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*Original article*

# Immunolocalization of sodium-dependent glucose co-transporter 1 and sodium-dependent glucose co-transporter 2 in chicken's *(Gallus gallus domesticus)* kidneys

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## Abstract

In homeostasis, which plays an important role in the proper functioning and maintenance of the internal functioning of the body, kidneys play a key role in being responsible for the proper homeostasis of glucose. Among glucose transporters, sodium-dependent glucose co-transporters (SGLTs) have a major role in the kidney's ability to reabsorb glucose. Although the localization of these transporters has been extensively studied in mammals, there are still gaps in knowledge of the localization of SGLTs in birds of different age groups. The aim of this study was to immunolocalize in kidneys of hen chickens of different ages the sodium-dependent glucose co-transporters SGLT1 and SGLT2, comparing the localization between different age groups.

The kidneys derived from 32 hen chickens (*Gallus gallus domesticus*) were devided equally into four age groups: 3, 7, 14, and 20 day old broilers, 8 indivuals in each group. The polyclonal primary antibodies Rabbit anti- SGLT1 and Rabbit anti-SGLT2 (Abcam, UK) were used together with the corresponding IHC kit (Abcam, UK). The results were visualized photographically using an AxioCam HRc camera (Germany) connected to a Zeiss Axioplan-2 Imaging microscope (Germany).

The study revealed similar immunolocalization of SGLT1 and SGLT2 in the apical part of cells of proximal renal tubules in hen chickens' kidneys in all age groups. Strong staining of SGLT2 was noted also in the cytoplasm of epithelial cells of the proximal straight and convoluted tubules. Based on our study, the kidney tissue of newly hatched chickens is ready immediately after hatching for glucose reabsorption and transport, similarily to that of three-week-old chicks.

**Keywords:** chicken, immunohistochemistry, renal glucose transport, renal tubules, sodium-dependent glucose co-transporter-1, sodium-dependent glucose co-transporter-2

## Introduction

As glucose is the primary energy source for every living organism, glucose homeostasis is particulary important (Hruby 1997). Glucose transport across cell membranes is mediated by two families of glucose transporters that have been identified as facilitated diffusion glucose transporters (GLUTs) and the Na (+) -dependent glucose co-transporters (SGLT family) (Takata 1996). These transporters vary in their substrate specificity, regulatory mechanisms and distribution (Sano et al. 2020). While the GLUTs transport glucose across the plasma membrane by means of a facilitated diffusion mechanism (Navale and Paranjape 2016), the sodium-dependent glucose co-transporters are a family of glucose transporters which simultaneously transport sodium and glucose by a concentration gradient (Sano et al. 2020).

In the body's glucose homeostasis the kidneys play a central role in filtering and absorbing glucose (Mota et al. 2015). Glomerulus, the primary filtration unit in the kidneys (König et al. 2016), filters glucose, which is later reabsorbed in the kidney's proximal convoluted tubule (Mather and Pollok 2011). SGLT1 and SGLT2 reabsorb glucose in the kidneys (Vallon and Thomson 2012, Haas et al. 2014). SGLT2 is the main co-transporter involved in glucose reabsorption in the kidney and is responsible for 80–90% of glomerular filtered glucose reabsorption (You et al. 1995, Bonora et al. 2020). Most of the remaining glucose absorption, approximately 10%, is carried out by SGLT1 (Horiba et al. 2003, Vallon and Thomson 2012). Up to now most of the experiments on SGLTs have been performed in mammals - rats and mice (Takata 1996, Hussar et al. 2004, Vrhovac et al. 2015), and less is known about the molecular basis of glucose transport in birds. The comparative study of glucose transporters GLUT2 and GLUT5 immunolocalization in the gastrointestinal tract of hen chickens and ostriches has been carried out (Hussar et al. 2016, 2017). In hen chickens of different age groups after hatching the immunolocalization of the glucose transporters GLUT2 and GLUT5 appeared in similar strength, whereas the experiments on ostrich intestines revealed changes in the immunolocalization of the glucose transporters during the first weeks after hatching, showing the staining for both studied antibodies to be weaker in all parts of the gastrintestinal tract of ostriches during the first week after hatching compared to 30 day-old ostriches. Due to the difference in staining between these age groups in the previously mentioned study with ostriches, this article focused on similar age groups.

