteolysis leading to generation of amino acids and glycogenolysis generating glucose phosphate. Following cell swelling, cell volume is restored by ion exit (activation of K\(^{+}\) channels and/or anion channels, KCl cotransport, parallel activation of K\(^{+}\)/H\(^{+}\) exchange and Cl\(^{-}\)/HCO\(_{3}\)\(^{-}\) exchange), release or degradation of organic osmolytes as well as stimulation of protein synthesis and of glycogen synthesis. The activity of cell volume regulatory mechanisms is modified by hormones, transmitters and drugs, which thus influence protein and glycogen metabolism. Moreover, alterations of cell volume modify generation of oxidants and the sensitivity to oxidative stress. Deranged cell volume regulation significantly contributes to the pathophysiology of several disorders such as liver insufficiency, diabetic ketoacidosis, hypercatabolism, ischemia, and fibrosing disease.

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Introduction

Avoidance of excessive alterations of cell volume is an obvious prerequisite for cell survival [1]. Undue cell shrinkage or swelling interferes with the integrity of cell membrane and cytoskeletal architecture. Moreover, cell hydration has a profound influence on cytosolic proteins. Proteins and protein-bound water occupy a large fraction of the intracellular space (macromolecular crowding)
leaving only little space for free water [1]. Loss or gain of water approaching only a few percent of cell volume thus exerts a profound effect on protein function and cellular performance.

Water channels allow rapid movement of water across the plasma membrane [2], which is driven by osmotic pressure gradients [1]. Hydrostatic pressure gradients across mammalian cell membranes are negligibly low. To achieve cell volume constancy, cells have to accomplish osmotic equilibrium across the cell membrane. If intracellular osmolarity exceeds extracellular osmolarity across the cell membrane. If intracellular osmolarity exceeds extracellular osmolarity across the cell membrane. If intracellular osmolarity exceeds extracellular osmolarity, water enters following its osmotic gradient and the cell swells. Conversely, if extracellular osmolarity exceeds intracellular osmolarity water exits leading to cell shrinkage [1, 3–7].

Intra- or extracellular osmolarity and thus osmotic equilibrium across the cell membrane are challenged by alterations of extracellular osmolarity, extracellular ion composition, transport across the cell membrane and cytosolic generation or disposal of osmotically active cytosolic components. Cells employ a variety of mechanisms to maintain cell volume constancy, including altered transport across the cell membrane and metabolism. Hormones and mediators may modify the activity of these cell volume regulatory mechanisms and thus influence cell volume sensitive functions. Accordingly, cell volume regulatory mechanisms are an integral part of the signaling mediating cellular effects of hormones and mediators [1].

Following untoward cell swelling, volume regulatory mechanisms decrease intracellular osmolarity and cell volume, thus accomplishing regulatory cell volume decrease. Following cell shrinkage, cell volume regulatory mechanisms increase intracellular osmolarity and cell volume thus accomplishing regulatory cell volume increase [1, 3–7]. Cell volume regulation is most rapidly accomplished by ion transport across the cell membrane [5]. Following cell swelling, cellular ions are released; upon cell shrinkage, ions are accumulated (fig. 1). However, high cytosolic inorganic ion concentrations interfere with the stability of cytosolic proteins and alterations of ion gradients across the cell membrane may interfere with cell function. To circumvent those problems, cells utilize in addition organic osmolytes for osmoregulation [8]. Organic osmolytes are particularly important in kidney medulla with its excessive hypertonicity [9].

In the following paper, cell volume regulatory mechanisms and several factors challenging cell volume constancy will be described. Moreover, the impact of cell volume on metabolism will be briefly reviewed.

**Balance of Cytosolic Osmolarity**

The cellular accumulation of organic substances, such as amino acids generates cytosolic osmolarity. Osmotic balance across the cell membrane is accomplished by lowering of the cytosolic inorganic ion concentration below that of
extracellular fluid [1]. To this end, cells extrude Na⁺ in exchange for K⁺ by the Na⁺/K⁺-ATPase. The cell membrane is largely impermeable to Na⁺ but highly permeable to K⁺. The chemical K⁺ gradient drives K⁺ exit through K⁺ channels leading to a cell-negative potential difference across the cell membrane which drives Cl⁻ exit. The low cytosolic Cl⁻ concentration outweighs the high concentration of osmotically active organic substances.

