Salt in the body: a newly discovered pathway of handling sodium

Bachelor thesis

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1. Summary

For this bachelor thesis, a literature study has been performed. Salt has had huge influences on humankind, but modern intake rates unfortunately have some adverse effects. Sodium as well as chloride are important electrolytes for the body. This thesis focuses on sodium. Sodium enters the body through the diet and is mostly excreted through the urine. There are a lot of hormones responsible for the maintenance of the sodium contents of the body. These act on different signals, where the change of sodium contents sometimes is merely an effect instead of the main goal. For a long time, high intake of salt was associated with the retention of water. However, Titze et al proved that this was not the only way that the body handles sodium levels. They found that the body is capable of storing the sodium in an osmotically inactive way. This is done with proteoglycans, negatively charged molecules in the extracellular matrix. To be able to store the salt, new proteoglycans are synthesized and the sulphation patterns change. The osmotically inactive sodium storage is associated with a higher risk of autoimmune diseases, but it also functions as a microbial barrier and prevents increase in blood pressure. Salt sensitive people are less capable of this kind of sodium storage compared to salt resistant people. The mechanisms of osmotically inactive sodium storage are partially understood, as well as what happens to chloride during this storage. When the mechanisms will be fully understood, this could have great clinical implications in for example the treatment of hypertension or autoimmune diseases.

2. Introduction

This review will discuss the way the body handles salt. The functions both sodium and chloride have in the body will be explained first. Thereafter, the way the body absorbs and excretes sodium will be discussed. Recently a new way of storing sodium in the body is discovered, namely the osmotically inactive storage. This will be reviewed and because proteoglycans play a major role in this process, they will be discussed afterwards. This sodium storage by proteoglycans has major consequences, which will be reviewed next. Finally a conclusion is drawn.

Salt is composed of the positive charged Na^+ and the negative charged Cl^- ions. There are two main sources of salt, namely sea water and rock salt. Salt has been an important substance throughout history. Due to it's never decaying property, it was a symbol for immortality. It's antibacterial properties, ionic strength and ability to induces osmosis were considered to have healing properties[1]. Salt has influenced our society greatly. For example, the French word 'salut', a greating, comes from the word salt and is originally used to wish someone health. In ancient Rome, salt was used as an equivalent of money. This is also where our word 'salary' derives from[1]. Salt hunger is proposed to be the reason some wildlife could be tamed into cattle. This wildlife was drawn towards human settlements for the salt in the human urine[1].

Salt intake differs throughout time and location. Ancient Romans used on average 25 grams of salt a day. In France around 1725, 13-15 grams of salt a day were consumed. In Scandinavian countries, salt intake was high. In the 16th century, 50 grams a day was consumed in Denmark and up to 100 grams a day in Sweden. This salt intake derived mainly from the intake of salted fish and meats[1]. Nowadays, the average adult consumes about 9-12 grams of salt a day[2,3]. This intake is constant over 5 decades and represents 49 countries, using 69,011 participants[3]. The intakes vary between countries. Lowest salt intakes of less than 3 gram were measured in remote populations such as Papua New Guinea

Highlanders, Luo in rural Kenya or Xingu Indians of Brazil[2]. The highest recorded salt intake was in Tianjin, China of 14.4 grams. The overall salt consumption is much more than the advised maximum of 5 grams a day, by the World Health Organisation (WHO)[4], and the physiological need of 1.2 grams a day[5]. The major part of the salt intake derives from processed foods[6]. Only 15 % of the salt intake comes from the use of table salt or during cooking and 10 % from natural occurring salts in unprocessed food.

High intake of salt is related to several diseases. The best known condition correlating with high salt intake is a raise in blood pressure[7]. Dietary salt increases arterial constriction and peripheral vascular resistance. Hereby the blood pressure is raised. Salt sensitivity and salt resistance are conditions present in humans. For salt-sensitive individuals, blood pressure rises with a high salt intake whereas salt-resistance individuals show no increase in blood pressure due to increased salt intake[8]. Salt-sensitive individuals show, amongst others, a decreased glomerular filtration rate and a reduced natriuresis[8]Other pathological conditions are associated with high levels of calcium in the urine (hypercalciura) and low levels of citrate in the urine (hypocitraturia)[9]. Citrate in the urine helps dissolve calcium to prevent stone formation[10]. High salt intake thus increases the risk of nephrolithiasis (calcium stone formation)[9]. High salt intake is also associated with an increased risk of gastric cancer by increasing the risk of a *Helicobacter pylori* infection. Salt can also act synergistically (synergy means two components together have a greater influence then the two apart, also known as 1 + 1 = 3) to further increase the risk of developing gastric cancer[11].

It may be clear a high salt intake is associated with several diseases. Therefore, the way sodium acts in the human body is very important. The main questions in this thesis are: How does the body handle salt, how is sodium stored and what are the consequences of this storage?

3. Salt handling

Sodium as well as chloride exert different functions in the human body. These will be shortly highlighted. The absorption as well as the secretion will be explained. The sodium concentrations remain under hormonal control, which will be shortly explained. The classical view of the way the sodium status of the body is seen will be explained, as well as some indications this view may be wrong.

Osmosis

Diffusion and osmosis are important processes to the body. They are both based on the need for communicating solutions to be equal. Diffusion describes the movement of particles from a relatively high concentration to a relatively low concentration. Osmosis occurs when two compartments are divided by a membrane permeable to water, but not to the solutes. Osmosis therefore describes the movement of water from a compartment with a relatively low concentration of osmotically active particles to a compartment with a relatively high concentration of osmotically active particles. The amount of osmotically active particles is described as osmoles per liter, osmol/L or OsM. Tonicity is a term to describe how a solution would affect the cell if a cell was placed in this solution. An isotonic solution has the same osmolarity as the cell. The human body has a osmolarity of 280-296 mOsM. Under physiological conditions, osmolarity inside and outside the cell is about equal. However, the Gibbs-Donnan effect causes an uneven distribution of small ions with a small excess in the plasma

compared to the interstitial fluid and intracellular fluid of 0.5 mOsM[12]. The Gibbs Donnan effect is caused by negatively charged proteins in the vascular compartment, creating an electrical charge[13].

Sodium and chloride are both nonpenetrating solutes, meaning they cannot pass the cell membrane passively without specific ion channels. Therefore they can induce osmosis. Sodium is mostly found in the extracellular fluid (ECF) (range 135-145 mM), and less in the intracellular fluid (ICF) (15 mM). Chloride is also mostly found in the ECF (range 100-108 mM versus 5-15 mM for ICF). This means if the osmolarity in the cells is higher than in the ECF, the cells would shrink, and if it is less, the cells would swell up.

Na^+ and Cl^- in the body

Sodium and chloride are important ions to the human body. The most important function of sodium is regulating the blood pressure, which will be explained in chapter 6. They exert different functions, of which a few examples will be given below:

Example 1: action potential

Sodium and chloride together with calcium (Ca^{2+}), which is mostly present in the extracellular fluid, and potassium (K^+), which is mostly present in the intracellular fluid, a membrane potential is created. For neurons, this potential is -70 mV. This means the inside of the cell is more negative compared to the outside. This potential is needed to conduct an action potential in a neuron to pass on a signal (Fig. 1).[14]

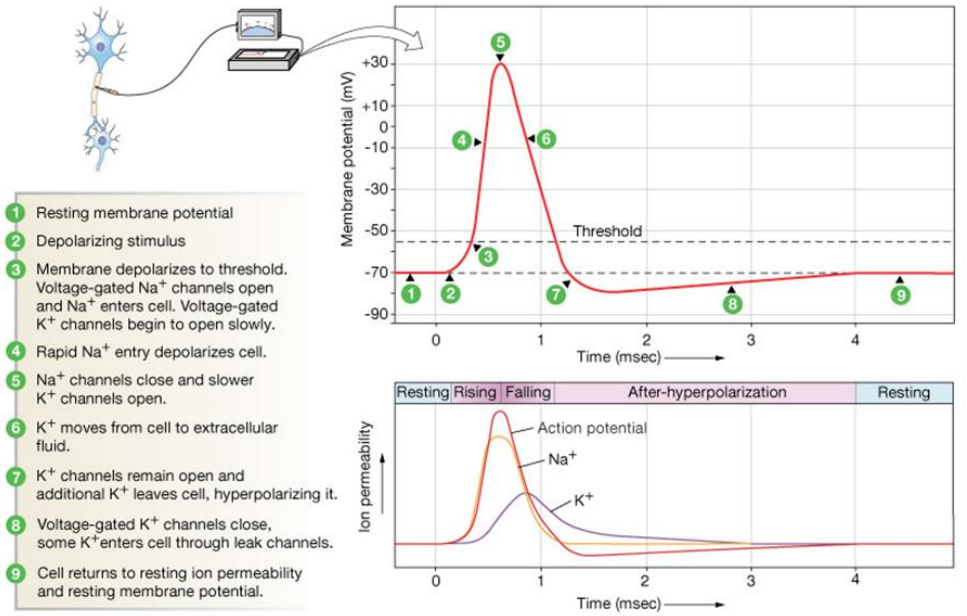

Figure 1: Action potential in a neuron[14]. This figure describes the movement of ions and the membrane potential throughout an action potential.

At first, a neuron has a resting membrane potential of -70 mV. A stimulus depolarizes the membrane until the threshold of -55 mV. After this threshold the voltage-gated sodium channels open and sodium can enter the cell. This causes a rapid entry of sodium, due to the concentration difference between the ECF and ICF and the negative membrane potential inside the cell. At the same time, voltage-gated potassium channels start to open, but these open slower compared to the sodium channels. The rapid entry of sodium causes the cell to

depolarize and even reverse polarity to a potential of +30 mV. This is called an overshoot. At the top of the peak, the sodium channels close and the potassium channels are opened. This causes potassium to move out of the cell, due to the high concentration in the ICF compared to the ECF and the positive potential at this point. The potential eventually reaches the -70 mV state again, but at that point the potassium permeability has not returned to its resting state. Potassium continues to leak out of the cell to generate a hyperpolarisation of -90 mV, the undershoot. After the undershoot, the voltage-gated potassium channels close and some potassium enters the cell through leak channels. Hereafter, the cell returns to its resting membrane potential and ion permeability. The ions are restored to their original compartments through the Na^+-K^+-ATPase. This is a pump that uses ATP to exchange intracellular sodium for extracellular potassium.[14]

Example 2: gastric acid

The stomach has three different functions: storage, digestion, and protection[14]. The stomach protects the body from pathogens swallowed by secreting gastric acid (HCl). The parietal cells lie deep in the gastric glands and secrete gastric acid. Gastric acid denaturates proteins by breaking the hydrogen bonds. It also kills bacteria and other microorganisms. The pH in the lumen of the stomach can be as low as 1. The H^+-K^+-ATPase secretes H^+ in exchanges for potassium. Cl^- passively follows[14].