As the kidneys of avians differ significantly from those of mammals, especially due to the fact that birds possess two types of glomeruli: the mammalian type and the reptilian glomeruli type (Liebich 2019), studies of SGLT1 and SGLT2 and their immunolocalization in the kidneys of chicken of different ages – to detect and examine their peculiarities – are neccessary.

The aim of this study was to immunolocalize in the kidney tissue of 3, 7, 14 and 20 day-old hen chicken the sodium-dependent glucose co-transporters SGLT1 and SGLT2 and to compare their immunolocalization and staining intensities in the kidneys of chickens in different age groups.

## Materials and Methods

Kidney tissue from 32 healthy domestic hen chickens (*Gallus gallus domesticus)* divided equally into four age groups – 3, 7, 14 and 20 day-old, was used. The hen chickens were killed by cervical dislocation and the sample material, which consisted of the hen chicken kidneys, was entirely removed. The kidney specimens, 1.0 cm in diameter, were fixed in 10% neutral buffered formalin solution for 72h at room temperature and embedded into paraffin. Thereafter 7 μm thick slices were cut using a Microm HM360 (USA) microtome, floated on Poly-L-Lysine coated slides (O. Kindler GmbH,Freiburg, Germany), dried at 44°C for 12 h, followed by deparaffinization with xylene. The rehydration of the slices was carried out in a graded series of ethanol followed by the methods of routine histology and immunohistochemical staining using an immunohistochemistry kit (IHC kit, Abcam, UK) according to the manufacturer's guidelines.

### Routine histology

For overall histological assessment, the tissue samples were stained using the hematoxylin and eosin method (Carson 1997).

### Immunohistochemistry

The slices for immunohistochemical staining were pre-treated for 20 minutes using the method of heat-mediated antigen retrieval, and were then covered with TRIS buffer for 20 minutes and incubated with primary polyclonal antibodies Rabbit anti-SGLT1 and Rabbit anti-SGLT2 (Abcam, UK) at 1/1000 dilution for 30 min at 37 C. Biotinylated secondary antibody and streptavidin-conjugated peroxidase were used for detection using DAB (3.3'-diaminobenzidine tetrahydrochloride) as chromogen. Human kidney tissue sections for identifying SGLT-2 and SGLT-1 were used as positive controls, available for comparison on the Abcam antibody producer's homepage as examples for the antibodies'

immunohistochemistry on paraffin-embedded tissues (IHC-P) (Official website of Abcam (2022). Retrieved from http://www.abcam.com). Negative controls contained diluent (Dako, S0809) instead of primary antibodies.

After staining a quality control of each slide was carried out to ensure consistent, reproducible and reliable results. These quality control steps followed a scheme by Lin and Zongming, 2014, including checking the slides for tissue and staining quality. A standard light microscope by Zeiss, Germany was used for examination and Zeiss Axioplan-2 Imaging was used for photography of the slides.

This study did not require an Ethics Agreement (Estonian Animal Experimentation Permit Committee decision nr.1.2-17/40).

## Results

For the overall histological assessment of the chicken kidneys, routine histology staining by Hematoxylin and Eosin was carried out. The morphofunctional units of the kidneys- nephrons- including the proximal and distal renal tubules were determined (Fig. 1).

Our study revealed immunolocalization of SGLT1 and SGLT2 in the kidneys of hen chickens at four different ages – at 3, 7, 14 and 20 days old. In all studied age groups the antibodies were localized similarily; however, comparatively, SGLT1 staining was found to be more intense than the staining for SGLT2.

### Immunolocalization of SGLT 1

In all age groups the apical parts of the epithelial cells of the renal straight proximal tubules stained strongly (Fig. 2) for SGLT1. The other renal tubules and the renal corpuscles remained unstained .

### Immunolocalization of SGLT2

In all age groups SGLT2 was localized in the renal cortical proximal straight and convoluted tubules, while other tubules remained unstained (Fig. 3 A-C). In addition to the apical parts, SGLT2 was observed also to be stained in the cytoplasm of the tubules epithelial cells.