The operation of the Na⁺/K⁺-ATPase and thus the establishment of the ionic gradients require expenditure of energy. Thus, cell volume constancy is challenged by cellular energy depletion, which impairs the function of the Na⁺/K⁺-ATPase, dissipates the Na⁺ and K⁺ gradients, depolarizes the cell membrane and leads to cellular accumulation of Cl⁻ and cell swelling [1]. During ischemia, the cellular K⁺ loss leads to increase in extracellular K⁺ concentration, which decreases the driving force for K⁺ exit and depolarizes the cell further. Moreover, excessive formation and reduced removal of lactate leads to cellular acidification, which in turn stimulates the Na⁺/H⁺ exchanger and thus augments cellular Na⁺ accumulation and cell swelling. The time course of cell swelling during energy depletion depends on the rate of Na⁺ entry [1]. In theory, in a completely Na⁺ impermeable cell, K⁺ and Cl⁻ approach an equilibrium which does not require any expenditure of energy for the maintenance of cell volume constancy. In some cells, energy depletion leads to transient cell shrinkage, preceding the eventual cell swelling. In those cells, the increase in intracellular Na⁺ concentration reverses the driving force for the Na⁺/Ca²⁺ exchanger and thus leads to Ca²⁺
entry, activation of Ca\(^{2+}\)-sensitive K\(^{+}\) channels and/or Cl\(^{-}\) channels, KCl exit and thus cell shrinkage.

**Regulatory Cell Volume Increase**

Following exposure of cells to hypertonic extracellular medium or cellular loss of osmolytes, the cytosolic osmolarity is lower than the extracellular osmolarity, and water exits leading to cell shrinkage. Regulatory cell volume increase in shrunken cells is mainly accomplished by cellular ion uptake [5]. Cell shrinkage triggers activation of the Na\(^{+}\), K\(^{+}\), 2Cl\(^{-}\) cotransporter and/or of the Na\(^{+}\)/H\(^{+}\) exchanger in parallel to the Cl\(^{-}\)/HCO\(_{3}\)\(^{-}\) exchanger [5]. H\(^{+}\) and HCO\(_{3}\)\(^{-}\) extruded by the Na\(^{+}\)/H\(^{+}\) exchanger and the Cl\(^{-}\)/HCO\(_{3}\)\(^{-}\) exchanger, respectively, are replenished in the cell from CO\(_{2}\) via H\(_{2}\)CO\(_{3}\). The activation of the carriers thus accomplishes NaCl entry. Na\(^{+}\) accumulated by either Na\(^{+}\), K\(^{+}\), 2Cl\(^{-}\) cotransport or Na\(^{+}\)/H\(^{+}\) exchange is pumped out by the Na\(^{+}\)/K\(^{+}\)-ATPase in exchange for K\(^{+}\). Thus, the transporters eventually accomplish cellular KCl uptake. The two Na\(^{+}\), K\(^{+}\), 2Cl\(^{-}\) cotransporters NKCC1 and NKCC2 [4] and the Na\(^{+}\)/H\(^{+}\) exchanger isoforms NHE1, NHE2 and NHE4 are activated, whereas NHE3 is inhibited by cell shrinkage [4].

Regulatory cell volume increase could be further achieved by activation of Na\(^{+}\) channels and depolarization, which in turn dissipates the electrical gradient for Cl\(^{-}\) and thus leads to Cl\(^{-}\) entry [10]. Some cells inhibit K\(^{+}\) channels and/or inhibit Cl\(^{-}\) channels upon cell shrinkage to avoid cellular KCl loss [1]. Cell shrinkage is further counteracted by cellular uptake or generation of organic osmolytes, such as sorbitol, myoinositol, betaine, glycerophosphorylcholine (GPC) and taurine [4, 8]. Sorbitol is generated from glucose, a reaction catalyzed by aldose reductase, which is expressed following osmotic cell shrinkage [8]. The expression of the protein is slow and the appropriate sorbitol concentrations are reached only within hours to days. GPC is produced from phosphatidylcholine by a phospholipase A\(_{2}\) and degraded by a phosphodiesterase. Cell shrinkage interferes with the degradation, thus leading to cellular accumulation of GPC.

Myoinositol (inositol), betaine and taurine are accumulated by the respective Na\(^{+}\)-coupled transporters SMIT, BGT and NCT [4]. The transporters accumulate Na\(^{+}\) and BGT and NCT Cl\(^{-}\) in parallel to organic osmolytes. Cell shrinkage stimulates and enhances the cellular accumulation of the osmolytes by the expression of the respective transporters. The expression of the transporters is slow and full adaptation requires hours to days. Beyond that, the osmolyte uptake depends on the availability of osmolytes in extracellular fluid.

Cellular concentration of osmotically active amino acids can be accomplished by both, cell volume-sensitive Na\(^{+}\)-coupled transport or degradation of intracellular proteins [1]. Organic osmolytes counteract the destabilizing effects
of inorganic ions, organic ions (e.g. spermidine) and urea, as well as the destabilizing effects of heat shock, desiccation and presumably radiation [1].