Example 3: chloride shift

CO_2 from oxidation in the body diffuses out of the tissue into the blood and then into the red blood cell[14]. Carbonic anhydrase convertes it into HCO_3^- by the following reaction: $CO_2 + H_2O <-> H^+ + HCO_3^-$. HCO_3^- is removed from the cell into the blood by the chloride shift, an antiport protein exchanges HCO_3^- for Cl^-. In the pulmonary capillaries the process is reversed. Cl^- is exchanged for HCO_3^-. The reaction is performed to create CO_2 again, which will diffuse out of the red blood cell into the alveoli[14]. These mechanisms can also compensate in case of metabolic alkalosis or acidosis.

The dietary advisements for chloride are equal to that of sodium, because both derive mostly from dietary salt ingestion[15]. There are indications that chloride, along with sodium, plays a role in the pathologies caused by salt, and may even be more important than sodium in the onset of hypertension[16-21]. Even though there are indications for its importance, the effects of chloride are far less studied. Therefore, this thesis will only consider the effects of sodium.

Absorption, secretion and excretion of sodium

The total amount of sodium in the body of a 70 kilogram man is around 4000 mmol[13]. 3000 mmol is exchangeable: 2000 mmol is found in the ECF, 700 mmol in the bone and 300 mmol in the ICF. The remaining 1000 mmol is probably bound in crystal structures in the bone and therefore not exchangeable[13]. There are also indications sodium could be stored in the connective tissue of the skin, tendon and cartilage[22]. Sodium is largely excreted trough the urine, for about 90-95%. The rest is lost through the faeces. Sodium can also be lost through sweating, the amount which is lost through sweat depends on the amount of sweat produced[13].

Absorption

Sodium enters the human body through the diet. Under normal conditions, the human body is capable of absorbing up to 1 mol (58 g NaCl) of sodium. Less than 20% of this sodium derives from the diet, the rest is sodium secreted into the lumen[13]. In the duodenum, the acid from the stomach, as discussed in example 2, has to be neutralized. The acid is neutralized by the base bicarbonate. Bicarbonate is secreted in the form of $NaHCO_3$ [14]. There is also an isotonic NaCl solution secreted in the small intestine and colon that mixes with the mucus to help lubricate the lumen[14]. The absorption of sodium occurs for about 90% in the small intestine, the rest is absorbed in the large intestine[13]. The absorption in the small intestine from the lumen to the cell is in exchange for H^+ or cotransported with organic molecules (glucose or amino-acids)[13]. Then the sodium is actively transported from the cell to the interstial fluid. In the large intestine, sodium absorption occurs mainly through selective channels. These channels are up regulated by aldosterone[13].

Secretion and excretion

The major part of sodium is thus excreted into the urine. This is regulated by the kidney. The kidney consists of nephrons, which are the functional units (smallest unit to be able to exert its function) of the kidney (Fig. 2)[14].

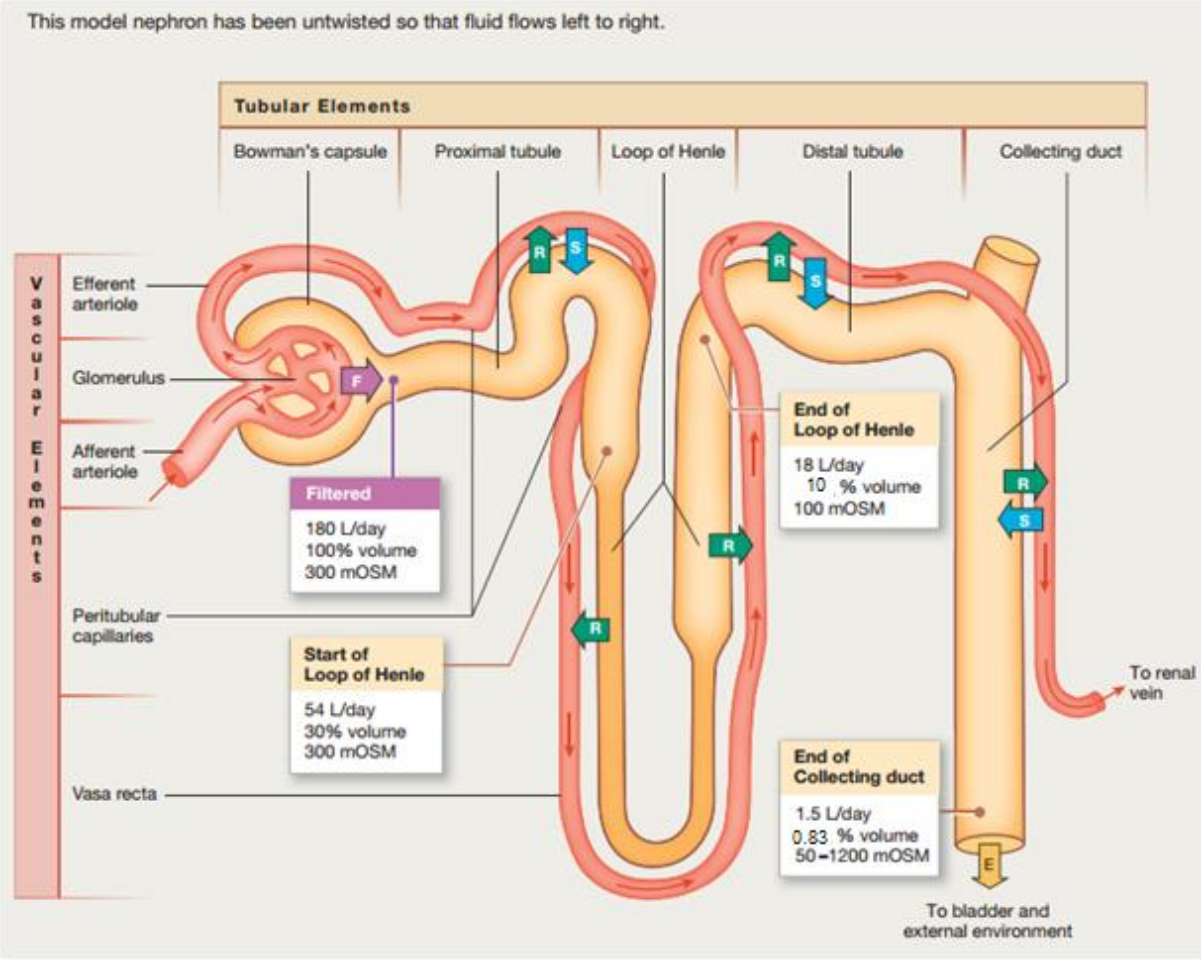

Figure 2: schematic display of a nephron[14 edited]. F = Filtration: movement from blood to lumen, R = Reabsorption: from lumen to blood, S = Secretion, from blood to lumen, E = Excretion: from lumen to outside of the body.

The tubuli of all nephrons modify the fluid volume and osmolarity. As seen in Fig.2, the osmolarity of the urine can greatly differ from 50-1200 mOsM. Everyday about 180 litres of fluid is filtered. The majority of this fluid is reabsorbed again. The rate of filtration in the glomerulus depends on pre- and post-glomerular pressures. The pressure which forces fluid to leave the circulation is the blood pressure (hydrostatic pressure, P_H). Opposing pressures are the colloid osmotic pressure gradient due to proteins in plasma but not in Bowman's capsule (π) and the fluid pressure created by fluid in the Bowman's capsule (P_{fluid}). This gives the formula: net filtration pressure = $P_H - \pi - P_{fluid}$ = 55 – 30 – 15 = 10 mmHg. The blood pressure of an individual varies throughout the day. For a healthy individual, in general, if the blood pressure varies between 80 and 180 mm Hg, the glomerular filtration rate (GFR) stays at 180 litre a day. Afferent vasoconstriction decreases the GFR where as efferent vasoconstriction increases the GFR. Within the kidney, in each nephron, the distal tubule turns back in between the afferent and efferent arteriole of its own glomerulus. Here is the macula densa located, which contains granular cells. If the NaCl delivery passing the macula densa increases as a result of increased GFR, the cells secrete a paracrine message to the afferent arteriole, inducing its constriction[14].

The reabsorption in the nephron can occur because of osmosis[14]. As seen in Fig. 2, the osmolarity of the fluid in the lumen changes over the course of the nephron. The fluid in the Bowman's capsule and the proximal tubule is isosmotic (Fig. 3). This means the osmolarity is equal to the osmolarity of the surroundings (actual 280-296 mOsM, 300 mOsM for easier reference). The loop of Henle and the collecting duct are located in the medulla instead of the cortex. The cortex is isosmotic to the plasma, but the medulla is more concentrated towards the centre of the kidney. In the descending limb, fluid leaves the proximal tubule due to osmosis. The ascending limb is not permeable to water, so no osmosis occurs. Solute is removed from the fluid to create hyposmotic (osmolarity of about 100 mOsM) fluid. In the distal tubule and the collecting duct, the permeability of water is under hormonal control. Some additional solutes can be obtained from the collecting duct, further reducing the osmolarity to 50 mOsM. At maximum water permeability, a lot of water is reabsorbed and the osmolarity can rise up to 1200 mOsM, equal to its surroundings[14].

