Original Article

Insights into the Kidney Tissue Proteins Signaling Responded to Sodium Intake Using Multiplex Quantitative Proteomics

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Abstract

Dietary sodium intake has been linked to the amount of kidney proteins in the body. High sodium intake can lead to an increase in the amount of proteins found in the kidneys, which can in turn lead to an increased risk of hypertension and other kidney-related health issues. In this study, the difference levels of sodium rat chow were used to alter rats' kidney tissue. Body weight was not affected by sodium intake. Urine volume was increased in high sodium group, and urine osmolality was decreased in low sodium intake compared to control. The homogenized kidney tissues were analyzed by multiplex quantitative proteomics. Quantitative analysis showed that total proteins data revealed 4054 proteins in kidney tissue at $p<0.05$. Among this, low sodium diet showed 259 significantly difference proteins compared to control. High sodium diet showed 357 significantly difference proteins compared to control. Protein-proteins interaction analysis showed the predominant signaling pathway are serine/threonine metabolism and proteins trafficking.

Keywords: Kidney tissue proteome, Sodium intake

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INTRODUCTION

Salt, or sodium chloride, is an essential nutrient that the body needs in small amounts to help regulate fluid balance, nerve and muscle function, and blood pressure. However, consuming too much salt can have adverse effects on health, particularly on the kidneys. The average American consumes about 3,400 milligrams of salt per day, which is much higher than the recommended daily intake of 2,300 milligrams. [1] Eating a diet high in salt can lead to a number of health problems, including an increased risk for developing high blood pressure, stroke, and heart disease. It can also have a negative effect on kidney health. [2-4] A high-salt diet can cause damage to the kidneys by increasing the amount of salt and water retained in the body. This can lead to an increase in blood volume, which can put a strain on the kidneys and lead to high blood pressure. High blood pressure can damage the delicate filtering system of the kidneys, leading to a decrease in kidney function and an increased risk of developing kidney disease. The kidneys are a vital organ, responsible for filtering and eliminating waste from the body. Low salt diets have become increasingly popular in recent years as a way to reduce the risk of high blood pressure and other health conditions. However, there is some evidence to suggest that a low salt diet may have an adverse effect on the kidneys. [5-7]

The kidneys regulate the amount of salt in the body by eliminating excess salt through the urine. Therefore, when salt intake is reduced, the kidneys work harder to maintain the necessary balance. This can lead to a decrease in the ability of the kidneys to filter waste,

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which can lead to a buildup of toxins and other unwanted substances in the blood. In addition, the kidneys may be unable to effectively absorb certain nutrients, leading to deficiencies. There are other potential risks associated with a low salt diet, as well. A decrease in salt intake can increase the risk of dehydration, as the body needs salt to help retain water. In addition, a low salt diet may lead to an electrolyte imbalance, which can cause serious health problems. Although a low salt diet may have some potential benefits, such as reducing the risk of high blood pressure, it is important to consider the potential risks associated with this type of diet.

The proteome of kidney tissue is a complex network of proteins that is essential for normal kidney function. It is also affected by changes in dietary intake of sodium. When sodium levels in the body become too high, the kidneys must work harder to excrete the extra sodium, which can lead to an increase in blood pressure and an increase in the risk of developing hypertension. Conversely, when sodium levels in the body become too low, the kidneys must work to absorb more sodium, which can lead to an increase in blood pressure and an increased risk of developing hyponatremia. The proteome of kidney tissue is affected by changes in dietary intake of sodium in several ways. Proteomes are composed of proteins, which are a critical component of normal kidney function. Sodium is an essential electrolyte, and it affects the regulation of water balance, acid-base balance, osmotic pressure, and cellular signaling in the body. Therefore, changes in dietary intake of sodium can significantly affect the proteome of kidney tissue. [8, 9]

