

**METABOLISM AND NUTRITION**

**Nutrient Transport in the Small Intestine: Na⁺,K⁺-ATPase Expression and Activity in the Small Intestine of the Chicken as Influenced by Dietary Sodium**

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**ABSTRACT** The Na⁺-K⁺-ATPase, localized in the basolateral membrane of enterocytes plays a major role in nutrient transport in the small intestine by transferring K⁺ ions into- and Na⁺ out of the cell. Within the enterocyte, homeostasis is maintained by active exclusion of Na from the cell by the Na⁺, K⁺-adenosine triphosphatase (ATPase) or sodium pump. Because much of the intestinal nutrient transport is by Na cotransporters, Na⁺, K⁺-ATPase may be used to evaluate nutrient uptake. In this study, nutrient transport was evaluated by determining expression and activity of Na⁺-K⁺-ATPase in the jejunum of chicks fed diets with different concentrations of Na.

Expression of the chicken Na⁺-K⁺-ATPase gene was examined following isolation of an 1,140 bp cDNA fragment of the α-subunit using a reverse transcription (RT)-PCR reaction with specific primers. This fragment was sequenced and showed 95 to 98% homology with the mammalian α-subunit of the Na⁺-K⁺-ATPase genes. This cDNA fragment was used as a specific probe in Northern blot hybridization for determination of expression in the chicken jejunum. Expression of mRNA of Na⁺-K⁺-ATPase was enhanced at low dietary Na but was unchanged at high dietary Na concentrations. In contrast, activity of the enzyme was low with low dietary Na and unchanged at high dietary Na. The Vmax of the Na⁺-K⁺-ATPase was unchanged, but affinity was altered by dietary Na concentrations. Thus, determination of expression and activity of intestinal Na⁺-K⁺-ATPase allows clearer understanding of changes in intestinal uptake due to dietary Na.

*(Key words: chicken, intestine, gene expression, Na⁺-K⁺-ATPase)*

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**INTRODUCTION**

Intestinal uptake of nutrients from the lumen across the enterocyte is by active and passive mechanisms. Much of the active transport across the brush border is affected by membrane-anchored transporters. Transport of glucose into the enterocyte is primarily by the Na glucose transporter (SGLT1) (Wright, 1993), which transfers two molecules of Na together with each molecule of glucose. Many amino acids are also transported by Na cotransporters with overlapping specificity (Nakanashi et al., 1994; Munck and Munck, 1999). Once within the enterocyte, much of the transport into the circulation is by passive means for both glucose and amino acids (Olson and Pes-sin, 1966; Verrey et al., 2000). Within the enterocyte the Na cotransported with the glucose and amino acids must be removed, and homeostasis is maintained by active exclusion of Na from the cell by the Na⁺, K⁺-adenosine triphosphatase (ATPase) or Na pump (EC 3.6.1.3). This ubiquitous membrane protein is instrumental in maintaining the electrochemical gradient of Na⁺ and K⁺ across the plasma membrane of animal cells (Glynn, 1993). Park et al. (1998) determined that approximately 33% of the total small intestinal O₂ uptake was used by the Na⁺-K⁺-ATPase. Previous studies have suggested use of the activity of this enzyme to estimate intestinal uptake (Croom et al., 1999; Sklan and Noy, 2000).

The Na⁺-K⁺-ATPase comprises two essential subunits, α and β. The catalytic α-subunit encompasses the sites of nucleotide and cation binding and undergoes conformational transitions associated with the coupling of ATP hydrolysis to translocation of Na⁺ and K⁺. The β-subunit is required for correct insertion and stability of the enzyme in the plasma membrane and also has a role in modulating cation affinity (Chow and Forte, 1995). Multiple isoforms of the α- (α1, α2, α3 and α4) and β- (β1, β2 and β3) subunits are expressed in tissue- and development-specific manners (Blanco and Mercer, 1988).