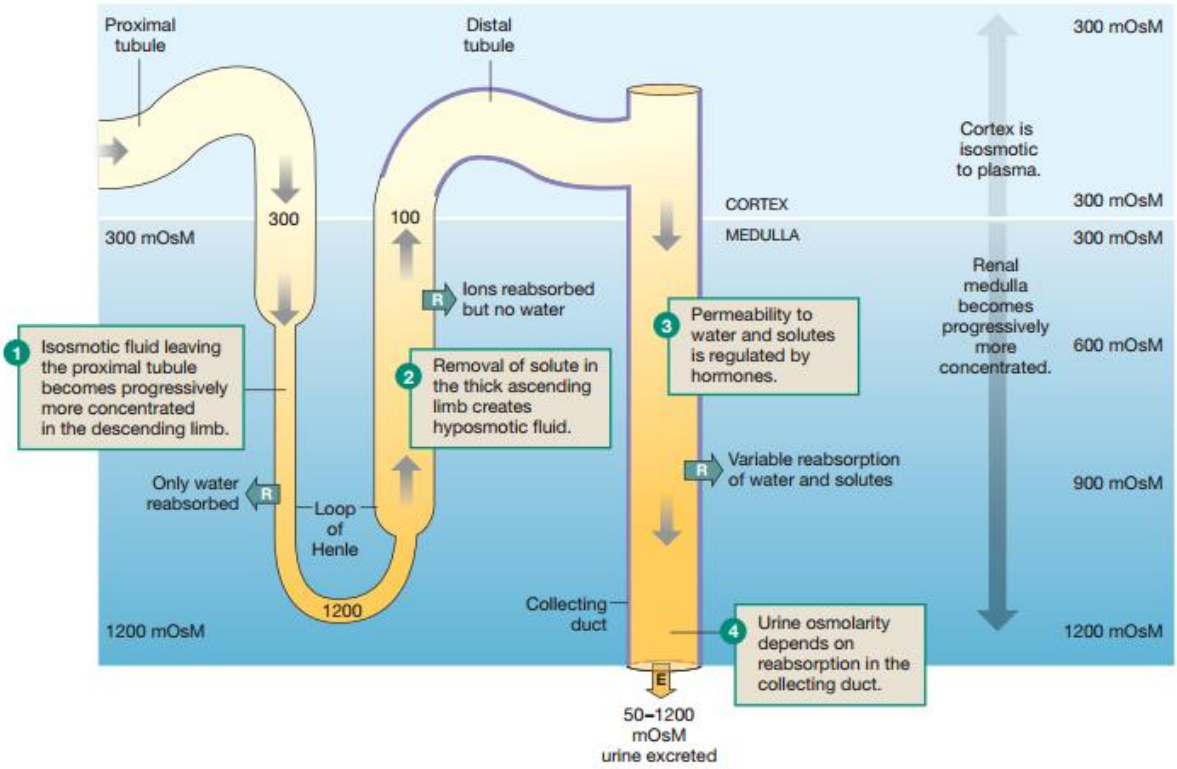

Figure 3: osmolarity changes through the nephron[14].

Hormonal control of sodium and water

Vasopressin

The hormone vasopressin (also called arginine vasopressin of antidiuretic hormone) controls the permeability of the distal tubule and the collecting duct[14]. Vasopressin induces the translocation of aquaporines (water pores) from the cytosol towards the plasma membrane, where they are inserted. Vasopressin is released from the posterior pituitary. There are 3 important triggers for vasopressin release, namely:

1. Decreased blood pressure, measured by the carotid and aortic baroreceptors
2. Decreased arterial stretch due to low blood volume, measured by the arterial stretch receptor
3. Plasma osmolarity greater than 280 mOsM, measured by the hypothalamic osmoreceptors. This is the most important stimulus.

Aldosterone and RAAS pathway

The absorption of sodium in the distal tubule and the collecting duct is regulated by aldosterone[14]. More aldosterone equals more absorption of sodium. One of the targets of aldosterone is the Na^+-K^+-ATPase. This means more aldosterone equals more potassium secretion. Aldosterone is secreted by the adrenal cortex. A low blood pressure activates the renin-angiotensin system (RAS) pathway which induces aldosterone secretion. A high potassium concentration also induces aldosterone secretion and a very high osmolarity inhibits aldosterone secretion.

The RAAS pathway is a quite complex system, see Fig. 4. It is stimulated by a low blood pressure[14]. It starts when cells in the afferent arterioles of a nephron secrete renin (an enzyme). In converts the

inactive plasma protein angiotensinogen into angiotensin I (ANG I). ANG I in the blood is converted to angiotensin II (ANG II) by the angiotensin-converting enzyme (ACE). ANG II has many effects:

1. Increases vasopressin secretion
2. Stimulated thirst
3. Vasoconstriction
4. Increases sympathetic output to the heart and blood vessels
5. Increases proximal tubule sodium reabsorption.

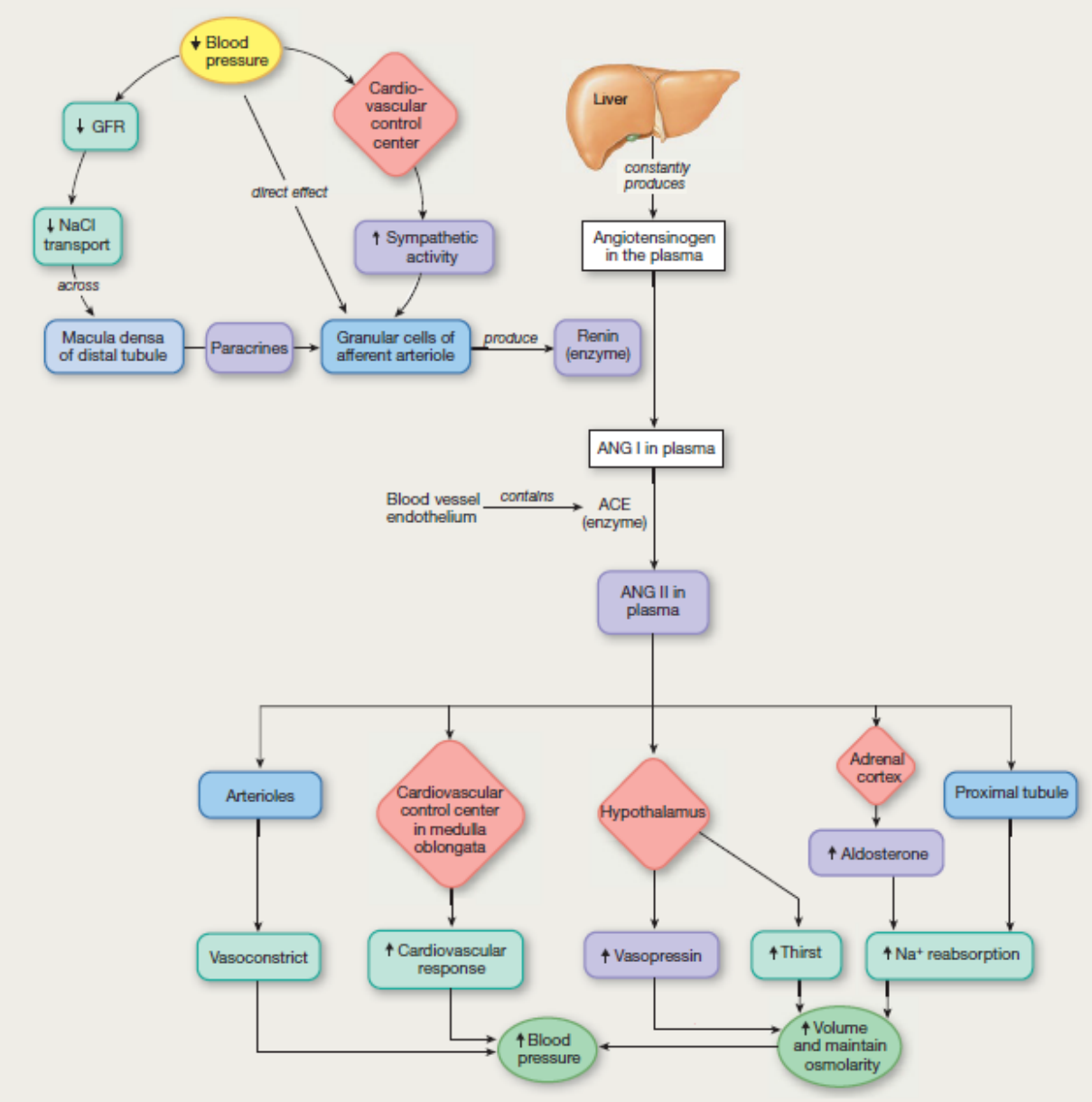

Figure 4: regulation of the RAAS pathway[14] **edited**

Arterial natriuretic peptide (ANP)

The last important hormone for sodium regulation is the arterial natriuretic peptide (ANP, also known as atriopeptin), produced by myocardial cells in response to arterial stretch[14]. Increased blood volume induces the production of ANP. ANP also has different functions, all to increase sodium and water secretion:

1. Directly influences the sodium and water secretion
2. Suppressing the release of renin

3. Suppressing the release of aldosterone
4. Suppressing the release of vasopressin

Altogether, the body has a complex Na^+ and water regulatory system, which is meant to keep the milieu interior as constant as possible.

Classical view

The way clinicians think about sodium excretion is mostly based on the classical view[23]. This view is displayed in Fig. 5. It is assumed that if someone would consume a high level of salt for a certain amount of time, the excretion of sodium would slowly increase. This increase of excretion, shown as a stair in Fig. 5, does not match the intake for a few days. In this example, the excretion matches the intake after 5 days. The excretion equals the intake for the time the high intake continues, suggesting some sodium must remain in the body. When the intake decreases to the original level, the excretion slowly decreases to match intake again. At this point, the remaining sodium is excreted. During the higher sodium intake, body weight is increased, due to water retention. According to the two compartment model, sodium is largely confined to the extracellular space, counteracted by potassium in the intercellular space[24]. These balance the amounts of water in each compartment other by osmosis. This can be displayed in a formula: $\left(\frac{(Na^+ + K^+)_{extra}}{ECF} \approx \frac{(Na^+ + K^+)_{intra}}{ICF}\right)$[24]. This indicates that an increase in extracellular sodium amounts will increase the amount of the ECF. A total body sodium content increase of 140 mmol sodium would lead to 1 litre water retention [25].