**Regulatory Cell Volume Decrease**

Following exposure of cells to hypotonic extracellular fluid or cellular excess of osmolytes, the cytosolic osmolarity is higher than the extracellular osmolarity, and water enters leading to cell swelling. Regulatory cell volume decrease could be accomplished by release of cellular ions by activation of K\(^+\) channels and/or anion channels [10, 11]. Cell volume regulatory K\(^+\) channels include Kv1.3, Kv1.5, Kv4.2,3, KCNE1/KCNQ1,4,5 TWIK1, TASK2/KCNK5, TREK1/KCNK2, TRAAK/KCNK4, intermediate or MaxiK (Kca) [4]. Cell volume regulatory anion channels are ClC-2, ClC-3, phospholemman and bestrophins. The involvement of I\(_{\text{Cl}}\), P-glycoprotein (MDR) and CFTR has been a matter of controversy [4, 12]. In any case, several distinct ion channels contribute to cell volume regulation. In some cells, swelling activates unspecific cation channels with subsequent entry of Ca\(^{2+}\) and activation of Ca\(^{2+}\)-sensitive K\(^+\) channels and/or Cl\(^-\) channels [4]. Cell volume regulatory ion exit could be further accomplished by activation of KCl cotransport [4]. In some cells, KCl exits via parallel activation of K\(^+\)/H\(^+\) exchange and Cl\(^-\)/HCO\(_3\) exchange. The H\(^+\) and HCO\(_3\) thus taken up by those transporters react via H\(_2\)CO\(_3\) to CO\(_2\) which easily passes the cell membrane and is thus not osmotically relevant.

Cell swelling stimulates the exit of GPC, sorbitol, inositol, betaine and taurine [13]. The mechanisms mediating the release of organic osmolytes are ill-defined and may involve several transporters and/or channels in parallel.

**Influence of Cell Volume on Metabolism**

Cell volume regulates a wide variety of functions including metabolism (fig. 2). For instance, cell shrinkage stimulates proteolysis and inhibits protein synthesis. The amino acids generated by net protein degradation are osmotically more active than the respective proteins. Accordingly, prevailing proteolysis generates cellular osmolarity. Conversely, cell swelling stimulates protein synthesis and inhibits proteolysis and glycogenolysis, thus converting the intracellular amino acids into the osmotically less active macromolecules [1]. Similarly, cell shrinkage stimulates and cell swelling inhibits glycogen degradation [1].

Cell volume further influences glucose and amino acid metabolism [1]. Cell swelling decreases glycolysis, increases flux through the pentose phosphate pathway, enhances lipogenesis from glucose, and inhibits transcription of phosphoenolpyruvate carboxykinase, a key enzyme for gluconeogenesis. Cell swelling stimulates oxidation of glycine and alanine, degradation of glutamine as well
as generation of NH$_4$\textsuperscript{+} and urea from amino acids. Cell swelling increases ketoscaproate oxidation, acetyl CoA carboxylase and lipogenesis, decreases carnitine palmitoyltransferase I activity, lowers cytosolic ATP and phosphocreatine concentrations, enhances respiration and fosters RNA and DNA synthesis. Cell shrinkage exerts opposite metabolic effects.

The increased flux through the pentose phosphate pathway following cell swelling enhances NADPH production and thus increases the formation of glutathione (GSH). Conversely, NADPH production and GSH formation are decreased by cell shrinkage. Cell swelling thus increases, and cell shrinkage decreases cellular resistance to oxidative stress [1]. By the same token, cell shrinkage decreases the activity of NADPH oxidase and thus impedes cellular O$_2$\textsuperscript{-} formation. Thus, cell swelling increases and cell shrinkage decreases the formation of reactive oxygen species [1, 14].

The volume sensitivity of metabolism is exploited by hormones. Insulin swells liver cells by activation of both Na\textsuperscript{+}/H\textsuperscript{+} exchange and Na\textsuperscript{+}, K\textsuperscript{+}, 2Cl\textsuperscript{-} cotransport and glucagon shrinks hepatocytes, presumably by activation of ion channels [14]. Insulin-induced cell swelling accounts for the antiproteolytic effect of the hormone and glucagon induced cell shrinkage accounts for the proteolytic effect of that hormone. Growth factors increase cell volume by stimulation of Na\textsuperscript{+}/H\textsuperscript{+} exchange and partially of Na\textsuperscript{+}, K\textsuperscript{+}, 2Cl\textsuperscript{-} cotransport, an effect required for the stimulation of cell proliferation [5]. Along those lines, osmolyte flux is in the brain regulated by transmitters [15]. Given the impact of cell volume in hormonal action, the sensitivity of target cells to hormonal influence is expected to be influenced by alterations of cell volume.