One-way dietary intake of sodium can affect the proteome of kidney tissue is by altering the protein profile. Sodium is a key factor in maintaining normal kidney function, and changes in sodium intake can lead to changes in the expression of various proteins. [10] In particular, changes in dietary sodium can alter the expression of proteins involved in renal tubular reabsorption and secretion, as well as proteins involved in the regulation of blood pressure. This can lead to changes in the overall protein profile of kidney tissue and, in turn, to changes in its proteome. [11] Another way dietary intake of sodium can affect the proteome of kidney tissue is by altering the metabolic activity of the tissue. [12] Sodium is necessary for the normal functioning of the kidneys, and changes in dietary intake can lead to changes in the metabolic activity of the kidney. This study explores changed of tissue proteome in responded to difference sodium intake even high or low level of dietary sodium.

MATERIALS AND METHODS

Animals and experimental group

Sprague Dawley male rats weighing between 200-250 grams were obtained. All rats were acclimatized and treated in the metabolic cages (Tecniplast™ Metabolic Cage Systems for Rodents, Thermo Scientific™, Canada), day-night cycle 12 hours, temperature 25°C. Rats were divided into 3 groups, the difference composition of the rat chow consisting of dietary sodium restriction rat chow (0.001% Na+) for low sodium (LS) group, regular rat chow (0.4% Na+) for control (Con) group, and high sodium rat chow (4% Na+) for high sodium (HS) group were used. All rats were treated with difference rat chow for 4 days. At the end of experiment the kidney cortexes were harvested and prepared according to the In-solution digestion and mass spectrometry sample preparation protocol.

In-solution digestion

Homogenized Renal cortical tissue was lysed with 5% sodium deoxycholate (SDC)(D6750, Sigma) in 50mM Triethylammonium bicarbonate (TEAB) containing 1x protease inhibitor cocktail (Halt™, Thermo Scientific™) and 1x phosphatase inhibitor cocktail (Pierce™, Thermo Scientific™). All samples were quenched with DTT for 15 minutes at room temperature and incubated with trypsin solution (Sequencing Grade Modified Trypsin, V511A, Promega, Wisconsin, USA) at concentration 0.1ug/µl at 1:50 ratio at 37oC for 12-16 hours. The digested peptides amount of samples were measured with the Pierce Quantitative Fluorometric Peptide Assay.

Tandem mass tag (TMT10plex™)

Tandem Mass Tag reagent kit (TMT10plex™ Isobaric Label Reagent, Thermo Scientific™) was chose to label digested peptides due to number of samples. LC/MS run. TMT10plex™Reagent was prepared according to the manufacturer's instructions. Briefly, dissolve reagent with 99% anhydrous acetonitrile (ACN) for 5 minutes with occasional vortex. Add equal amount of each sample into TMT10plex™ label reagent tube, one sample per one amine-reactive number (Figure 3.12) The samples were mixed by vortexing follow by centrifugation, then incubated the reaction for 1 hour at room temperature. After incubation, quench the reaction by added 8 µl of 5% hydroxylamine to the sample and incubated for 15 minutes.

LC-MS/MS and data analysis

All tagged samples were analyzed by a Q-Exactive Orbitrap mass spectrometer (Thermo Scientific™) via the

electrospray ionization (ESI) technique couple with EASY-nLC1000 system (Thermo Scientific™) for total protein analysis and phosphoprotein analysis, respectively. For total protein analysis, 33 µg of sample were divided into 8 fractions. Each fraction was resuspended in 0.1% FA to the final concentration 27.5 ng/µl. The injected sample contained 302.5 ng of peptide. Samples were pick up and loading into an EASY-nLC1000 system eluted with 4 difference gradients and times, at flow rate 300 nl/min (1) 4-20% acetonitrile in 0.1% FA for 60 minutes followed by (2) 20-40% acetonitrile in 0.1% FA for 15 minutes, (3) 40-95% acetonitrile in 0.1% FA for 10 minutes, and (4) 95-95% acetonitrile in 0.1% FA for 5 minutes, 90 minutes in total. Relative abundance and distribution of signal were determined, then small distribution or flat or sporadic signal was repeated with new gradients and times. Quantitative MS data were analyzed by MaxQuant v1.6.10 version against 8,050 proteins on "Rat proteomes reviewed (Swiss-Prot)", FASTA (canonical & isoform) data base on UP000002494, 26 Jan, 2019. The searching parameters were following setup 1) peptide-spectrum match (PSM) false discovery rate (FDR): 0.01, 2) protein FDR: 0.01.