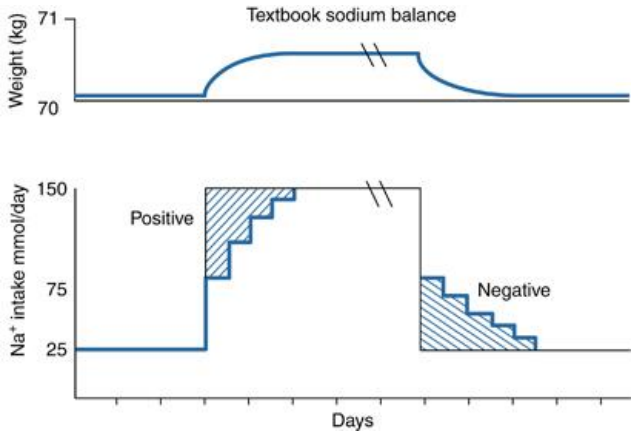

Figure 5: sodium intake, weight and excretion[23]. The upper graph shows the weight change if a person abruptly changes its diet from a sodium intake of 25 mmol/day (1.4 gram salt a day) to 150 mmol/day (8.8 gram salt a day). The lower graph shows the intake and the accompanying excretion (thick blue line and shaded area's)

Indications against the classical view

Heer et al[26] challenged this classical view. They controlled the salt intake in the subjects by assigning them different salt intake levels. They found a dose-effect relation between salt intake and plasma volume. The increase in salt intake did not influence the extracellular fluid compartment as would be expected by the formula.

To test if the classical view indeed is wrong, Titze et al performed a terrestrial space study[27]. For 135 days, three subjects had a free choice of food. On average they consumed 267 mmol sodium (21.7 gram salt) a day. Urine samples were taken and total body sodium contents were calculated. During the study, subjects had accumulated 2,973, 6,180, and 7,324 mmol of sodium. In all of the subjects body weight increased. For two subjects this was solely fat and for the third it was largely fat and little water. So for all the three subjects, the total body sodium contents were increased whereas virtually no water was retained, contrary to the classical view. Titze et al deemed it possible the subjects stored the sodium in an osmotic inactive way [27].

In an actual space study, Rakova et al[28] controlled the salt intake of subjects on a space flight for 105 or 205 days. They also found no relationship between extracellular fluid and sodium intake. They state that if they only did this experiment for one week, the classical view would be applicable here. There are other studies which suggest that the retention of sodium is not in balance with water retention, suggesting the formula of the classical view is incorrect[29,30].

4. Osmotically inactive sodium storage

Discovery of osmotically inactive sodium storage

Titze et al wanted to further examine the possibility of osmotically inactive sodium storage. They fed male salt-sensitive (SS) Dahl rats, salt-resistance (SR) Dahl rats and normal Sprague-Dawley (SD) rats a high (8% NaCl) or salt-free diet (<0.1% NaCl)[31]. Salt-sensitive Dahl rats develop hypertension when fed a high salt diet whereas salt-resistance Dahl rats do not develop hypertension under the same conditions[32]. After sacrificing the animals, total body water (TBW) content and total body sodium (TBS) content was determined. From the TBW, the portion sodium which would have been active could be calculated (TBS_a) and the osmotically inactive sodium content (TBS_i) could be calculated with the formula: $TBS_i = TBS-TBS_a$. The TBS was significantly increased in the SS strain, compared to the SR or SD strains. They found osmotically inactive sodium in all the strains. The amount of osmotically inactive sodium differs between them, see Fig 6. The portion sodium of TBS in the bone increased in SS Dahl rats, but not in the SR or SD rats. Although sodium is largely stored in the bone, the sodium contents in the bone are not responsible for the differences between the different strains.

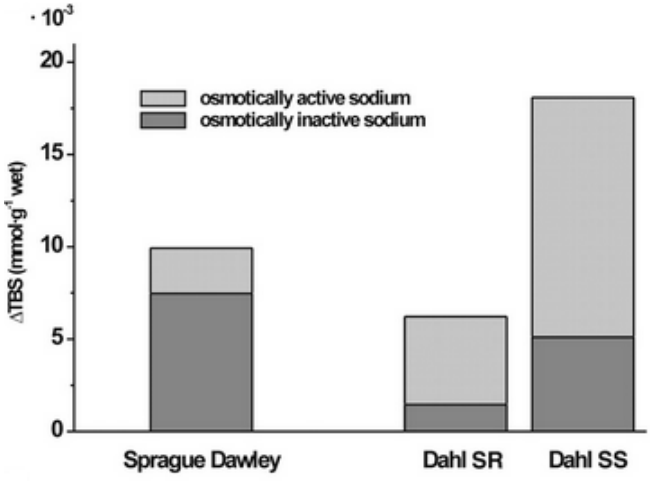

Figure 6: TBS distribution in different strains[31]. Results are from rats fed with a high salt diet.

If the bone is not responsible for the osmotically inactive sodium storage, another compartment must be. The attention from Titze et al got focussed on the skin. Therefore, he performed a study where he fed male, female and ovariectomized (OVX) female rats a high or low salt diet[33]. After menopause, women are

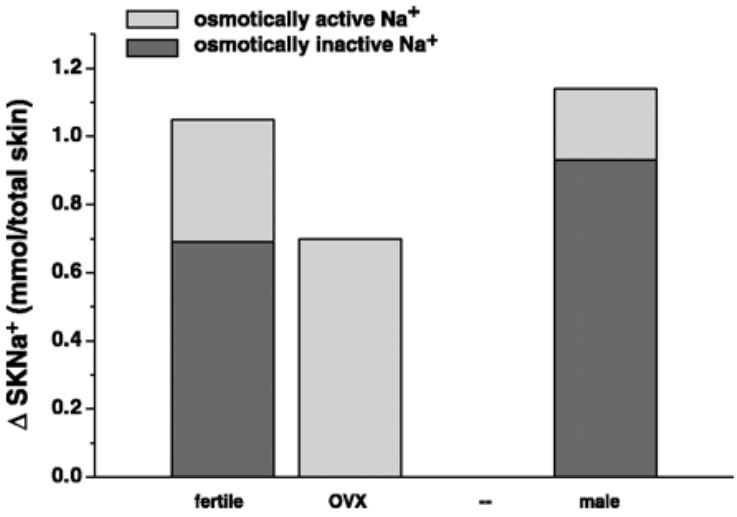

Figure 7: osmotically active and inactive sodium storage in the skin[33]. Fertile: fertile female rats, OVX: ovariectomized female rats, male: male rats.

more sensitive to hypertension caused by dietary salt than before menopause[34]. Therfore they also used ovariectomized female rats. After eight weeks they sacrificed the rats and determined the osmotically inactive sodium contents of the skin. He found that sodium is osmotically inactively stored in the skin, but only in male and fertile female rats. The OVX female rats displayed no osmotically inactive sodium storage, see Fig 7. The fertile rats had a shift towards skin sodium.

In hindsight, reviewing the results of their previous experiment[27], both the SS and SR Dahl strains were not capable of inducing osmotically inactive sodium storage in the skin. The increased amount of sodium in the body in SS rats must be distributed in the osmotically active compartments.

At this point, it is unknown which structure is responsible for the osmotically inactive sodium storage. Proteoglycans (chapter 4) (anchoring molecules present in the extracellular matrix, amongst others in the skin and cartilage) are negatively charged and therefore attract cations and repel anions[35]. This results in a concentration of 250-350 mmol/L sodium in cartilage. Titze et al therefore proposed proteoglycans as responsible for the increased osmotically inactive sodium storage in the skin[36]. They fed female Sprague-Dawley rats a high or a low salt diet for eight weeks. They found no osmotically inactive skin sodium in the rats fed a low salt diet and they did found osmotically inactive skin sodium in the rats fed a high salt diet. They then performed a Western Blot (technique to determine different protein contents of a sample) on skin and cartilage samples from rats with low, medium and high skin sodium contents and a control. The results revealed that a higher skin sodium content correlated with higher proteoglycan contents in skin and cartilage[36].

At this point, not everyone is willing to accept this theory. Seeliger et al performed a study to test this hypothesis[37]. They used female beagle dogs and fed them diets with different salt amounts. They found changes in the total body weight of the dogs, corresponding with changes in salt intake. This supports the idea that the body adjust the total body weight according to the sodium and potassium concentration. High sodium excretion is correlated with low potassium excretion and vice versa[25]. Seeliger suggests that it is possible that Titze did not consider the total body potassium and therefore misinterpreted his results[37]. In his next study, Seeliger specifically searched for osmotically inactive sodium storage[38]. Again, he found a strong correlation of total body sodium and total body weight. Therefore he again concluded that sodium is not stored in an osmotically inactive form, but in an active form.

Characteristics of osmotically inactive sodium storage

In the mean time, Titze continued his investigation of the osmotically inactive sodium storage. If sodium were to be stored osmotically inactive, regulatory mechanisms would be available[39]. High levels of cortisol induce the apparent mineralocorticoid excess (AME) syndrome[40]. This induces sodium retention, hypokalemia and hypertension. This phenomenon is used to induce hypertension in laboratory animals. This is done by implanting DOCA (deoxycorticosterone acetate, a precursor of mineralocorticoids, these induce more aldosterone release) pellets with or without increasing the salt intake[41]. DOCA pellets only induce transient sodium retention, a phenomenon also called 'mineralocorticoid escape' [42]. Laboratory animals quickly achieve a sodium balance without further sodium retention. This escape is external. Titze et al suggested an internal escape in the form of osmotically inactive sodium storage might exist[39]. They gave female Sprague-Dawley rats DOCA or no DOCA pellets, tap or saline water. In the DOCA-salt rats they found osmotically inactively stored

sodium in the skin and in the muscle as well. This implies an 'internal escape' for sodium. They also found that the increase in total body sodium was associated with potassium loss, described as an osmotically neutral Na^+/K^+ exchange. As a result, the $Na^+ + K^+$-to-water ratio remained unchanged in bone and slightly changed in muscle and skin, meaning more sodium is gained compared to the potassium lost[39].

