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MINIREVIEW

Physical Water Compartments: A Revised Concept of Perinatal Body Water Physiology

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Summary

This review presents experimental data on the perinatal significance of the recently developed concept of physical water compartments. This concept implies that in addition to the compartmentalization of body water into the intra- and extracellular spaces, motionally distinct water fractions – designated as physical water compartments – are also of importance in the neonatal body fluid redistribution. H^1-NMR spectroscopy provides a quantitative estimate of tissue water fractions with different mobility as multicomponent analysis of the T_2 relaxation decay curves allows us to determine the fast and slow relaxing components of the curves corresponding to the bound and free fractions of tissue water. Using this method, free and bound water fractions were measured in fetal and neonatal rabbit tissues (skin, skeletal muscle, liver, brain, lung) at different stages of maturity and under conditions of various fluid intake. It has been demonstrated that water mobility in individual fetal/neonatal tissues varies greatly and there is a general tendency of increasing free water at the expense of bound water fraction with progressing maturation. This tendency appears to be accelerated in the immediate postnatal period when the tissue water content is markedly reduced. The importance of hyaluronan in this process has also been addressed as the hyaluronan content is markedly elevated in the fetal/neonatal tissues and due to its polyanionic, hydrophilic nature it has been claimed to play a prominent but not clearly defined role in the control of tissue hydration.

Key words

Tissue water mobility • Proton nuclear magnetic resonance • Hyaluronan • Perinatal period

Background

According to the traditional concept of body fluid physiology, body water is distributed in well-defined compartments with strictly controlled volume and composition. The water movement between the interrelated fluid compartments are mostly regulated by physical forces.

Newborn infants are born in a state of relative hyperhydration which is inversely proportional to the maturity of the neonate. Expressed as percentage of body weight total body water (TBW) and extracellular water

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(ECW) decrease whereas intracellular water (ICW) increases with advancing gestation (Friis-Hansen 1961). Soon after birth a redistribution of fluid compartments occurs that is a subject of controversy. Most authors agree that the early postnatal weight loss reflects isotonic contraction of extracellular fluid (ECF), whereas the volume of intracellular fluid (ICF) and circulating plasma is maintained, the reduction in ECF being thus confined to the interstitial space (Shaffer *et al.* 1986, Bauer and Versmold 1989, Bauer *et al.* 1991, Heimler *et al.* 1993). Others claim unchanged or slightly increased ECF and a decrease in ICF, suggesting that during the period of initial weight loss the plasma volume is preserved at the expense of ICF (Cheek 1961, MacLaurin 1966). In support of this contention Coulter and Avery (1980) and Coulter (1983) reported a paradoxical reduction in tissue hydration with increasing fluid intake in newborn rabbits and concluded that ICF served as a fluid reservoir to maintain circulation when fluid intake is restricted. In response to adequate fluid administration, however, the superfluous cellular water was rapidly released presumably through a mechanism that involved prolactin regulation (Coulter and Avery 1980, Coulter 1983).

To reconcile these apparently conflicting observations, a concept has been recently put forward implying that not only the compartmentalization but also the mobility of tissue water is of importance in neonatal body fluid redistribution (Sulyok 1994). Accordingly, motionally distinct water fractions have been established - the free bulky water and the relatively constrained, slow-motion bound water. Water can be liberated from this latter fraction in a regulated manner according to the actual need irrespective of its location in the cellular or extracellular phase.

The principle of physical water compartments

The term *physical water compartments* designates the physical state of tissue water and implies interactions between dipole water molecules and tissue biopolymers including proteins and glycosaminoglycans. The interaction of polar solid surface of intra- or extracellular macromolecules with water results in the formation of a dynamic structure of polarized water multilayer. The degree of water polarization depends on the number of the exposed active, polar groups of the water polarizing macromolecules. The first oriented layer of water molecules on the surfaces can induce a second layer to orient, the second will likewise influence the third and so on. As a result, a picture of hydrophilic surfaces bounded by a coat of structured water emerges. The range of interactions generating the polarized water multilayer has been variously suggested to extend from nanometers to several micrometers. Parallel arrangement of the linear biopolymer chains with alternating positive and negative sites enhances the degree of polarization and motional restriction and increases stability of the multilayer. With respect to the electrical polarization and spatial orientation of tissue water, intra- and extracellular macromolecules therefore creates microcompartments of different size and stability. The extent of water polarization is assumed to be proportional to the limitation of tissue water mobility (Ling 1992, Israelachvili and Wennerström 1996).