**Cell Volume-Sensitive Genes**

Cell volume modifies the expression of a wide variety of genes [8, 16]. Altered gene expression may serve to adjust cellular osmolarity. For instance, cell shrinkage stimulates the expression of the Na\textsuperscript{+}, K\textsuperscript{+}, 2Cl\textsuperscript{-} cotransporter, the Na\textsuperscript{+}/K\textsuperscript{+}-ATPase $\alpha_1$-subunit and enzymes or transporters engaged in cellular formation or accumulation of osmolytes including the aldose reductase as well as the Na\textsuperscript{+}-coupled transporters for betaine (BGT), taurine (NCT), myoinositol (SMIT) and amino acids. Other cell volume-sensitive genes encode proteins involved in the signaling of cell volume regulation. For instance, cell swelling stimulates the expression of the extracellular signal-regulated kinases ERK1, ERK2 and the Jun kinase-1, and cell shrinkage enhances the expression of the serum and glucocorticoid-inducible kinase SGK1 and cyclooxygenase-2 [4, 8, 16, 17]. Other proteins are expressed to protect cells against excessive osmolarity [1]. For instance, cell shrinkage stimulates the expression of protein-stabilizing heat shock proteins [1]. Cell shrinkage stimulates expression and release of antidiuretic hormone ADH, a hormone retaining water and thus counteracting dehydration [1].
Several cell volume-sensitive genes are seemingly unrelated to cell volume regulation [1]. For instance cell swelling stimulates the expression of β-actin and tubulin, of the immediate early genes c-jun and c-fos and the enzyme ornithine decarboxylase. Conversely, cell shrinkage stimulates the expression of the cytokine TNF-α, the Cl⁻ channel CIC-K1, P-glycoprotein, the immediate early genes Egr-1 and c-fos, the GTPase inhibitor α₁-chimaerin, the CDβ antigen, the enzymes phosphoenolpyruvate carboxykinase, arginine succinate lyase, tyrosine aminotransferase, tyrosine hydroxylase, dopamine β-hydroxylase, matrix metalloproteinase-9 and tissue plasminogen activator, as well as matrix proteins including biglycan and laminin B₂.

The stimulation of transcription is in part mediated by respective promoter regions in the cell volume-sensitive genes. Expression of the genes encoding aldose reductase, BGT and SGK1 are governed by osmolarity-responsive, tonicity-responsive (TonE) or cell volume-responsive elements. TonE binds the transcription factor TonE-binding protein, which is activated by cell shrinkage [16].

**Signaling of Cell Volume Regulation**

Sensors of cell volume and hydration remained elusive. Some evidence points to cellular protein content or macromolecular crowding [1]. The protein density
may influence a serine/threonine kinase (see below), which in turn regulates the activity of cell volume regulatory KCl and Na\textsuperscript{+}, K\textsuperscript{+}, 2Cl\textsuperscript{−} cotransport by respective phosphorylation of the transport proteins [4]. Alternatively, the cell could sense ionic strength or the concentration of individual ions such as Cl\textsuperscript{−} [4], stretch on the cytoskeleton and/or cell membrane [4], change of the cell membrane curvature as well as activation of cytokine, Ca\textsuperscript{2+}-sensing receptors [18] and/or integrins [4].

The sensors stimulate a myriad of cellular signaling pathways, depending on cell type and functional state of a given cell [4]. In many cells, swelling increases intracellular activity of Ca\textsuperscript{2+}, which may enter through nonselective Ca\textsuperscript{2+} channels, Ca\textsuperscript{2+}-permeable members of the TRP (transient receptor potential) channel family, such as TRPV4, and L-type voltage-gated Ca\textsuperscript{2+} channels [4]. Ca\textsuperscript{2+} subsequently activates volume-regulatory K\textsuperscript{+} channels and Cl\textsuperscript{−} channels and influences other cell volume-sensitive cellular functions [4].

Cell volume affects cytoskeletal architecture and expression of cytoskeletal proteins [19]. Microtubules and actin filaments may participate in cell volume regulation, and their disruption may interfere with cell volume regulation. Alterations of cell volume modify the phosphorylation of a variety of proteins [4, 20]. Kinases activated during cell swelling include tyrosine kinases, protein kinase C, adenylate cyclase, MAP kinases, focal adhesion kinase (p121\textsuperscript{FAK}), phosphoinositide 3 (PI3) kinase and extracellular signal-regulated kinases ERK-1 and ERK-2 [4, 20]. Activation of PI3 kinase is followed by stimulation of protein kinase B (Akt) and the serum and glucocorticoid-inducible kinases which modify a wide variety of carriers and channels [17]. Expression of SGK1 is, however, downregulated by cell shrinkage, which disrupts SGK1-dependent signaling [17].

Osmotic cell shrinkage stimulates WNK (with no lysine kinase) 1 and 4, which in turn activate Ste-20-related proline alanine-rich kinase (SPAK) and oxidative stress-responsive kinase (OSR1) [21, 22]. SPAK and OSR1 activate the Na\textsuperscript{+}, K\textsuperscript{+}, 2Cl\textsuperscript{−} cotransporters NKCC1 and NKCC2 [23]. Conversely, WNK4 inhibits KCl cotransporters [4]. WNK1 activates SGK1, which in turn inhibits WNK4 [4, 24]. Osmotic cell shrinkage further activates the tyrosine kinase Fyn, several MAP (mitogen-activated protein) kinase cascades, SAPK, p38 kinase, myosin light-chain kinase, Jun kinase, p21-activated kinases PAKs Rho kinase, LIM kinase and casein kinase [4, 20]. The kinases may influence cell volume regulation by modulating cytoskeleton, cell volume regulatory ion transport or activation of transcription factors governing expression of cell volume-regulated genes.