Titze et al did not consider the non-sodium and non-potassium solutes which are osmotically active. If these would decrease during the presumed osmotically inactive sodium storage, it is possible there is no such storage[43]. Their group calculated the amount of osmotically active sodium and potassium on the concentration of these solutes in the serum[33,36,39]. Therefore, due to osmosis, they assume these concentrations are equal to the concentrations in ICF and interstitial fluid (ISF). Continuing on this idea, they would also assume:

$$\frac{Total\ body\ sodium\ +\ potassium}{TBW} = [Na^+ + K^+]_{serum}$$

but it is already known that the serum concentration of sodium + potassium is greater than the concentration in the ICF and ISF[43], due to the Gibbs Donnan effect.

High salt intake is associated with an increase in the proteoglycan contents of skin and cartilage[36]. Long-term salt deprivation is associated with a decreased negative charge density of the GAGs in the skin[44]. The total body sodium content can change without concomitant changes in the serum sodium concentration. Growth leads to the mobilization of osmotically inactively stored sodium in the bone and the skinned and deboned carcasses, but not in the skin and is not balanced out by potassium[44]. Dietary salt intake influences the osmotically inactive sodium content of the skin. A high salt intake is associated with less unsulfated GAGs, compared to a low salt intake[44].

Osmotically inactive sodium in the muscle and skin is found in men as well. The sodium amount in the skin and muscle of the calf increases with age[45,46] . The amount of water does not increase between the same subjects, meaning sodium is osmotically inactively stored (Fig. 8)[45]. This age dependent increase occurs inside or directly under the keratinocyte layer of the skin[46,47]. Active sodium transport is likely involved in the storage of sodium in the skin[47]. In men, increasing skin sodium contents is also associated with increasing glycosaminoglycans content in the skin. Dietary salt loading is associated with increased chondroitin synthase mRNA content[45].

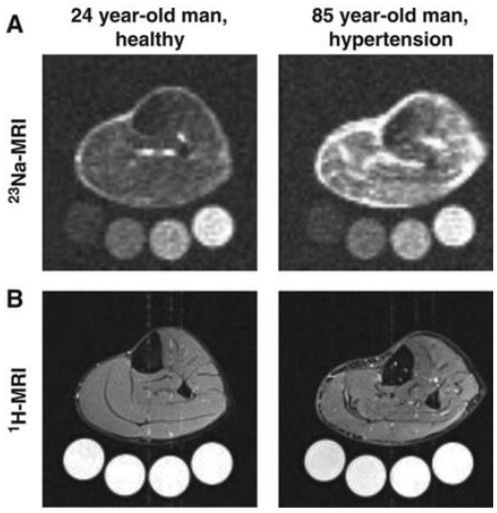

Figure 8: ^{23}Na magnetic resonance imaging (^{23}Na-MRI) of tissue Na^+ [45]. A: ^{23}Na-MRI of a 24 year-old healthy male and a 85 year-old man with hypertension. Lower circles are tubes with solutions of 10, 20, 30, and 40 mmol/L of NaCl, allowing tissue sodium to be calibrated. B: tissue water in the same subjects.

5. Proteoglycans

Proteoglycans seem to be the molecules mainly responsible for osmotically inactive sodium storage. To get a better understanding of these molecules, they will be discussed in this chapter.

Function of proteoglycans

Animal cells lack a cell wall, which is present in the cell of plants[49]. To compensate for this lack, animal cells have an elaborate extracellular matrix (ECM). For years it was thought that the ECMs only function was to keep the cells together. Evidence now shows that the ECM plays a vital role in may physiological processes such as growth, development and cell death[14]. The ECM consists of glycoproteins (proteins with covalently bound polysaccharide) and other carbohydrate-containing molecules, which are secreted by cells. The most abundant molecule is collagen. This forms strong fibers outside the cells. The collagen fibers are embedded in a network of proteoglycans. A proteoglycan is a glycoprotein molecule and consists of a backbone (core protein, Fig. 9) with many carbohydrate chains covalently attached[49]. These carbohydrate chains are called glycosaminoglycans (GAGs)[50]. One of the two sugars is always an amino sugar[50]. In most cases, GAGs are sulphated. They are also strongly hydrophilic, so the proteoglycan proteins form a gel-like substance. These proteins in connective tissue are produced by fibroblasts. In cartilage, the cells are called chondrocytes and osteoblasts form bone tissue[50]. There are four main groups of GAGs: (1) hyaluronan,(2) chondroitin sulphate and dermatan sulphate, (3) heparan sulphate and heparin, and (4) keratan sulphate. These differ in their sugars and type of linkage between the sugars[50]. The polysaccharides are stiff, this means they make up a lot of volume relative to their mass. All GAGs (except hyaluronan) are conjugated to a core-protein in the Golgi apparatus of the cell and released by exocytosis[50] or embedded in the plasma membrane.

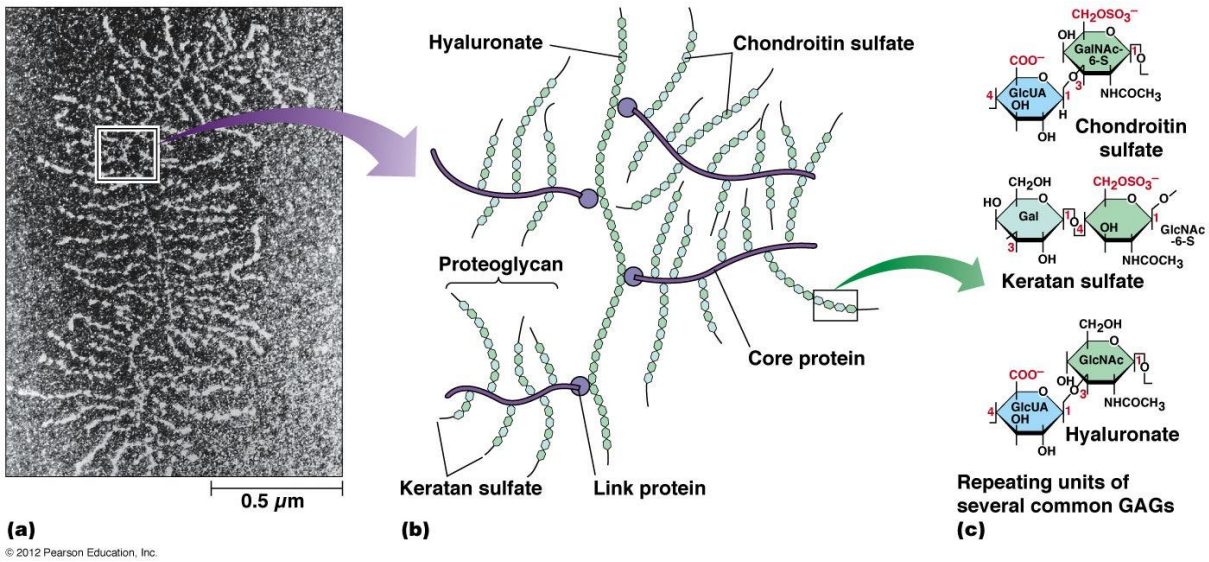

Figure 9: structure of proteoglycans[51]. A: microscopical image of a proteoglycan. B: structure of a proteoglycan with different GAGs shown. C: example of the repeating units of several common GAGs.

Different types of proteoglycans can assembly to form even bigger aggregates[50]. For instance, molecules of aggrecan can bind with hyaluronan to form aggregates as big as bacteria. Because proteoglycans are so divers, they can exert different functions. They can form gels and regulate the traffic of molecules and cells according to their size and charge. They also influence the chemical

signaling between cells. They bind secreted molecules, such as growth factors and in this way control the diffusion through the matrix, the range of action and the lifetime of the molecules. They can also enhance of inhibit the signal. During an inflammatory response, heparan sulphate proteoglycans immobilize secreted chemokines on the endothelial surface of a blood vessel. This way the chemokines stay here, causing the white blood cells to leave the bloodstream and migrate into the tissue. Heparan sulphate proteoglycans can also bind fibroblast growth factor (FGF), which stimulates different cell types to proliferate. Signal molecules such as TGF-β can be bound by proteoglycans. Vascular endothelial growth factor (VEGF) can be bound by fibronectin[50].

Instead of being part of the ECM, proteoglycans can also be integral components of plasma membranes[50]. They have their core protein attached to or through the membrane. Some proteoglycans act as a coreceptor. The syndecans are the best-characterized plasma membrane proteoglycans. Among others, syndecans are located on the surface of epithilial cells and fibroblasts. In fibroblast they modulate integrin function by interacting with fibronectin on the cell surface. They also bind FGFs and present them to a receptor on the same cell. Betaglycan can do the same for TGFβ[50].

The best characterized proteoglycans are those present in the cartilage[52], which consists mainly of aggrecan. Aggregan has three globular domains, between domains 2 and 3 are chondroitin sulphate and keratan sulphate bound. The first domain is noncovalently bound to hyaluronan[52]. See Fig. 10

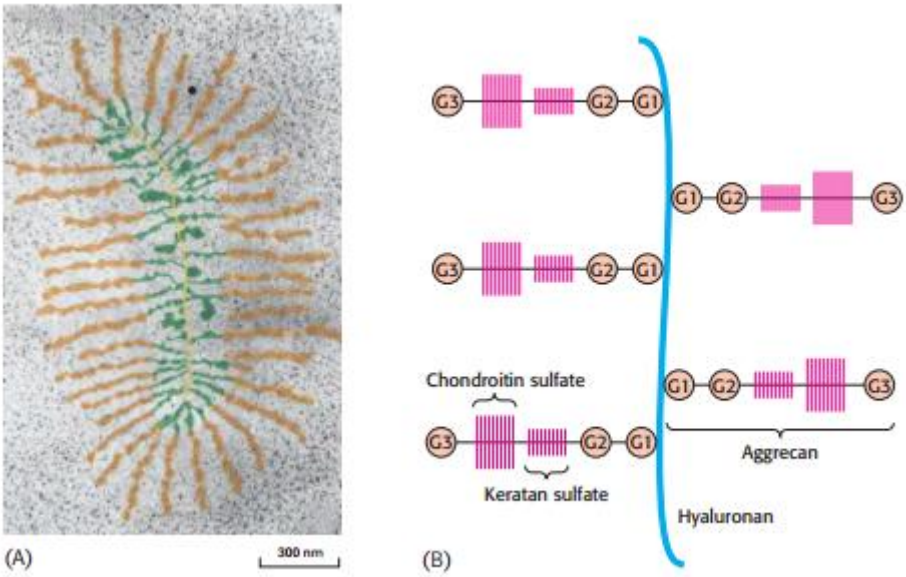

Figure 10: structure of proteoglycan from cartilage[52]. A: electron micrograph of a proteoglycan from cartilage (colour added). B: schematic representation

Biochemical properties of proteoglycans

The sugar molecules in the proteoglycans are attached either to the oxygen atom in the side chain of serine or threonine (O-linkage) or to the amide nitrogen atom in the side chain of asparagines (N-linkage)(Fig 11)[52]. Which sides are glycosylated depend on the other aspects, such as protein

structure and on the cell type in which the protein is expressed.