Determination of tissue water fractions with different mobility

Proton nuclear magnetic resonance (H^1-NMR) measurements have been applied to assess quantitative changes in tissue water mobility since it provides an estimate of the physical state of tissue water including the volume fraction, proton residence time and intrinsic magnetic relaxation rate within the compartments. Theoretical basis of this estimate is that the magnetic relaxation rates for ordered (bound) water protons are faster than those for non-ordered (free) water protons. For quantitative assessment of tissue water fractions with different mobility multicomponent analysis of the T_2 relaxation decay curves have been applied. The free induction decay of the proton relaxation process follows an exponential function. This function can be described by a multiexponential equation provided that in the tissues studied there are water compartments with different rates of relaxation and these compartments are not interdependent at the time of measurement. The process of proton T_2 relaxation can be derived from the following expression:

$$F = k_1 \times e^{-t/T_{21}} + k_2 \times e^{-t/T_{22}} + ...k_n \times e^{-t/T_{2n}}$$

where k_1, k_2, and k_n represent the relative contribution of each set of protons; T_{21}, T_{22} and T_{2n} are the relaxation times of the different components.

Biexponential analysis of the T_2 relaxation curves allows us to estimate the bound and free water fractions by determining the fast and the slow

components of the curves. Using triexponential analysis, further partition of the T_2 curves is possible and a distinction can be made between the fast, intermediate and slow components corresponding to the tightly bound, loosely bound and the free water fractions (Mulkern *et al.* 1989).

Physical water compartments during the early postnatal period

In a series of recent studies we attempted to quantitate the free and the bound water fractions in the skin, skeletal muscle, brain and liver in two groups of newborn rabbits during the first 3-4 days of life. Rabbit pups of one group were nursed conventionally by their mothers (suckling *ad libitum*), while the other group included pups separated from their mothers and completely withheld from fluid intake (Berényi *et al.* 1996, 1998).

Biexponential analysis of the T_2 relaxation curves revealed that the bound water fraction amounted to 42-47 % in the skin, 50-57 % in the muscle and 34-40 % in the liver respectively, of the total tissue water. This pattern of distribution did not change either with age or fluid intake. By contrast, the percentage contribution of bound water fraction in the brain fell progressively from 61 % at birth to 3 % at the age of 72-96 hours. In response to complete fluid deprival the reduction of bound water fraction was accelerated to attain a value of as low as 4 % already on the first day of life (Fig. 1).

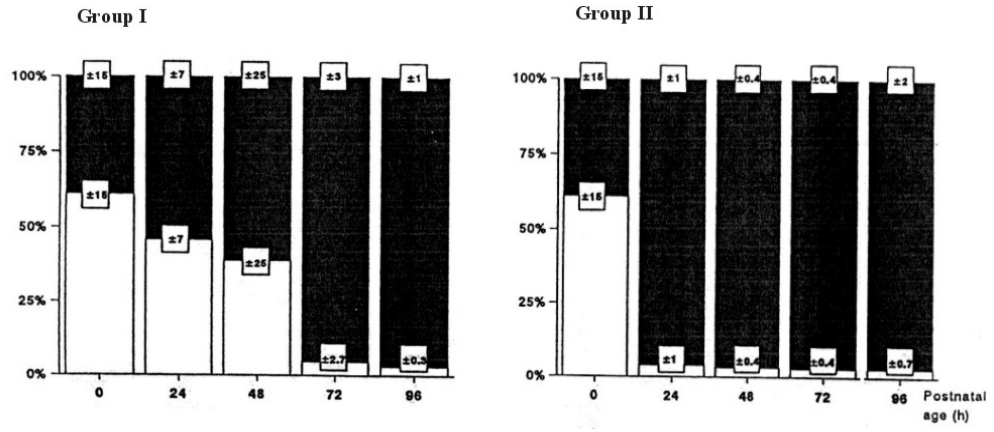

Fig. 1. Partition of brain water fractions according to their mobility as derived from biexponential analysis of T_2 decay curves in newborn rabbits nursed with their mothers and fed *ad libitum* (group I) or completely deprived from fluid intake (group II). Symbols □ are for fast-bound and ■ for slow-free components. Data are given as mean ± S.E.M.