In some cells, swelling activates phospholipase A\textsubscript{2} resulting in the formation of the 15-lipoxygenase product hepxoxilin A\textsubscript{3} and the 5-lipoxygenase product leukotriene LTD\textsubscript{4} [4]. The eicosanoids in turn stimulate cell volume-regulatory K\textsuperscript{+} and/or Cl\textsuperscript{−} channels and/or taurine release. Cell shrinkage may stimulate formation of PGE\textsubscript{2}, which in turn may activate PGE\textsubscript{2}-sensitive Na\textsuperscript{+} channels [25]. Cell volume signaling may further involve nitric oxide [4, 26].
Cell swelling decreases and cell shrinkage increases the formation of phosphatidylinositol 4,5 bisphosphate [PtdIns(4,5)P$_2$] [4] by influencing the phosphatidylinositol 4-phosphate 5-kinase-β isoform (PIP5KIβ) [4]. PtdIns(4,5)P$_2$ stimulate some channels (TRPV1, ENaC), Na$^{+}$/H$^{+}$ exchange (NHE1) and Na$^{+}$/Ca$^{2+}$ exchange and they inhibit TRPC6 [4, 27]. PtdIns(4,5)P$_2$ further stimulates actin polymerization [4, 28].

Cell swelling increases and cell shrinkage decreases the pH in acidic cellular compartments, including endosomes, lysosomes and secretory granules. The alkalinization of the acidic cellular compartments decreases protease activity and thus autophagic proteolysis [1]. By this means, cell swelling inhibits autophagic proteolysis [1].

**Influence of Metabolism on Cell Volume**

Cellular degradation of proteins to amino acids, glycogen to glucose phosphate, or triglycerides to glycerol and fatty acids results in an increased number of osmotically active particles, increased cellular osmolarity, and thus cell swelling. The degradation of the substrates to CO$_2$ and H$_2$O decreases cellular osmolarity and thus fosters cell shrinkage [1]. Glycolysis fosters cell swelling by cellular accumulation of lactate and H$^{+}$ and subsequent activation of the Na$^{+}$/H$^{+}$ exchanger [1].

Metabolic pathways may influence cell volume indirectly through alteration of transport across the cell membrane. In some cells, a decrease in cellular ATP could activate ATP-sensitive K$^{+}$ channels and thus lead to cell shrinkage. Formation of reactive oxygen species may shrink cells by activation of oxidant sensitive K$^{+}$ channels or by inhibition of oxidant sensitive Na$^{+}$, K$^{+}$, 2Cl$^{-}$ cotransport. By the same token, it could swell cells by inhibiting Kv1.3 K$^{+}$ channels and KCNE1/KCNQ1 K$^{+}$ channels [1].

In liver insufficiency, impaired formation of urea leads to accumulation of NH$_3$, which enters the brain, is taken up by glial cells, stimulates cellular formation and accumulation of glutamine and thus triggers glial cell swelling. Glial cells release myoinositol to counteract swelling and exhaustion of the osmolyte is followed by severe dysfunction leading to development of hepatic encephalopathy [14, 29].

In diabetic ketoacidosis, cell swelling may result from cellular accumulation of organic acids and cellular acidity activating the Na$^{+}$/H$^{+}$ exchanger as well as from hyperglycemia stimulating cellular formation and accumulation of sorbitol from glucose through aldose reductase [1]. Hyperglycemia further leads to formation of advanced glycation end products which upregulate SGK1 and similarly induce cell swelling [17]. To counteract cell swelling, cells release osmolytes such as myoinositol. Cell swelling inhibits proteolysis, fostering together with SGK1 the disposal of matrix proteins [30].

Hypercatabolic states, such as burns, acute pancreatitis, severe injury, or liver carcinoma may be caused by a decrease in muscle cell volume, which correlates...
with urea excretion, an indicator of protein degradation [1]. Conversely, hypercatabolism can be reversed by glutamine, which swells cells by Na\textsuperscript{+}-coupled cellular uptake.

**Cell Volume and Athletic Performance**

A goal of exercise training is to produce cellular adaptations that, in turn, improve athletic performance. Given the considerable evidence from in vitro studies that cell shrinkage or swelling induces gene transcription and can impact carbohydrate and protein metabolism, it seems plausible to use hydration as a tool to manipulate cell signaling. There is supportive evidence, in vivo, that cell swelling can affect cell metabolism. For example, Keller et al. [31] reported that expansion of total body water (and induction of hyponatremia) produced modest reductions in resting protein breakdown (–6%) and protein catabolism (–20%) – observations consistent with in vitro cell swelling experiments.

The induction of overhydration also had effects on glucose and fat metabolism, as glucose disposal was reduced and glycerol appearance increased during an euglycemic hyperinsulinemic clamp. Together, the in vitro and in vivo observations suggest that manipulation of cellular hydration state might be a tool to promote favorable cellular adaptations.

Specific strategies exploiting cellular hydration to augment training adaptations, however, have not been established. A challenge in vivo is producing cell swelling and downstream metabolic signaling; water intake does not equate to cell swelling. The in vivo setting is also much more complex than in vitro, as hormones, cytokines, oxidative stress, etc. can potentially complicate the cellular metabolic responses to cell swelling and cell shrinkage. Strategies that exploit the effects of substances such as glutamine on cell volume may be more effective than simple manipulation of drinking behavior.