Although the localization of SGLT2 and SGLT1 in the apical parts of the renal proximal tubules was noted to be similar, strong staining for SGLT2 throughout the cytoplasm of epithelial cells of the proximal straight and convoluted tubules was also noted.

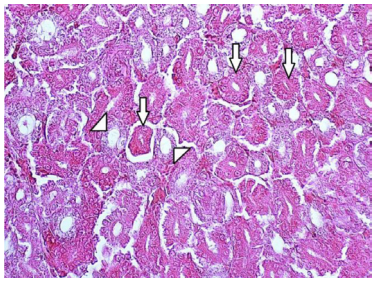

Fig. 1. Proximal- (arrows) and distal (arrowheads) tubules in 3 day-old hen chicken kidney. Hematoxylin-eosin, x400

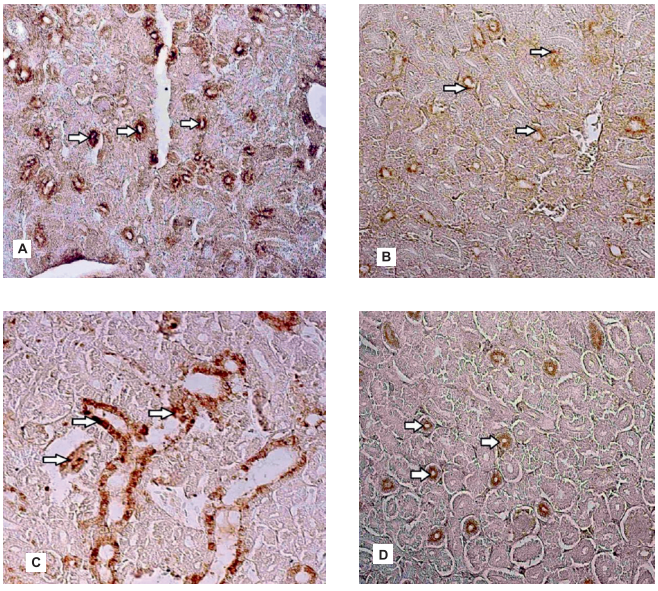


Fig. 2. Immunolocalization of SGLT1 in 3(A), 7(B), 14(C) and 20(D) day-old chicken renal tubules: note the strong staining in the apical parts of the epithelial cells of the straight proximal tubules (arrows). Immunohistochemistry (IHC), x200

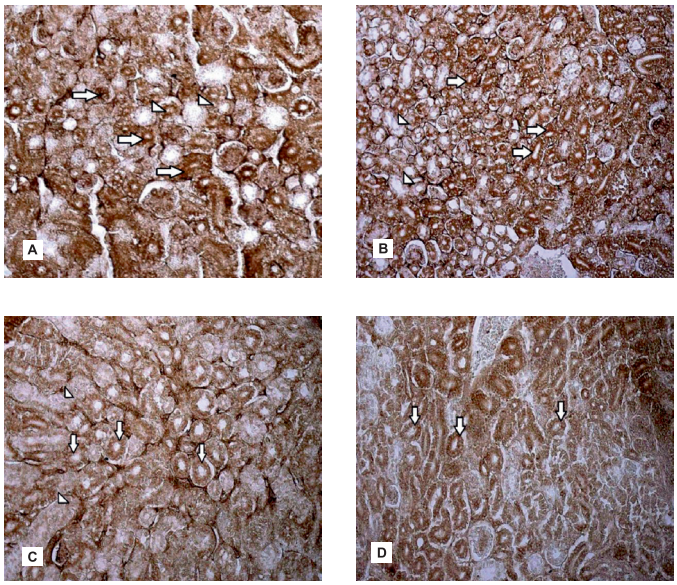


Fig. 3. Immunolocalization of SGLT2 in 3(A), 7(B), 14(C) and 20(D) day-old chicken kidneys: note the strong staining of the renal proximal tubules (arrows) and the unstained distal tubules (arrowheads). IHC, x200

## Discussion

Glucose serves as the central source of energy for most living cells; however the glucose molecules, being unable to traverse the lipid membrane of the cell by simple diffusion, require transport proteins known as glucose transporters for the entry of glucose molecules into the cells (Navale and Paranjape 2016). The main types of glucose transporters identified so far are facilitated diffusion glucose transporters (GLUTs) and sodium – glucose linked transporters (SGLTs). In the glucose homeostasis in the body the kidneys have a central role (Mota et al. 2015). In healthy kidneys, 100% of the glucose filtered by the glomeruli is reabsorbed by the nephrons while healthy nephrons do not excrete glucose.