Depending on the cell type, the activity of the Na pump may be coupled to other crucial functions of the cell, such

**Abbreviation Key:** HS = high sodium diet; LS = low sodium diet.
as regulation of cell volume, nerve and muscle excitability, pH regulation, and uptake of carbohydrates, amino acids, and vitamins. In intact cells, Na pump activity may be modulated by alteration of intrinsic kinetic behavior, cell surface expression, and de novo protein synthesis (Bertorello and Katz, 1993; Ewart and Klip, 1995). The adult mammalian small intestine expresses only the \( \alpha \) and \( \beta_1 \) isoforms (Giannella et al., 1993; Wild et al., 1999).

The sequence of Na\(^{+}\)K\(^{-}\)-ATPase has been reported for some species: cDNA of Na\(^{+}\)K\(^{-}\)-ATPase was isolated from chicken kidney (Takeyasu et al., 1990), human placenta (Chehab et al., 1987), pig kidney (Broude et al., 1987; Monastyrskaia et al., 1987), and rat brain (Hara et al., 1987).

Because the intestinal Na\(^{+}\)K\(^{-}\)-ATPase is instrumental in Na and hence in glucose and much of the amino acid uptake, this study reports on the expression and transcriptional regulation of chicken Na\(^{+}\)K\(^{-}\)-ATPase in the small intestine as influenced by dietary Na concentrations. In order to determine expression, a 1,140-bp cDNA fragment of the chicken small intestine \( \alpha \)-subunit of the Na\(^{+}\)K\(^{-}\)-ATPase gene was isolated and used as a probe, and then expression was compared with the Na\(^{+}\)K\(^{-}\)-ATPase activity in chicks fed diets with different Na concentrations.

**MATERIALS AND METHODS**

**Birds and Diets**

Male chicks (Ross \( \times \) Ross) were obtained from a commercial hatchery on the day of hatch and were maintained under standardized temperature and humidity conditions. Chicks were blocked to three dietary treatments (three replicates of 10 chicks/treatment), equalizing BW variance. Chicks received a starter feed meeting or exceeding NRC (1994) requirements (control) or a diet formulated similarly but without addition of NaCl (LS) or with 0.55% added NaCl (HS) (Table 1). The diets contained 0.05, 0.14, and 0.32% Na, respectively. Chicks had access to water and food ad libitum. At 13 d of age, chicks fed diets with different Na concentrations.

**Sampling and Collection of Tissues**

The intestines were removed immediately and weighed (\( n = 9 \)). The jejunum was separated and opened longitudinally, intestinal digesta were removed, and the tissue was washed with a solution of 50 mM tris HCl, 50 mM mannitol, and 10 mM choline chloride, pH 7.2. The mucosal layer was gently scraped with a slide, frozen in liquid nitrogen, and stored at \(-80^\circ C\) until analysis.

**Measurement of Na\(^{+}\)K\(^{-}\)-ATPase Activity**

Na\(^{+}\)K\(^{-}\)-ATPase kinetic measurements were conducted as described by Del Castillo and Robinson (1985), and phosphate was measured by spectrophotometry at 690 nm using Sigma Diagnostics Kit (catalog no. 670).\(^2\) Na\(^{+}\)K\(^{-}\)-ATPase activity was expressed as nanomoles of Pi per gram of protein per minute and was determined as the difference between total ATPase activity and ouabain-insensitive ATPase activity.

**Chemical Analyses**

Protein concentration was measured with a protein assay kit (6 mg/mL). Measurement of Na in the plasma was by flame photometry.

**RNA Preparation**

Total RNA was isolated from the jejunal tissues using TRI reagent (1 mL/100 mg tissue) according to the manufacturer’s protocol.\(^4\)