There is little doubt that glutamine decreases catabolism in vivo, and as shown in in vitro experiments, the antiproteolytic effect of glutamine is completely lost if glutamine-induced cell swelling is prevented. The experiments conducted to date have been focused on furthering our understanding of the physiological consequences of cell swelling and shrinkage, and the mechanisms responsible for these effects. Very little research has been done to date to examine if cell swelling can be exploited to facilitate exercise training.

**Conclusions**

Cells are equipped with cell volume-regulatory mechanisms adjusting cell volume to the functional demands. The mechanisms are under the control of diverse signaling pathways. Conversely, cell volume and cell volume-sensitive
cellular functions participate in a wide variety of physiological and pathophysiological mechanisms including regulation of metabolism. Understanding the interplay of cell volume and metabolic regulation may open novel opportunities for favorably influencing several clinical disorders such as diabetes, renal or hepatic insufficiency, catabolic states and fibrosing disease. Strategies that exploit cell volume regulation may also have applications in exercise training adaptation.

References

**Discussion**

*Dr. Montain*: Your observations that cell volume can alter cell metabolism leads to the idea that manipulating hydration to produce swelling or shrinking could be a tool to promote adaptation. In your in vitro model, are the cells responsive to osmotic challenges in the 270–300 mosm range? How much ‘slop’ does the cell accommodate before a response is produced?

*Dr. Lang*: That’s a very good question. As you may have seen, only a few percent of cell volume changes are required to modify cell function. The effect of insulin on cell volume is only 8% cell swelling. That extent of cell swelling is sufficient to stop proteolysis. I should add that effects of insulin are not exclusively cell volume mediated, and there is extensive signaling of insulin which is not directly related to cell volume but is only modified by alterations of cell volume. The effect of insulin on proteolysis, however, is almost completely due to cell swelling. The sensitivity of cells to alterations of cell volume is exquisite, and only a 1% change of cell volume may activate channels and transporters.

*Dr. Montain*: So, the cell is responsive to an osmolarity step change between 280–290, 280–300 mosm?

*Dr. Lang*: If you force the cell to swell by reducing extracellular osmolarity, it regulates its volume rapidly and the cell swelling is only transient. Frequently, the cells do not regulate completely and thus, a residual change of cell volume persists. If you add glutamine, you observe sustained cell swelling. Under those conditions, volume regulation prevents further increase in cell volume but, in that case, some 4% cell swelling is maintained, which is not necessarily true if you change osmolarity. Similarly, if you add insulin or any other hormone which upregulates cell volume, then you observe a sustained effect.

*Dr. Montain*: Often, in the cell culture experiments, a step change of a fairly large magnitude is applied. Do you see different cell responses if the stress is smaller and applied in a graded manner?

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**Dr. Lang:** There is the so called isovolumetric cell volume regulation: If you change osmolarity very slowly, then the cell may not swell or shrink appreciably, because it regulates fast enough to avoid cell volume changes to the extent that we can measure it. The cells are better in measuring their volume than we are, so our methods are only able to pick the cell volume changes when the cell is already stimulated extensively. Accordingly, upon very slow osmolarity change, you do not see any changes in cell volume, yet you stimulate cell volume regulation.

**Dr. Zemel:** Can you comment on the consequences of cell volume changes that are iso-osmotic, for example cell volume changes consequent to increases or decreases in fat droplet size – how might this impact SGK1? Also, what about the tissue distribution of SGK1?

**Dr. Lang:** For many years, I have wondered whether or not fat droplets in a cell are relevant for cell volume. I do not believe they are, but I have no data to show that. The belief is based on the consideration that the protein concentration does not change when you accumulate triglycerides in the cell, and to the extent that cell volume regulatory mechanisms are triggered by alterations of the cytosolic protein concentration, addition of triglycerides would not stimulate cell volume regulation. However, this is mere speculation.

**Dr. Haschke:** What is the effect on the cell if somebody is treated with IGF-I, which stimulates insulin secretion? Is there a visible effect, and if so, does this explain the symptom of headache observed during treatment?

**Dr. Lang:** IGF-I stimulates SGK1, which in turn stimulates sodium potassium cotransport. This leads to cell swelling; that is to say, IGF-I may lead to cell swelling. Whether or not that occurs in glial cells and in neurons to the extent that intracerebral pressure increases, I don't know, but that's an interesting comment.

**Dr. Sprriet:** My question relates to skeletal muscle, particularly during exercise. I noticed that you presented one slide from a patient population where you appear to have an estimate of cell water in muscle. Do you have any information on the impact of whole-body dehydration on skeletal muscle? In other words, how well defended is the cell volume of active skeletal muscle during exercise?

**Dr. Lang:** The skeletal muscle volume measurements I showed were the work of Dr. Roth in Vienna. He calculated the intracellular water content from the chloride concentration. We do not have data during exercise or dehydration.