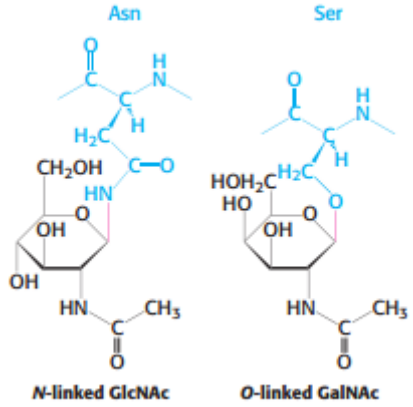

Figure 11: N-linkage and O-linkage[52]

Proteoglycans are highly negatively charged, Fig. 12. The negative charge is located on the COO^- and/or OSO_3^- (sulphated) groups. The amount of negative charges depends on the type of GAG. For instance, heparin is strongly negative compared to hyaluronate or keratan sulphate. These negative charges attract cations, such as Na^+ [50]. Due to the negative charge, large amounts of water are attracted. This creates turgor (swelling pressure) and enables the matrix to withstands great forces, for example in cartilage matrix.

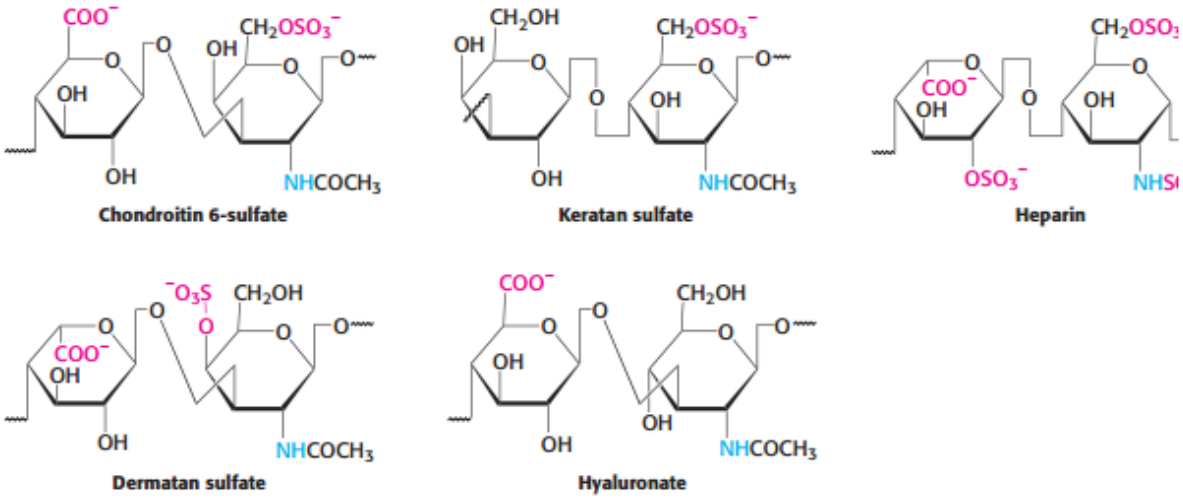

Figure 12: repeating units in glycosaminoglycans[52]. Structural formulas for the five most common GAGs. The negative charges are displayed in red, aminogroups are shown in blue. For clarity, hydrogen atoms have been omitted.

Proteoglycans and osmotically inactive sodium storage

During high dietary salt intake, sodium is osmotically inactively stored. This storages is coupled with increased proteoglycan and GAG content in the skin, higher sulphation rate of the GAGs and increased chondroitin synthase[44,45]. Together, these factors create a higher negative charged density in the skin. This negative charge can attract and trap cations. One condition for molecules to induce osmosis is the ability to freely move in a solution[14]. If sodium ions are trapped by proteoglycans, they cannot move freely anymore and therefore cannot induce osmosis, meaning they are osmotically inactive.

6. Consequences of osmotically inactive sodium storage

The osmotically inactive sodium storage has effects on the human body.

Immuno modulation

Osmotically inactive sodium storage modulates different components of the immune system.

High sodium concentrations in the skin induce serum glucocorticoid kinase 1 (SGK1) expression[53]. SGK1 is a downstream target of TonEBP[54] (which regulates osmoprotective genes in response to osmotic stress[55]) and NFAT5[56] (which regulates osmoprotective genes as well). SGK1 induces the proliferation of pro-inflammatory Th17 cells. These Th17 cells are associated with several autoimmune diseases[57]. Mice fed a high salt diet develop a more severe form of experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis (MS)[56]. Through this pathway, high dietary salt intake could be an environmental risk factor for the development of autoimmune diseases[54].

High dietary salt intake leads to a two-fold increase in lymph capillary number in the skin[58] (Fig. 13). The diameter of the lymph capillary was increased as well. This increase in lymph capillary density was associated with an increase in mononuclear phagocyte system (MPS) cell infiltration (MPS cells include: monocytes and macrophages[14]). Macrophages migrate towards NaCl in a dose-dependent fashion, suggesting NaCl-dependent chemotaxis[59]. 50% of these MPS cells expresses dendritic cell markers and 90% is VEGF-C positive[58]. VEGF-C (vascular endothelial growth factor C) induces lymphatic endothelial proliferation and vessel enlargement, but not that of vascular[60]. Treatment with clodronate (induces macrophage apoptosis) resulted in no VEGF-C positive MPS cell infiltration and lymph capillary hyperplasia[58]. The same relationship was present for expression of the transcription factor TonEBP and eNOS[58], a vascular relaxation agent[14]. MPS cells decrease salt induced hypertension and water retention[58]. High levels of sodium accumulation in the skin is found in bacterial skin infections[61]. Osmotic stress in the skin, due to sodium accumulation boosts the antimicrobial defence of the host and thereby strengthens the anti-infectious barrier function of the skin[61]. This function is possibly due to the increase in macrophage activity in the skin, induced by osmotically inactive sodium storage[61].

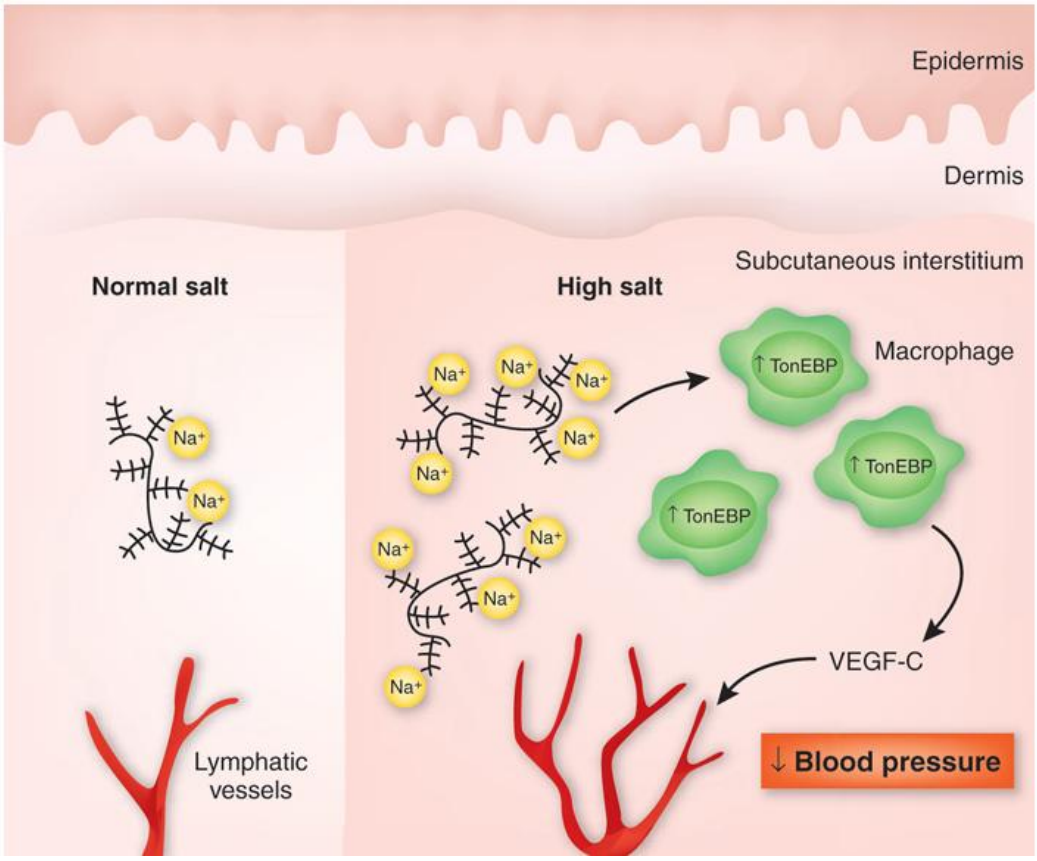

Figure 13: osmotically inactively stored sodium causes an increase in lymphatic vessel density[62]. Left is the normal situation, with a normal salt intake. Right is the situation of a high salt intake. More sodium is osmotically inactively stored in the skin, inducing a higher expression of TonEBP in macrophages. These release VEGF-C, which causes an increase in lymphatic vessel density and a decrease in blood pressure.