**Isolation and Sequence of Fragment from Chicken Na\(^{+}\)K\(^{-}\)-ATPase Gene**

A comparison of five different published sequences of the Na\(^{+}\)K\(^{-}\)-ATPase gene from different sources [chicken kidney (GeneEMBL chkatpas, chknapat), rat liver and brain (GeneEMBL ratnalpH1, ratnakat), human placenta (GeneBank/EMBL hspat4), pig kidney (GeneEMBL pigatpas), and dog kidney (GeneBank/EMBL dogntaka)] enabled us to identify common regions. Two primers, (forward: 5′-GACCCTTCCTCCTGCGTCGTTGCGGCG-3′, and reverse: 5′-CCACCAGGTTGGCTTGGAGGCGG-3′) were chosen from conserved regions of the \( \alpha \)-subunit and were subjected, with jejunal RNA, to a reverse transcription-PCR reaction (2 min at 94°C, 30 s at 60°C for primer annealing, 1 min at 68°C for 30 cycles followed by 7 min at 68°C) using Promega Access RT-PCR System.\(^5\) The reverse transcription-PCR products were examined on a 1.5% agarose gel, visualized by staining with ethidium bromide, excised from the gel, and purified with a gel extraction column using Wizard PCR Prep DNA purification system.\(^5\)

The chicken Na\(^{+}\)K\(^{-}\)-ATPase cDNA fragment was subjected to automated sequencing using an Applied Biosystem 373A DNA sequencer.\(^6\) Nucleic acid sequences were analyzed using the GCG suite of programs (Devereux et al., 1984) on a VAX 4000-300 computer. The homology between chicken and other Na\(^{+}\)K\(^{-}\)-ATPase sequences was calculated using DNAMAN.\(^7\)

**Northern Blot**

For Northern blot analysis, 30 µg of total RNA from the jejunum was denatured and separated by electropho-

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\(^2\)Sigma Chemical Co., St. Louis, MO.

\(^3\)BioRad, Hercules, CA.

\(^4\)Tri-Reagent Molecular Research Center, Inc., Cincinnati, OH.

\(^5\)Promega Corp., Madison, WI.

\(^6\)Applied Biosystems, Foster City, CA.

\(^7\)DNAMAN, 1997, Version 4, Lynnon Biosoft, Quebec, Canada.
described by Garay and Garrahan (1973): $V = V_{\text{max}}/(1 + K_{\text{Na}}/[\text{Na}])^n$, where $K_{\text{Na}}$ is the apparent affinity for Na+, and [Na] is the Na concentration.

## RESULTS

### Isolation of a Na<sup>+</sup>-K<sup>+</sup>-ATPase Gene Fragment

Using jejunal mRNA an 1,140-bp RT-PCR product was isolated and sequenced, and the predicted amino acid sequence exhibited a translation product of 380 amino acids. Homology of 95 to 98% was found between the chicken and the mammalian α-subunit of the Na<sup>+</sup>-K<sup>+</sup>-ATPase genes (Figure 1). The use of this cDNA fragment as a probe revealed a transcript of approximately 3.5 kb in the jejunal tissues (Figure 2). The nucleotide sequence data appear in GMLG GenBank and DDBJ Nucleotide Database under the accession number a9293621.

### Effect of Dietary Na Levels on Expression and Activity of Na<sup>+</sup>-K<sup>+</sup>-ATPase

Body weight, intestinal weight, feed intake, and Na<sup>+</sup> in plasma of the chicks fed LS were significantly lower than at 14 d than the control and HS chicks (Table 2). Feed efficiency was lowest in the LS birds, slightly higher in HS birds, and highest in the control chicks (Table 2). Plasma Na concentrations were lower in the LS birds compared with the control and HS chicks (Table 2). Expressions of mRNA of Na<sup>+</sup>-K<sup>+</sup>-ATPase and β-actin were determined by Northern blot analysis, and the ratio was calculated (Figure 3 and Table 3). This result indicated that expression of Na<sup>+</sup>-K<sup>+</sup>-ATPase mRNA was enhanced in chicks fed the LS diet as compared to control and HS chicks ($P < 0.05$). In contrast, determination of the activity of the Na<sup>+</sup>-K<sup>+</sup>-ATPase indicated that this was depressed in chicks fed the LS diet as compared to control and HS chicks. Kinetic parameters of the enzymatic activity were determined, and the calculated apparent affinity of Na to the Na<sup>+</sup>-K<sup>+</sup>-ATPase indicated that this was depressed in chicks fed the LS diet as compared to control and HS chicks. Kinetic parameters of Na<sup>+</sup>-K<sup>+</sup>-ATPase activity were determined, and the calculated apparent affinity of Na to Na<sup>+</sup>-K<sup>+</sup>-ATPase ($K_m$) was lowest in control chicks (0.71 mmol/L), higher in LS chicks (2.61 mmol/L), and highest in HS chicks (4.63 mmol/L). Values of the $V_{\text{max}}$ were not different in the different treatments (Table 3).