**Dr. Maughan:** Many years ago, we did some studies on the osmotic fragility of the red blood cell because you can easily incubate them and measure cell swelling as osmolarity is manipulated. Some of the cells were much more sensitive to changes in osmotic pressure than others because they didn't all burst at the same osmolarity, and exercise seemed to increase the osmotic fragility of the red cells. What is it that exercise is doing to the cell that is changing its ability to tolerate that swelling?

**Dr. Lang:** We have no experience with cell volume regulation of erythrocytes following exercise. However, there is a powerful machinery linking volume and survival of erythrocytes. Similar to nucleated cells, erythrocytes undergo suicidal death upon excessive cell shrinkage, a phenomenon called eryptosis. They express a cation channel, which is calcium permeable. If you shrink them, they open that cation channel leading to increases in cytosolic calcium, calcium-sensitive scrambling of the cell membrane, and phosphatidylserine exposure on the cell surface. Phosphatidylserine-exposing erythrocytes are eventually removed. This mechanism is important for the removal of injured cells.
Dr. Maughan: We certainly saw a big increase in the osmotic fragility after a marathon race – so that's a very acute effect on the cells. A second question: you say there are no cells with a significant hydrostatic pressure, but in the situation of hyponatremic encephalopathy where individuals drink so much that plasma sodium concentration falls, the brain swells, and there is increased intracranial pressure that may lead to circulatory occlusion. Why isn't there a regulatory mechanism that overrides the osmotic effect and prevents the increase in pressure?

Dr. Lang: It usually works very well; otherwise, we would face this problem all the time. Of course, the single glial cell which regulates its volume doesn't think about the circulation and about the pressure. The small increase in pressure, sufficient to stop cerebral perfusion, is negligible as compared to osmotic pressure gradients. Accordingly, you cannot squeeze out neuronal cells by increasing intracerebral pressure. There is another problem: during cerebral cell volume regulation, cellular potassium is not released into the blood, but rather into the cerebral interstitial fluid, where it leads to local increases in extracellular potassium concentration. As a result, the brain is not very well able to maintain osmotic equilibrium, making it extremely important for the brain to be bathed in an isotonic environment. Cerebral cells regulate their volume mainly utilizing organic osmolytes, a mechanism which is very slow.

Dr. Baar: With regard to insulin and swelling effects, how much is dependent on stretch-activated channels rather than some indirect effect of opening or closing of channels?

Dr. Lang: The stretch-activated channels are the last line of defense. They usually are not important for daily life, and not for insulin effects. Nevertheless, the stretch-activated channels were the first discovered direct mechanism of cell volume regulation. Thus, the scientific community was very excited about those channels. ADH-releasing cells express stretch-inactivated channels, so that when the stretch reduces they are activated. But otherwise, the mechanosensitive channels are not so important for cell volume regulation and they are not involved in the effects of insulin.

Dr. Phillips: In clinical situations like burns and sepsis, treating the patients with osmolytic solutions containing glutamine as well as other substances, produces positive outcomes. In the sports supplement market, people have tried to exploit these data using osmolytic compounds like glutamine, taurine and creatine for example, but normal skeletal muscle doesn't seem very responsive. In your cell culture model, do these approaches affect protein synthesis or protein breakdown such that the cells appear to be in a better state?

Dr. Lang: This is a very interesting question. We almost failed to see the insulin effect by looking at resistant cells. When Dieter tried the effect of insulin in fed rats, he did not see anything. Only when he took livers of starved rats, did he discover the effect of insulin. Obviously, you need prior shrinkage to see the effect of insulin. We now know that SGK1, which is required for the full effect of insulin on cell volume, is a cell volume-regulated gene which is downregulated in a swollen cell. To see a full effect of insulin, SGK1 must be expressed. Along those lines, possibly only a dehydrated skeletal muscle expresses high levels of SGK1. That is just a speculation at the moment. As long as SGK1 is high, the swelling effect is apparent. If SGK1 is low, no effect is observed. Possibly the resting normal skeletal muscle does not respond because it has very low SGK1 levels. Again, we need to do the measurements to test whether this explanation is correct.
**Dr. Phillips:** Is it fair to conclude that so called osmolytic substances like creatine or taurine have limited effect in normal skeletal muscle?

**Dr. Lang:** The effect of creatine would depend on how much creatine transporter is expressed and that depends on how much SGK1 is expressed. So, I think it's not as simple that you consume the substances, then the cells take up the substance and swell. You may need to go further upstream in the signaling pathway to modify taurine uptake or creatine uptake in a skeletal muscle which is not shrunken.

**Dr. van Loon:** With exercise, there are massive changes in osmolarity in the muscle cell. The increase in cell volume will trigger cell volume regulation mechanisms, but may also stimulate metabolism. Based on your observations, do you think these changes in osmolarity lead to changes in the myonuclear domain and therefore contribute to muscle hypertrophy?