Statistical analysis

Data were expressed as mean (±SD). The normal or non-normal distribution of all data was investigated using Shapiro-Wilk test. Statistical analysis that were applied for MS data consist of normalized peak intensity log2 ratios of each sample to reference intensity of its injection set was compared, ANOVA at threshold p-value <0.05 followed by Benjamini–Hochberg procedure to control false discovery rate (FDR) at q-value 0.05 were applied.

RESULTS AND DISCUSSION

Effects of salt restricted diet and high salt diet on rat body weight and urine output

Body weight (mean ± SD) at start date (before treatment) (Con: 275.78±19.13, LS: 270.33±22.16, HS: 276.11±32.05), 2 day and 4 day after treatment were measured. The mean body weight of low salt diet and high salt diet treated groups both day2 (Con: 274.67±21.25, LS: 270.00±24.94, HS: 273.56±28.87) and day4 (Con: 293.33±20.95, LS: 305.50±22.27, HS: 274.95±31.06) are comparable to that of control (Fig. 1 A). 24hr urine volume that was collected daily of control group and low salt group was not changed since day1 to day4 of experiment (Con day1: 17.83±9.70, Con day2: 18.00±7.92, Con day3: 14.83±8.54, Con day4: 17.16±5.98), low salt group (LS day1: 22.50±9.99, LS day2: 21.00±10.16, LS day3: 21.50±7.47, LS day4: 23.66±7.08). However, I found a significantly increased 24hr urine volume in High salt group on day2 to day4 at $p<0.05$ ((HS day1: 25.83±10.99, HS day2: 31.00±11.66, HS day3: 28.33±12.11, HS day4: 30.66±10.21) (Fig. 1 B) and 24hr urine volume of the high salt group on day 2 significantly increased compared to the low salt group at $p<0.05$. After treatment, urine osmolality was measured. Low salt group (1523.33±650.31) showed significantly decrease urine osmolality ($p<0.05$) compared to control (2599.66±856.56), while High salt group's urine osmolality (1840.00±652.38) was comparable to control (Fig. 1 C). After 4 days of treatments Low salt group