Blood pressure

High salt intake is associated with an elevated blood pressure, hypertension[7]. Although the exact mechanisms are poorly understood, it is known salt increased the blood pressure through different mechanisms[63]:

- a reduced endothelial function[64]. The production of NO (vasodilator) is reduced by a high salt diet, thus less vasodilatation and more vasoconstriction occurs.
- an increased arterial stiffness[65]. TGF-β is upregulated (due to low NO levels) and has profibrotic effects, increasing the arterial stiffness [66].
- a more sensitized sympathetic nervous system[67], activating the 'fight-or-flight' reaction[14]. Dietary salt sensitizes the neurons in the rostral ventrolateral medulla (RVLM), a site which is critical in for several sympathetic reflexes[68]

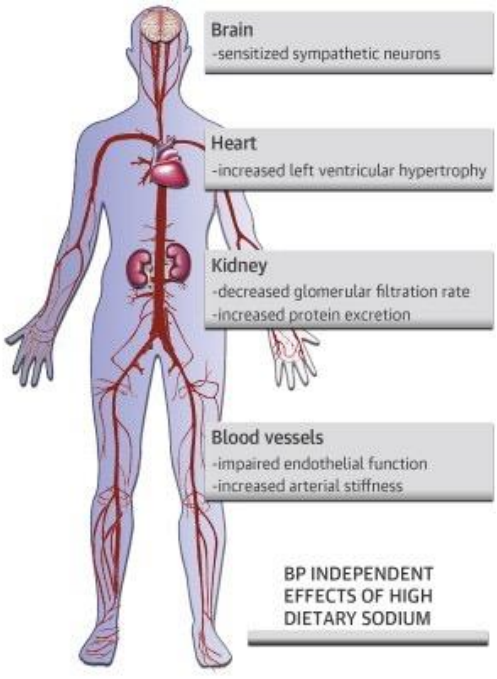

Figure 14: summary of the mechanisms through which high salt intake elevates blood pressure[63]. These effects occur as a result of a high salt intake, not as a result of a high blood pressure (Blood pressure independent).

- an increased left ventricular hypertrophy [69], which can cause hypertension[70]. This is a result of the increased sympathetic nervous system activation[71].
- a decreased glomerular filtration rate and an increased protein excretion[72].

These mechanisms occur independent of blood pressure, meaning the blood pressure does not cause these effects, but the hypertension is the result. They are summarized in Fig. 14.

The osmotically inactive sodium storage also has its effects on the blood pressure. Th17 cells, which are present in the skin as a result of osmotically inactive sodium storage, produces IL-17[73]. IL-17 is a critical mediator of angiotensin-II- induced hypertension and vascular dysfunction[74]. Originally, body fluids were considered to be divided over two compartments, according to the two-compartments model[75]. The lymph capillary in the skin create a third compartment to buffer the impact of sodium accumulation on intravascular volume and blood pressure[58].

Salt sensitivity versus resistance

Titze et al studied whether salt sensitive Dahl rats are as much capable of osmotically inactive sodium storage as salt resistant Dahl rats are[31]. Salt sensitive rats were less capable of storing sodium osmotically inactive and showed a higher blood pressure. This could be the case in humans as well.

7. Discussion

The sodium balance is quite complicated. It contains many hormones, which are regulated by different factors, such as blood pressure of water content of the body. The classical view of sodium balance could be seen as outdated and should be renewed. This seems to be recognized by Heer et al in 2000[26] and later developed by Titze et al in 2002[27] and later confirmed by Noakes et al[29], Palacios et al[30], and Rakova et al[28]. Titze has developed the new view of the osmotically inactive sodium storage. The exact mechanisms are yet unknown, but it is a promising view. Proteoglycans play a major role in this process. This storage has it effects on the human body. It may promote the occurrence of autoimmune diseases, but also provides a microbiological barrier, protecting the body against bacteria etcetera. This storage could be the link in human salt sensitivity and salt resistance and therefore have huge medical implications. If the mechanisms of osmotically inactive sodium storage will be known, they could potentially be clinically induced to convert salt sensitive patients to salt resistant patients. It could also be used to reduce blood pressure by creating a new fluid compartment. It could even have implications in the battle against bacterial infections, as shown by Jantsch et al[61]. As previously illustrated, sodium storage in the skin is linked to autoimmune diseases. This could have implications in the fight against these autoimmune diseases. If the mechanisms of clearing the sodium stored in the skin are known, this could potentially be induced to reduce the severeness of the autoimmune disease.

In line with the notion that chloride may be important for the clinical consequences of a high salt intake, what happens to the chloride when this sodium storage occurs, should be investigated.
The effects of chloride on the body seem to be largely underestimated. Most studies focus on the effects of sodium, without paying attention to chloride. Potassium chloride is a salt used to reduce the sodium intake. The apparent importance of chloride raises the question whether this substitute is beneficial or harmful to health.

Classical studies, such as the INTERSALT study of salt intake, have based their calculated intake on the sodium contents of the urine collected over 24 hours[76]. The existence of a storage of sodium in the body may indicate that the estimations of the intake are wrong. Besides, the excretion of sodium through the urine display a circaseptan (weekly) rhythm, evenly indicating the estimations are wrong[77].

Around the world, people consume too much salt. Most of this salt comes from processed foods, even you would not necessarily expect them in, like breakfast cereals, biscuits and cakes[78]. Salt decreases the taste perception of bitterness and increases the perception of a sweet taste. Reducing the salt contents in processed food will have flavour consequences, for instance: decreased salty and sweet taste and decreased appetitive aromas associated with salty and sweet taste , increased bitter taste and increased aversive aromas associated with bitter taste[78]. Recently, the adverse effects of salt is more and more recognized and great efforts are made worldwide to reduce salt intake to an acceptable level of 6 grams a day[79]. However, no country has achieved this level of salt intake yet.

8. References

1. Ritz E. The history of salt - aspects of interest to the nephrologist. *Nephrol Dial Transplant*. 1996;11(6):969-975.
2. Brown IJ, Tzoulaki I, Candeias V, Elliott P. Salt intakes around the world: Implications for public health. *Int J Epidemiol*. 2009;38(3):791-813.
3. McCarron DA, Kazaks AG, Geerling JC, Stern JS, Graudal NA. Normal range of human dietary sodium intake: A perspective based on 24-hour urinary sodium excretion worldwide. *Am J Hypertens*. 2013;26(10):1218-1223.
4. Diet, nutrition and the prevention of chronic diseases. *World Health Organ Tech Rep Ser*. 2003;916:i-viii, 1-149, backcover.
5. Holbrook JT, Patterson KY, Bodner JE, et al. Sodium and potassium intake and balance in adults consuming self-selected diets. *Am J Clin Nutr*. 1984;40(4):786-793.
6. James WP, Ralph A, Sanchez-Castillo CP. The dominance of salt in manufactured food in the sodium intake of affluent societies. *Lancet*. 1987;1(8530):426-429.
7. Blaustein MP, Leenen FH, Chen L, et al. How NaCl raises blood pressure: A new paradigm for the pathogenesis of salt-dependent hypertension. *Am J Physiol Heart Circ Physiol*. 2012;302(5):H1031-49.
8. Luft FC, Weinberger MH. Heterogeneous responses to changes in dietary salt intake: The salt-sensitivity paradigm. *Am J Clin Nutr*. 1997;65(2 Suppl):612S-617S.
9. Ticinesi A, Nouvenne A, Maalouf NM, Borghi L, Meschi T. Salt and nephrolithiasis. *Nephrol Dial Transplant*. 2014.
10. Sampath A, Kossoff EH, Furth SL, Pyzik PL, Vining EP. Kidney stones and the ketogenic diet: Risk factors and prevention. *J Child Neurol*. 2007;22(4):375-378.
11. Tsugane S. Salt, salted food intake, and risk of gastric cancer: Epidemiologic evidence. *Cancer Sci*. 2005;96(1):1-6.
12. Nguyen MK, Kurtz I. Determinants of plasma water sodium concentration as reflected in the edelman equation: Role of osmotic and gibbs-donnan equilibrium. *Am J Physiol Renal Physiol*. 2004;286(5):F828-37.
13. Petersen OH. *Lecture notes: Human physiology.* 5th ed. Oxford: Blackwell Publishing; 2007.

14. Silverthorn DU. *Human physiology, an integrated approach.* 6th ed. Glenview: Pearson; 2012.

15. Institute of Medicine I. DRI_Electrolytes_Water.pdf. http://www.iom.edu/~/media/Files/Activity Files/Nutrition/DRIs/DRI_Electrolytes_Water.pdf. Accessed 6/22/2015, 2015.

16. Boegehold MA, Kotchen TA. Importance of dietary chloride for salt sensitivity of blood pressure. *Hypertension*. 1991;17(1 Suppl):I158-61.

17. Boegehold MA, Kotchen TA. Relative contributions of dietary na+ and cl- to salt-sensitive hypertension. *Hypertension*. 1989;14(6):579-583.

18. Whitescarver SA, Holtzclaw BJ, Downs JH, Ott CE, Sowers JR, Kotchen TA. Effect of dietary chloride on salt-sensitive and renin-dependent hypertension. *Hypertension*. 1986;8(1):56-61.

19. Wyss JM, Liumsiricharoen M, Sripairojthikoon W, Brown D, Gist R, Oparil S. Exacerbation of hypertension by high chloride, moderate sodium diet in the salt-sensitive spontaneously hypertensive rat. *Hypertension*. 1987;9(6 Pt 2):III171-5.

20. Shah J, Jandhyala BS. Studies on the role(s) of cerebrospinal fluid osmolality and chloride ion in the centrally mediated pressor responses of sodium chloride. *Clin Exp Hypertens A*. 1991;13(2):297-312.

21. McCallum L, Lip S, Padmanabhan S. The hidden hand of chloride in hypertension. *Pflugers Arch*. 2015;467(3):595-603.

22. EDELMAN IS, LEIBMAN J. Anatomy of body water and electrolytes. *Am J Med*. 1959;27:256-277.

23. Titze J, Dahlmann A, Lerchl K, et al. Spooky sodium balance. *Kidney Int*. 2014;85(4):759-767.

24. Titze J. Water-free sodium accumulation. *Semin Dial*. 2009;22(3):253-255.

25. EDELMAN IS, LEIBMAN J, O'MEARA MP, BIRKENFELD LW. Interrelations between serum sodium concentration, serum osmolarity and total exchangeable sodium, total exchangeable potassium and total body water. *J Clin Invest*. 1958;37(9):1236-1256.