**Dr. Lang:** Osmolarity changes metabolism and metabolism may, of course, change the cell volume. Signaling is not a chain of events, but a network with mutual interactions. If you fully understand the system, then you can exploit that knowledge to manipulate cell volume and metabolism. However, you should not expect a simple means to elicit the desired effect. The underlying mechanisms are of course regulated by negative feedback, which adds complexity to the mutual interaction between osmolarity changes and metabolism.

**Dr. van Loon:** Is an increase in cell volume following exercise a setoff point for cell activation and ultimately greater myonuclear content and muscle hypertrophy?

**Dr. Lang:** I think if you swell a cell, you set the stage for net protein synthesis. The lipid metabolism is less sensitive to cell volume. The most sensitive metabolic pathway is certainly proteolysis and the second is glycogenolysis.

**Dr. van Loon:** So, if you design an experiment to pharmaceutically prevent rehydration or cell volume increase following exercise you would prevent the adaptive response to exercise? Is it so crucial?

**Dr. Lang:** The experiment has yet to be done, but my expectation would be yes, it has a major impact. Please remember that 8% of cell volume change is the full effect of insulin on proteolysis in the liver. Cell swelling is really a highly effective, very powerful mechanism regulating metabolism. We have not done the experiment with skeletal muscle, but my prediction would be you won't be able to produce net protein synthesis if you don't allow the cell to reswell and to overcome cell shrinkage.

**Dr. Maughan:** To follow on from this point, we have recently done a pilot study in which every other day during an 8-week training period, subjects ingested sufficient glycerol to drive up plasma glycerol concentrations by ~20 mM. Presumably, this glycerol dosing would cause a transient cellular dehydration. We don't have any muscle tissues to examine if the stimulus affected skeletal muscle signaling, but there has been no benefit in terms of muscle strength or muscle size.

**Dr. Lang:** I recommend an experiment determining whether or not exercise would have an effect in the SGK1 knockout mice or not. My expectation is that exercise will upregulate SGK1, and the SGK1 will enhance cell swelling, and the cell swelling sets the stage for more protein synthesis and less protein degradation.

**Dr. Baar:** Just to follow on from that, we have seen using downstream targets of SGK1 that mTOR seems to be an important part of SGK1 activation.

**Dr. Lang:** You are perfectly right, and the effect of mTOR for instance on creatine transporter depends on SGK1.
Dr. Baar: Can you comment on your preliminary studies of the physical activity of SGK1 knockout mice?

Dr. Lang: Yes, they run at the same speed but they become exhausted more rapidly. When we placed them on a running wheel, the knockout mice did not run as far as the wild type mice under otherwise identical conditions. But, I think I would need an expert like you to perform the respective experiments in a really professional way.

Dr. McLaughlin: A lot of the plasma membrane-mediated signaling events and channel activities are modulated by the composition of the plasma membrane and lipids in particular. Is there any evidence that the composition of the plasma membrane or altering the composition of the plasma membrane affects the cell response to volume regulation?

Dr. Lang: Yes, that has been done by others. Cell volume regulation is for instance influenced by cholesterol content of the plasma membrane. We have never done these types of experiments, so I can't say much more.

Dr. Baar: We are going to talk later about how you might try to accelerate a stress beforehand, for example by manipulating glycogen content, and how that can augment the adaptations that occur with exercise. Would there be any merit to an approach where you try to dehydrate prior to resistance exercise to sort of prime the protein synthesis response and then following the resistance exercise training, restore fluid and provide the amino acids?

Dr. Lang: I would go another way. Again you are the experts in skeletal muscle, I am just expert in SGK1 and in cell volume regulation, you have to do the experiments, but I would try to upregulate SGK1 without shrinkage. The shrinkage would be counterproductive. I would try to upregulate SGK1 and then add a stimulus for SGK1, and then you would get dramatic swelling and would have hopefully dramatic protein synthesis and inhibition of protein degradation. There are several means to stimulate SGK1 without shrinking the cells. SGK1 is regulated in two steps, first there is genomic regulation which is required for the activation, and then there is activation. Insulin is only activating, so if there is no SGK1 around, insulin cannot activate it.

Dr. Baar: I wonder if cell swelling isn't one of the mechanisms underlying the occlusion growth paradigm, where hypertrophy is induced by inflating a cuff to cut off blood flow during low-intensity exercise. This approach produces a period of osmotic stress which magnifies a very small exercise stimulus with a huge blood flow stimulus.

Dr. Lang: Ischemia is one of the mechanisms upregulating SGK1 expression, so that could be one practical way to do what I just suggested.

Dr. Montain: In your studies, have you examined whether there is adaptation to a repeated stimulus? Do the cells need a greater challenge to produce a response or do the cells basically have a very short memory so a pulsatile challenge repeatedly produces the same cellular activation?

Dr. Lang: We have not exactly done that experiment. When you shrink the cells continuously, then you have a sustained increase in SGK1 expression. Likewise, if you stimulate the cell with TGF-β, you have a sustained increase in SGK1 expression. However, if you stimulate expression with interleukin-4, then you have only a transient increase in SGK1 expression. So, whether SGK1 is upregulated transiently or in a sustained manner seems to depend on the stimulus.