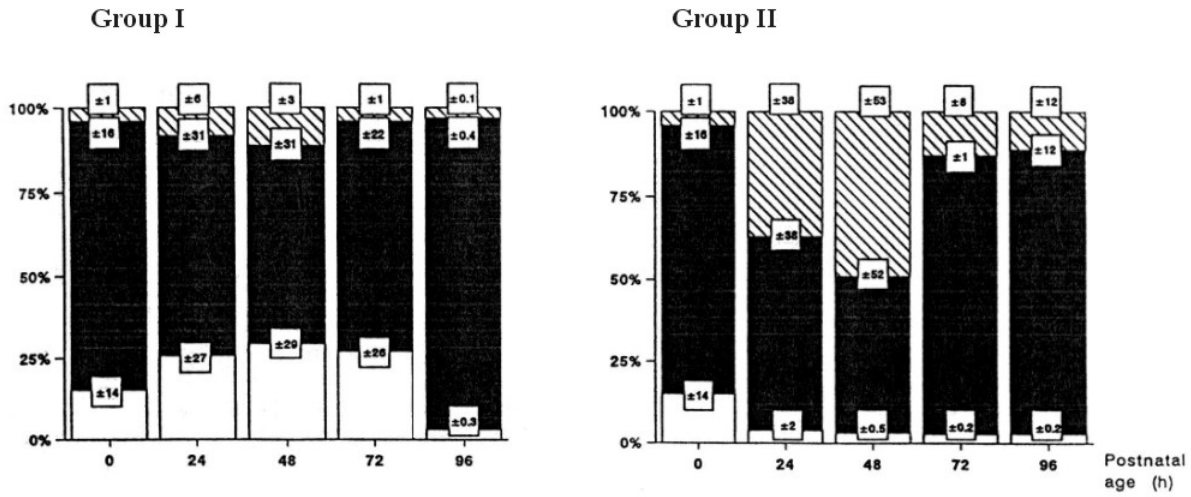

Fig. 2. Partition of brain water fractions according to their mobility as derived from triexponential analysis of T_2 decay curves in newborn rabbits nursed with their mothers and fed *ad libitum* (Group I) or completely deprived from fluid intake (Group II). Empty bars are for fast-tightly bound, full bars for middle-loosely bound, and hatched bars for slow-free components. Data are given as mean ± S.E.M.

Using triexponential analysis we found that most of the skin (48-64 %) and muscle water (54-64 %) is loosely bound followed by the free (skin: 26-45 %, muscle: 25-32 %) and tightly bound water fraction (skin: 6-14 %, muscle: 10-16 %). Postnatal age and fluid intake had no apparent influence on this partition of tissue water. Interestingly, more water was tightly bound in the liver than in the other tissues, this fraction increased from 14 % at birth to 26-33 % later on mostly at the expense of loosely bound water fraction. The postnatal increase of the tightly bound fraction proved to be more pronounced in the suckling than in the starving pups. In the brain loosely bound water (48-94 %) also predominated over the free (3-49 %) and tightly bound water fraction (3-29 %). Starving pups responded to fluid deprivation with a 3- to 6-fold decrease in the tightly bound water and with a simultaneous 4-fold increase in the free water fraction (Fig. 2). This observation can be regarded as a evidence for restructuring of brain water in order to maintain brain volume.

The different water mobility in individual newborn rabbit tissues and its response pattern to the complete withdrawal of fluid intake appear to be the result of differences in the water content, water-free chemical composition, qualitative or quantitative alterations in macromolecular compounds and metabolic activity of the tissues investigated.

Role of hyaluronan in the water metabolism of perinatal lung and brain tissue

Hyaluronan (HA) has been claimed to be the major macromolecular compound controlling water mobility and water balance in the lung (Hällgren *et al.* 1989). During the fetal and neonatal period, HA concentration in the lung tissue is elevated and inversely proportional to the maturity of the neonate. Its role as a determinant of tissue water content during pulmonary adaptation has been established (Allen *et al.* 1991, Sedin *et al.* 1994).

In a recent study using H^1-NMR relaxometry we simultaneously investigated parameters of lung water metabolism and lung HA concentrations in preterm and term rabbit pups. It was demonstrated that the T_2-derived free water fraction remained unchanged at a gestational age of 25-29 days (~ 67 %), but increased progressively to a value of 75.5±7.9 % on day 31 and to 83.4±9.4 % at postnatal age of 4 days. Opposite changes occurred in the bound water fraction. Lung HA concentration decreased with advancing gestation from 870.8±205.2 μg/g dry weight on day 25 to 162.6±32.4 μg/g dry weight on day 31 but there was a two-fold increase postnatally. HA correlated positively with total lung water but not with the bound water fraction.

It has therefore been suggested, that the elimination of lung fluid is associated with an increase in free water at the expense of the bound water fraction. The underlying mechanisms of the release of water molecules from macromolecular binding remain to be established as HA does not appear to be directly involved in this process (Sedin *et al.* 2000).