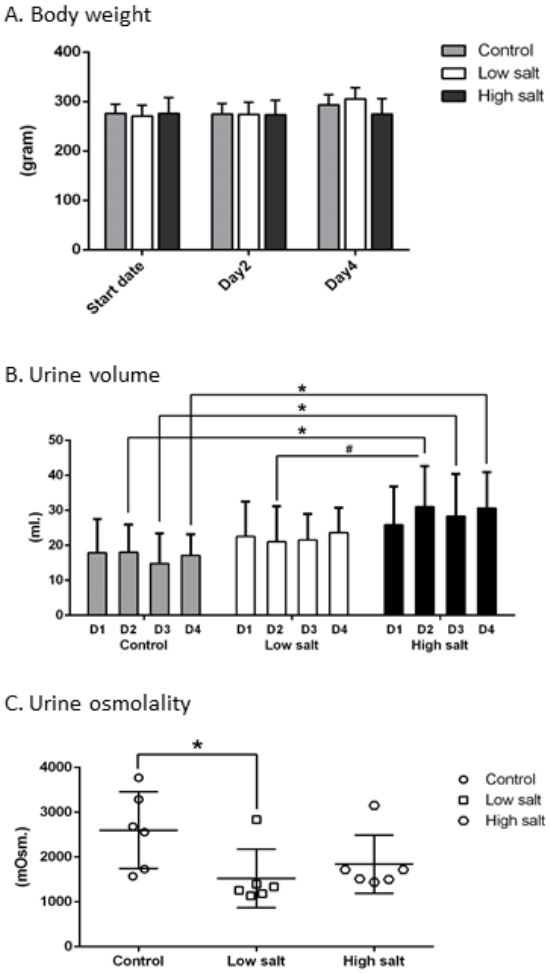

Figure 1. *Panel A.* Mean (±SD) rats body weight before treatment (Start date), day 2, and day 4 (end of experiment). *Panel B.* Mean (±SD) 24hr urine volume on day 1 to day 4. *Panel C.* Mean (±SD) urine osmolality after treatment, *, # $p<0.05$ compared to Control group, and Low salt group respectively.

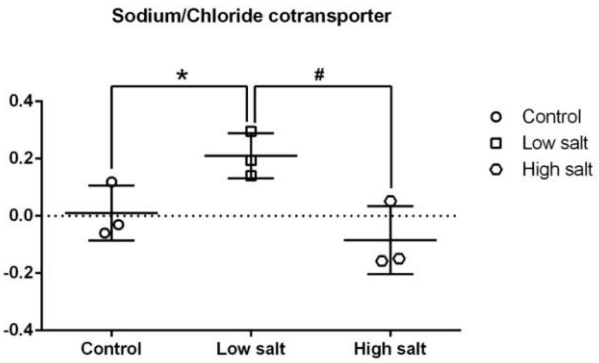

Figure 2. Mean (±SD) after treatment sodium/chloride cotransporter (NCC) abundance from mass spectrometry analysis. *, # $p<0.05$ compared to Control group, and Low salt group respectively.

showed decreased urine osmolality compared to regular diet. The mechanism for this change is control by reabsorption of sodium by the kidneys. This finding corresponding to increased expression of NCC in the kidney of Low salt group compared to Normal diet group. Urine osmolality of High salt group was comparable to control group but the increasing of urine volume since day-2 to day-4 could represent more sodium and water excretion than the control group.

Effects of salt restricted diet and high salt diet on rat kidney cortical tissue protein abundance

After 4 day of treatment 3 homogenized rat kidney cortical tissue samples (From N=6) from each group were randomly picked up to be a representative to proceed along TMT10plex labeling coupled with mass spectrometry. MS data was quantification and statistical analysis revealed that 0.001% sodium diet could raise sodium-chloride cotransporter (NCC) abundance in kidney cortical tissue (0.209850±0.078) compared to control (0.009678±0.095) and significantly increased when compared to high salt group at $p<0.05$. Moreover, NCC in rat kidney cortical tissue abundance in high salt group tended to decreased but not significant compared to control (Fig. 2). In this study low salt and high potassium diet could elevate serum aldosterone level. Combination of low salt and high potassium diet could induce aldosterone release in an addition pattern. ENaC response in the same manner as changes in serum