26. Heer M, Baisch F, Kropp J, Gerzer R, Drummer C. High dietary sodium chloride consumption may not induce body fluid retention in humans. *Am J Physiol Renal Physiol*. 2000;278(4):F585-95.

27. Titze J, Maillet A, Lang R, et al. Long-term sodium balance in humans in a terrestrial space station simulation study. *Am J Kidney Dis*. 2002;40(3):508-516.

28. Rakova N, Juttner K, Dahlmann A, et al. Long-term space flight simulation reveals infradian rhythmicity in human na(+) balance. *Cell Metab*. 2013;17(1):125-131.

29. Noakes TD, Sharwood K, Speedy D, et al. Three independent biological mechanisms cause exercise-associated hyponatremia: Evidence from 2,135 weighed competitive athletic performances. *Proc Natl Acad Sci U S A*. 2005;102(51):18550-18555.

30. Palacios C, Wigertz K, Martin BR, et al. Sodium retention in black and white female adolescents in response to salt intake. *J Clin Endocrinol Metab*. 2004;89(4):1858-1863.

31. Titze J, Krause H, Hecht H, et al. Reduced osmotically inactive na storage capacity and hypertension in the dahl model. *Am J Physiol Renal Physiol*. 2002;283(1):F134-41.

32. Dahl LK. Salt and blood pressure. *Lancet*. 1969;1(7595):622-623.

33. Titze J, Lang R, Ilies C, et al. Osmotically inactive skin na+ storage in rats. *Am J Physiol Renal Physiol*. 2003;285(6):F1108-17.

34. Ferrucci A, Pignatelli G, Sciarretta S, Tocci G. Hypertension in premenopausal women: Is there any difference? *High Blood Press Cardiovasc Prev*. 2014;21(3):195-199.

35. Lesperance LM, Gray ML, Burstein D. Determination of fixed charge density in cartilage using nuclear magnetic resonance. *J Orthop Res*. 1992;10(1):1-13.

36. Titze J, Shakibaei M, Schafflhuber M, et al. Glycosaminoglycan polymerization may enable osmotically inactive na+ storage in the skin. *Am J Physiol Heart Circ Physiol*. 2004;287(1):H203-8.

37. Seeliger E, Wronski T, Ladwig M, Rebeschke T, Persson PB, Reinhardt HW. The 'body fluid pressure control system' relies on the renin-angiotensin-aldosterone system: Balance studies in freely moving dogs. *Clin Exp Pharmacol Physiol*. 2005;32(5-6):394-399.

38. Seeliger E, Ladwig M, Reinhardt HW. Are large amounts of sodium stored in an osmotically inactive form during sodium retention? balance studies in freely moving dogs. *Am J Physiol Regul Integr Comp Physiol*. 2006;290(5):R1429-35.

39. Titze J, Bauer K, Schafflhuber M, et al. Internal sodium balance in DOCA-salt rats: A body composition study. *Am J Physiol Renal Physiol*. 2005;289(4):F793-802.

40. Palermo M, Quinkler M, Stewart PM. Apparent mineralocorticoid excess syndrome: An overview. *Arq Bras Endocrinol Metabol*. 2004;48(5):687-696.

41. Schenk J, McNeill JH. The pathogenesis of DOCA-salt hypertension. *J Pharmacol Toxicol Methods*. 1992;27(3):161-170.

42. Knox FG, Burnett JC,Jr, Kohan DE, Spielman WS, Strand JC. Escape from the sodium-retaining effects of mineralocorticoids. *Kidney Int*. 1980;17(3):263-276.

43. Nguyen MK, Kurtz I. Is the osmotically inactive sodium storage pool fixed or variable? *J Appl Physiol (1985)*. 2007;102(1):445-447.

44. Schafflhuber M, Volpi N, Dahlmann A, et al. Mobilization of osmotically inactive na+ by growth and by dietary salt restriction in rats. *Am J Physiol Renal Physiol*. 2007;292(5):F1490-500.

45. Kopp C, Linz P, Dahlmann A, et al. 23Na magnetic resonance imaging-determined tissue sodium in healthy subjects and hypertensive patients. *Hypertension*. 2013;61(3):635-640.

46. Linz P, Santoro D, Renz W, et al. Skin sodium measured with (2)(3)na MRI at 7.0 T. *NMR Biomed*. 2015;28(1):54-62.

47. Hofmeister LH, Perisic S, Titze J. Tissue sodium storage: Evidence for kidney-like extrarenal countercurrent systems? *Pflugers Arch*. 2015;467(3):551-558.

48. Hannon MJ, Verbalis JG. Sodium homeostasis and bone. *Curr Opin Nephrol Hypertens*. 2014;23(4):370-376.

49. Reece JB, Urry LA, Cain ML, et al. *Campbell biology.* 9th ed. Boston: Pearson; 2011.

50. Alberts BJ,A, Lewis J, Raff M, Roberts K, Walter P. *Molecular biology of the cell.* 5th ed. New York: Garland Science; 2008.

51. Proteoglycans. http://www.lookfordiagnosis.com/mesh_info.php?term=Proteoglycans&lang=1. Accessed 6/16/2015, 2015.

52. Berg JM, Tymoczko JL, Stryer L. *Biochemistry.* 7th ed. New York: W. H. Freeman and Company; 2011.

53. Wu C, Yosef N, Thalhamer T, et al. Induction of pathogenic TH17 cells by inducible salt-sensing kinase SGK1. *Nature*. 2013;496(7446):513-517.

54. Binger KJ, Linker RA, Muller DN, Kleinewietfeld M. Sodium chloride, SGK1, and Th17 activation. *Pflugers Arch*. 2015;467(3):543-550.

55. Miyakawa H, Woo SK, Dahl SC, Handler JS, Kwon HM. Tonicity-responsive enhancer binding protein, a rel-like protein that stimulates transcription in response to hypertonicity. *Proc Natl Acad Sci U S A*. 1999;96(5):2538-2542.

56. Kleinewietfeld M, Manzel A, Titze J, et al. Sodium chloride drives autoimmune disease by the induction of pathogenic TH17 cells. *Nature*. 2013;496(7446):518-522.

57. Singh RP, Hasan S, Sharma S, et al. Th17 cells in inflammation and autoimmunity. *Autoimmun Rev*. 2014;13(12):1174-1181.

58. Machnik A, Neuhofer W, Jantsch J, et al. Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat Med*. 2009;15(5):545-552.

59. Muller S, Quast T, Schroder A, et al. Salt-dependent chemotaxis of macrophages. *PLoS One*. 2013;8(9):e73439.

60. Jeltsch M, Kaipainen A, Joukov V, et al. Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. *Science*. 1997;276(5317):1423-1425.

61. Jantsch J, Schatz V, Friedrich D, et al. Cutaneous na+ storage strengthens the antimicrobial barrier function of the skin and boosts macrophage-driven host defense. *Cell Metab*. 2015;21(3):493-501.

62. Marvar PJ, Gordon FJ, Harrison DG. Blood pressure control: Salt gets under your skin. *Nat Med*. 2009;15(5):487-488.

63. Farquhar WB, Edwards DG, Jurkovitz CT, Weintraub WS. Dietary sodium and health: More than just blood pressure. *J Am Coll Cardiol*. 2015;65(10):1042-1050.

64. Tzemos N, Lim PO, Wong S, Struthers AD, MacDonald TM. Adverse cardiovascular effects of acute salt loading in young normotensive individuals. *Hypertension*. 2008;51(6):1525-1530.

65. Avolio AP, Clyde KM, Beard TC, Cooke HM, Ho KK, O'Rourke MF. Improved arterial distensibility in normotensive subjects on a low salt diet. *Arteriosclerosis*. 1986;6(2):166-169.

66. Sanders PW. Vascular consequences of dietary salt intake. *Am J Physiol Renal Physiol*. 2009;297(2):F237-43.

67. Stocker SD, Monahan KD, Browning KN. Neurogenic and sympathoexcitatory actions of NaCl in hypertension. *Curr Hypertens Rep*. 2013;15(6):538-546.

68. Stocker SD, Madden CJ, Sved AF. Excess dietary salt intake alters the excitability of central sympathetic networks. *Physiol Behav*. 2010;100(5):519-524.

69. Rodriguez CJ, Bibbins-Domingo K, Jin Z, Daviglus ML, Goff DC,Jr, Jacobs DR,Jr. Association of sodium and potassium intake with left ventricular mass: Coronary artery risk development in young adults. *Hypertension*. 2011;58(3):410-416.

70. Cuspidi C, Sala C, Negri F, Mancia G, Morganti A, Italian Society of Hypertension. Prevalence of left-ventricular hypertrophy in hypertension: An updated review of echocardiographic studies. *J Hum Hypertens*. 2012;26(6):343-349.

71. Jin Y, Kuznetsova T, Maillard M, et al. Independent relations of left ventricular structure with the 24-hour urinary excretion of sodium and aldosterone. *Hypertension*. 2009;54(3):489-495.

72. Smyth A, O'Donnell MJ, Yusuf S, et al. Sodium intake and renal outcomes: A systematic review. *Am J Hypertens*. 2014;27(10):1277-1284.

73. Takatori H, Kanno Y, Watford WT, et al. Lymphoid tissue inducer-like cells are an innate source of IL-17 and IL-22. *J Exp Med*. 2009;206(1):35-41.

74. Madhur MS, Lob HE, McCann LA, et al. Interleukin 17 promotes angiotensin II-induced hypertension and vascular dysfunction. *Hypertension*. 2010;55(2):500-507.

75. Guyton AC. Blood pressure control--special role of the kidneys and body fluids. *Science*. 1991;252(5014):1813-1816.

76. Brown IJ, Dyer AR, Chan Q, et al. Estimating 24-hour urinary sodium excretion from casual urinary sodium concentrations in western populations: The INTERSALT study. *Am J Epidemiol*. 2013;177(11):1180-1192.

77. Titze J. Sodium balance is not just a renal affair. *Curr Opin Nephrol Hypertens*. 2014;23(2):101-105.

78. Liem DG, Miremadi F, Keast RS. Reducing sodium in foods: The effect on flavor. *Nutrients*. 2011;3(6):694-711.

79. Webster JL, Dunford EK, Hawkes C, Neal BC. Salt reduction initiatives around the world. *J Hypertens*. 2011;29(6):1043-1050.