The pathophysiological and clinical significance of the above described reorganisation of lung water is emphasized by the demonstration of unaffected physiological lung dehydration in aquaporin-1, -4, and -5 knocked out mice where the airspace to capillary water permeability is markedly reduced (Verkman *et al.* 2000). It appears relevant to conclude that the channel-mediated water transport alone cannot be responsible for perinatal clearance of lung fluid but rather it may also be attributed to the redistribution of bound to free water fraction in the lung.

Brain water content and HA concentrations are also known to undergo developmental changes. Aquaporin-4, the predominant water fluxing membrane protein in the brain has been shown to have a major role in the control of brain water by facilitating water transport at the brain/blood and brain/cerebrospinal fluid interfaces (Nielsen *et al.* 1997). Developmental studies to reveal the ontogeny of brain aquaporin-4 protein expression in rats demonstrated very low protein levels (0.6-2.0 %) in the first postnatal week, its pronounced increase in the second week (25 %), followed by a further rise to 63 % of adult levels on day 28 (Wen *et al.* 1999). The perinatal course of brain dehydration is just opposite to that of aquaporin-4 expression (Lorenzo *et al.* 1989), it is unlikely therefore, that this protein contributes significantly to the process of physiological brain dehydration.

In an attempt to reconcile these apparently conflicting observations we have recently conducted a study to obtain more insight into perinatal brain water metabolism. We have found highly elevated water and HA contents in the preterm brain that decreased markedly with progressing fetal/neonatal age. Maturity-related changes also occurred in the T_2 relaxation-derived bound water fraction which amounted to 4-19 % of total brain water. The bound water fraction appeared to be

independent of total brain water suggesting restructuring of the freely moving and motionally constrained water fractions. Unexpectedly, in spite of the high HA concentrations in the developing brain (Jenkins and Bachelard 1988) and the polyanionic nature and gel-like properties of HA no association could be detected between tissue HA content and the bound water fraction (Sulyok *et al.* 2001). Our failure to document an apparent relationship of HA to bound water may hitherto be related to only partly defined molecular alterations of HA. These may include maturity-related changes in molecular size (Dahl *et al.* 1986) and electrical charge, variations in the HA conformation with subsequent (un)covering of active, polar sites of the molecular surface (Ling 1992), and also hormonally regulated enzymatic degradation (Ginetzinsky 1958).

Taken together during the perinatal period when aquaporine 4-mediated transmembrane water flux is limited, the water release from the brain tissue reservoir may contribute to the physiological reduction of brain water and at the same time to maintaining brain volume.

Conclusions

Intra- and extracellular macromolecules with active, polar sites on their surface polarize water and generate microcompartments with different size and stability. Water proton mobility derived from H^1-NMR measurements provides an estimate of the dynamic structure of polarized water multilayer. The limitation of tissue water mobility is assumed to be proportional to the water polarization.

Water bound to macromolecules in the intra- and extracellular compartments can be stored and released in a regulated manner to meet the actual need of maintaining plasma volume. The control of interactions between water fractions with different mobility remains to be determined.

Water mobility in individual fetal/neonatal tissues varies greatly and there is a general tendency of increasing free water at the expense of the bound water fraction with progressing maturation. Hyaluronan content is markedly elevated in the fetal/neonatal tissues and due to its polyanionic, hydrophilic nature it is claimed to play a prominent, but not clearly defined role in the control of tissue hydration.

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References

ALLEN SJ, SEDIN EG, JONZON, WELLS AF, LAURENT TC: Lung hyaluronan during development: a quantitative and morphological study. *Am J Physiol* **260**: H1449-H1454, 1991.

BAUER K, VERSMOLD HT: Postnatal weight loss in preterm neonates <1500 g is due to isotonic dehydration of the extracellular volume. *Acta Paediatr Scand* Suppl 360: 37-42, 1989.

BAUER K, BOVERMANN G, ROITHMAIER A, GOTZ M, PROLLS A, VERSMOLD HT: Body composition, nutrition, and fluid balance during the first two weeks of life in preterm neonates weighing less than 1500 grams. *J Pediatr* **118**: 615-620, 1991.

BERÉNYI E, SZENDRŐ ZS, RÓZSAHEGYI P, BOGNER P, SULYOK E: Postnatal changes in water content and proton magnetic resonance relaxation times in newborn rabbit tissues. *Pediatr Res* **39**: 1091-1098, 1996.

BERÉNYI E, REPA I, BOGNER P, DÓCZI T, SULYOK E: Water content and proton magnetic resonance relaxation times of brain in newborn rabbits. *Pediatr Res* **43**: 421-425, 1998.