Membrane proteins responsible for glucose reabsorption from the renal glomerular filtrate are the sodium-glucose cotransporters SGLT1 and SGLT2 (Vallon and Thomson 2012, Haas et al. 2014). SGLT2 is localized at the beginning of the proximal tubule in mammals and is responsible for the main reabsorption of glomerular-filtered glucose (You et al. 1995, Bonora et al. 2020). The remaining glucose absorption is mostly (ca. 10%) carried out by SGLT1 in the more distal parts of the renal proximal tubules (Horiba et al. 2003, Vallon and Thomson 2012). The low-affinity, high-capacity Na+-glucose co-transporter SGLT2 handles the major glucose uptake to the cytoplasm from the lumen and is found in the apical part of the S1/S2 segments of the proximal renal tubules (Vallon et al. 2011, Umino et al. 2018, Wilcox 2020). The remainder of the glucose that is not reabsorbed by SGLT2 is resorbed by SGLT1, GLUT1, and GLUT2 commonly found in the S2/S3 segments of proximal kidney tubules and additionally in intestinal brush border cells (Rieg et al. 2014, Navale and Paranjape 2016). Whilst the glucose transport mediated by GLUT's occurs by faciliated diffusion, the glucose transport mediated by SGLTs is sodium-dependent and therefore the transport relies on a Na+ concentration gradient (Navale and Paranjape 2016). Compared to SGLT2, SGLT1 is considered the minor transporter as it accounts for only 10% of all of the filtered glucose (Koepsell and Vallon 2020). The proximal renal tubule can be divided into two sections which are the proximal convoluted tubule and the proximal straight tubule. These two sections have ultrastructural divisions, so-called segments, that consist either of higher or lower cell complexity. The S1 segment corresponds to the convoluted proximal tubule while the S2 and S3 segments correspond to the straight proximal tubule (Boron and Boulpaep 2004, 2016). In previous studies on mammals, SGLT1 has been identified on the apical side of the epithelial cells of the renal proximal tubules and SGLT1 and SGLT2 have been localized in the S1/S2 and S3 segments of the proximal tubules respectively.

In birds, the localization of glucose transporters in their gastrointestinal tract has been extensively studied (Hussar et al. 2017, 2020); however the investigation of immunolocalization of sodium-dependent glucose cotransporters in the kidney tissue of 14-day-old birds started only recently (Hussar et al. 2020, 2022). Previous studies on the avian gastrointestinal system have shown differences of glucose transporter immunohistochemical staining in different age groups of hen chickens and ostriches, but up to now there has been

lack of similar comparative studies on sodium-dependent glucose transporters in kidney tissue.

The findings of previous studies on sodium-dependent glucose transport immunolocalization in mammalian kidneys (Vallon et al. 2011, Sano et al. 2020) are in correlation with the results of our present study, showing the localization of SGLT1 and SGLT2 in chicken renal proximal tubules. Control samples were used to assess the correctness of staining of the SGLTs (Lin and Chen 2014, Magaki et al. 2019).

In our present study the immunohistochemical localization of SGLT1 and SGLT2 in the kidneys of hen chickens of different ages was detected on the apical side of the epithelial cells of the proximal renal tubules. Although the localization of SGLT1 and SGLT2 in the apical parts of the renal proximal tubules was noted to be similar, strong staining for SGLT2 throughout the cytoplasm of the epithelial cells of the proximal straight and convoluted tubules was noted.

Comparatively, there were no differences in the immunolocalization of both studied antibodies found between the different age groups of hen chickens, indicating that the excretion of urine, glucose transport and filtering of glucose is ready immediately after hatching.

As the sodium-dependent glucose co-transporters are involved in a variety of processes and diseases (Wright et al. 2007, Nespoux et al. 2019, Wright 2021) their immunolocalization may have implications in future research with therapeutic indications.

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