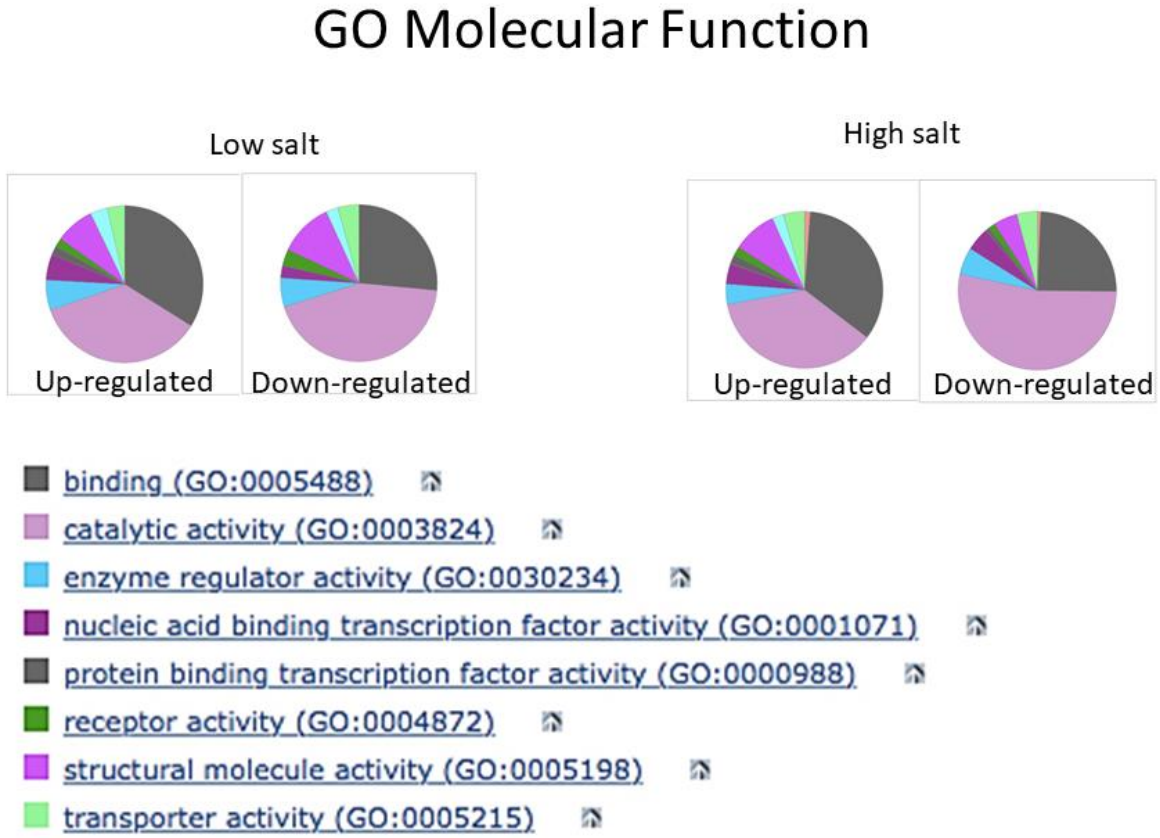

Figure 3. Ratio of the number of proteins categorized by GO molecular function that significant changed compared to control group