CHEEK DB: Extracellular volume: its structure and measurement of the influence of age and disease. *J Pediatr* **58**: 103-125, 1996.

COULTER DM: Prolactin: a hormonal regulator of neonatal tissue water reservoir. *Pediatr Res* **17**: 665-668, 1983.

COULTER DM, AVERY ME: Paradoxical reduction in tissue hydration with weight gain in neonatal rabbit pups. *Pediatr Res* **14**: 1122-1126, 1980.

DAHL LB, DAHL IMS, BORRESEN AL: The molecular weight of sodium hyaluronate in amniotic fluid. *Biochem Med Metab Biol* **35**: 219-226, 1986.

FRIIS-HANSEN B: Body water compartments in children. Changes during growth and related changes in body composition. *Pediatrics* **28**: 169-181, 1961.

GINETZINSKY AG: Role of hyaluronidase in the reabsorption of water in renal tubules: the mechanism of action of antidiuretic hormone. *Nature* **182**: 1218-1219, 1958.

HÄLLGREN R, SAMUELSSON T, LAURENT TC, MODING J: Accumulation of hyaluronan (hyaluronic acid) in the lung in adult respiratory distress syndrome. *Am Rev Respir Dis* **139**: 682-687, 1989.

HEIMLER R, DOUMAS BT, JENDRZEJCZAK BM, NÉMETH PB, HOFFMAN RG, NELIN LD: Relationship between nutrition, weight change, and fluid compartments in preterm infants during the first week of life. *J Pediatr* **122**: 110-114, 1993.

ISRAELACHVILI J, WENNERSTRÖM H: Role of hydration and water structure in biological and colloidal interactions. *Nature* **379**: 219-225, 1996.

JENKINS HG, BACHELARD HS: Developmental and age-related changes in rat brain glycosaminoglycans. *J Neurochem* **51**: 1634-1640, 1988.

LING GN: Cell water. In: *A Revolution in the Physiology of the Living Cell.* LING GN (ed.), Krieger Publishing Co., Malabar, Florida, 1992, pp 69-110.

LORENZO AV, JOLESZ FA, WALLMAN JK, RUENZEL PW: Proton magnetic resonance studies of triethyltin-induced edema during perinatal brain development in rabbits. *J Neurosurg* **70**: 432-440, 1989.

MACLAURIN JC: Changes in body water distribution during the first two weeks of life. *Arch Dis Childh* **41**: 286-291, 1966.

MULKERN RV, BLEIER AR, ADZAMIL IK, SPENCER RGS, SÁNDOR T, JOLESZ FA: Two-site exchange revisited: a new method for extracting exchange parameters in biological systems. *Biophys J* **55**: 221-232, 1989.

NIELSEN S, NAGELHUS EA, AMIRY-MOGHADDAM M, BOURONE C, AGRE P, OTTERSEN OP: Specialised membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. *J Neurosci* **17**: 171-180, 1997.

SEDIN EG, GERDIN B, JOHNSSON H, JONZON A, ERIKSSON L, HÄLLGREN R: Hyaluronan and water content in the lung of preterm and term infants who died less than 24 hours after birth. *Pediatr Res* **36**: 37A, 1994.

SEDIN G, BOGNER P, BERÉNYI E, REPA I, NYÚL Z, SULYOK E: Lung water and proton magnetic resonance relaxation in preterm and term rabbit pups: their relation to tissue hyaluronan. *Pediatr Res* **48**: 554-559, 2000.

SHAFFER SG, BRADT SK, HALL RT: Postnatal changes in total body water and extracellular volume in the preterm infant with respiratory distress syndrome. *J Pediatr* **109**: 509-514, 1986.

SULYOK E: Postnatal adaptation. In: *Pediatric Nephrology.* HOLLIDAY MA, BARRATT TM, AVNER ED (eds), Williams and Wilkins, Baltimore, 1994, pp 267-286.

SULYOK E, NYÚL Z, BOGNER P, BERÉNYI E, REPA I, VAJDA ZS, DÓCZI T, SEDIN G: Brain water and proton magnetic resonance relaxation in preterm and term rabbit pups: their relation to tissue hyaluronan. *Biol Neonate* **79**: 67-72, 2001.

VERKMAN AS, MATTHAY MA, SONG Y: Aquaporin water channels and lung physiology. *Am J Physiol* **278**: L867-L979, 2000.

WEN H, NAGELHUS EA, AMIRY-MOGHADDAM M, AGRE P, OTTERSEN OP, NIELSEN S: Ontogeny of water transport in rat brain: postnatal expression of the aquaporin-4 water channel. *Eur J Neurosci* **11**: 935-945, 1999.

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