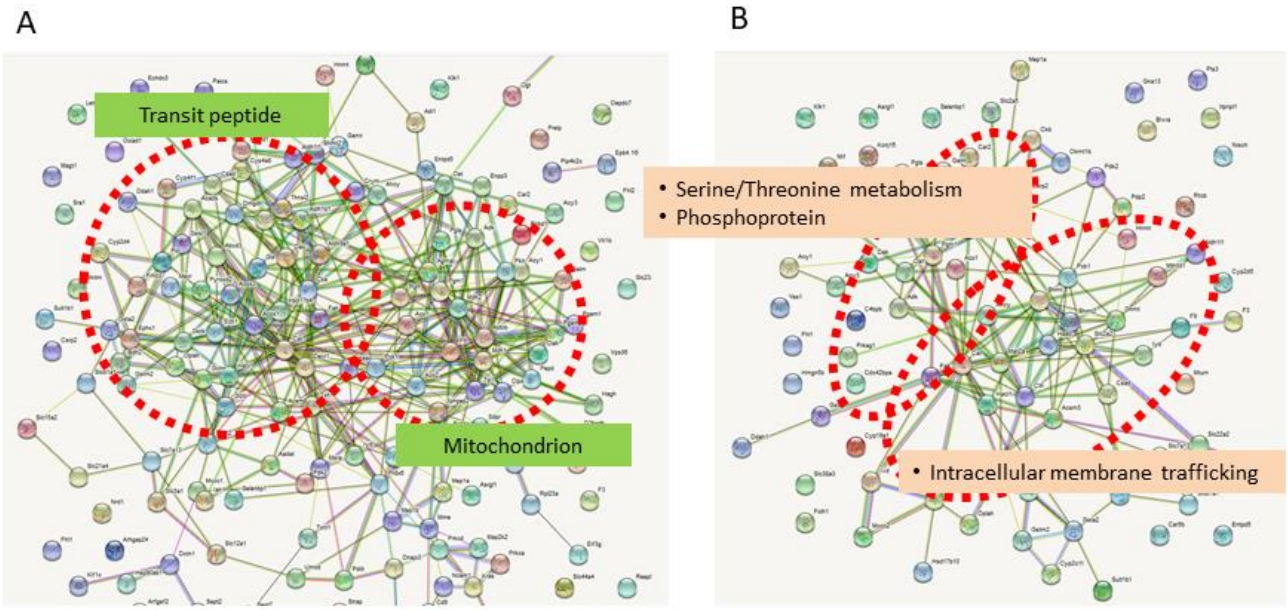

Figure 4. STRING algorithm shows the proteins interaction responses to difference dietary sodium intake. *Panel A.* Up-regulation proteins in Low sodium group compared to control group. *Panel B.* Up-regulation proteins in High sodium group compared to control group.

aldosterone. The results consistent with previous study by Nils van der Lubbe and coworkers, who study in rodent models with low salt and/or high potassium diet. They found ENaC expression corresponded with aldosterone level, but not NCC. [13] They also confirmed that NCC phosphorylation domain was essential for NCC expression level. On the other hand, NCC level were changed according to serum angiotensin II level. Angiotensin II regulated NCC via WNK-SPAK signaling pathway. Increasing angiotensin II will increase phosphorylate of WNK1 the further activate NCC by phosphorylation. In this study, I found that rat treated with combined diet had highest angiotensin II level and higher level of pNCC/NCC expression ratio from the immunoblot analysis than the other groups. [14]

Proteins Molecular function and cellular pathways changed

Quantitative analysis showed that total proteins data revealed 4054 proteins in kidney tissue at $p<0.05$. Among this, low sodium diet showed 259 significantly difference proteins compared to control. High sodium diet showed 357 significantly difference proteins compared to control. While 173 proteins are the significant proteins compared between low and high sodium treated rats at $p<0.05$. I categorized the significantly changed proteins of kidney tissue by low sodium and high sodium after 4 day compared to control group. Gene ontology (GO) molecular function revealed that the significantly changed proteins are the proteins involved in binding proteins, catalytic activity, enzyme regulator activity, nucleic acid binding transcription factor activity, protein binding transcription factor activity, receptor activity, structural molecular activity and transporter activity, respectively. (Fig. 3)

All significant proteins ID were further analyzed by Search tool for the retrieval of interacting gene/proteins (STRING) algorithm to explore the protein-protein interactions and cellular pathways. I found that transit peptide and mitrochondrion were up-regulated in low salt group. (Fig. 4A) Which might show the upregulation of the translocation of the channels in the kidney tissue especially NCC and ENaC in order to maintained serum osmolality within normal range. Previous study showed that vasopressin-increased NCC expression might be via inhibition of Nedd4-2 by PKA activation. [15] In high sodium treated rat tissue, 105 Significantly Up-regulated proteins were illustrated, then proteins that involved in Serine/Threonine metabolism, Phosphoprotein binding and intracellular membrane trafficking were found. (Fig. 4B) Several proteins involved in ENaC regulatory complex (ERC) were found significant increased consist of NHERF-1, NHERF-2, Adenosyl homocysteinase (AdoHcyase) that might involve in lessen ubiquitination process of both ENaC and NCC from apical membrane then allow channel to continue an action. [16]

CONCLUSION

Urine volume and urine electrolytes were tightly regulated to maintain serum osmolality when rats were treated with low salt diet, high potassium diet or combination of low salt with high potassium diet. Under low salt condition, rats can control serum osmolality via modulation of NCC and ENaC expression and possibly posttranslational modifications. The predominant signaling pathway that regulate ENaC found in this study is ERC. These proteins regulate cell surface expression and activities of ENaC. The predominant signaling pathways that regulate NCC found in this study are involved in serine/threonine metabolism.

Conflicts of Interest: The author has no conflict of interest to declare.

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