## DISCUSSION

In this study, we examined the chicken intestinal Na<sup>+</sup>-K<sup>+</sup>-ATPase expression and activity as a means of evaluating processes influencing intestinal Na-dependent absorption. This was done by isolating and sequencing an 1,140-bp portion of the α-subunit of the mRNA of Na<sup>+</sup>-K<sup>+</sup>-ATPase. This fragment was then used as a probe for measurement of the mRNA expression of Na<sup>+</sup>-K<sup>+</sup>-ATPase in jejunal tissues from chicks fed with differing dietary Na concentrations, and parallel activity of the enzyme was determined. Dietary Na status influenced performance of the chicks and mRNA expression and parameters of Na<sup>+</sup>-K<sup>+</sup>-ATPase activity in the jejunum.
Isolation of a fragment of the chick intestinal Na"-K"-ATPase amino acid sequence revealed that this was of high homology (95 to 98%) with that of human placenta (Kawakami et al., 1986; Chehab et al., 1987), pig kidney (Monastyrskaya et al., 1987), dog kidney (Xie et al., 1994), rat liver and brain (Herrera et al., 1987), and chicken...
kidney and liver (Takeyasu et al., 1988, 1990). The Na\(^{+}\)-K\(^{+}\)-ATPase protein fraction isolated in this study included the location of the predicted membrane spanning domains including five putative membrane-spanning domains (5 to 9) (Blanco and Mercer, 1988). The size of the chicken intestinal Na\(^{+}\)-K\(^{+}\)-ATPase gene (∼3.5 kb) was similar to the rat small intestine Na\(^{+}\)-K\(^{+}\)-ATPase (Barada et al., 1994).

Some kinetic parameters of the small intestinal Na\(^{+}\)-K\(^{+}\)-ATPase were determined in the chicks fed diets with different Na concentrations. The apparent affinity of Na\(^{+}\)-K\(^{+}\)-ATPase for Na\(^{+}\) ranged from 0.71 to 4.74 mmol/L in the chick jejunum. These values are within the ranges previously reported for rat intestine and kidney (1.46 ± 0.16 mM and 1.02 ± 0.09 mM) (Therien and Blostein, 2000). The affinity, however, was altered by the dietary Na concentrations, whereas maximal activity of the ATPase was similar among treatments and averaged 0.012 mmol per gram of protein per minute.

Expression of the Na\(^{+}\)-K\(^{+}\)-ATPase gene was upregulated by low dietary concentration of Na\(^{+}\) and was two-fold higher in LS chicks. Thus, dietary Na affected Na\(^{+}\)-K\(^{+}\)-ATPase expression, affinity, and maximal rate. The similar V\(_{\text{max}}\) values observed with different dietary Na status indicate the presence of similar numbers of active pumps on the enterocyte basolateral membrane. In contrast, mRNA for Na\(^{+}\)-K\(^{+}\)-ATPase α-subunit was expressed at higher levels in chicks fed low Na; however, this expression might not have been accompanied by increased transcription. Furthermore, changes in affinity also contributed to the decreased activity at low dietary Na concentrations. It should also be noted that because feed intake by the LS chicks decreased, it is not possible in this study to separate the effects of low dietary Na from those due to reduced feed intake. In chicks receiving high dietary Na the mRNA expression and V\(_{\text{max}}\) were similar to those of control chicks, although affinity was decreased in HS chicks. Chicks fed the HS diet increased Na intake by almost twofold, and despite the high dietary Na concentrations, growth and feed intake changed only slightly, although feed efficiency decreased. The greater Na absorbed by the HS chicks resulted in higher plasma Na

### Table 2. Performance at 13 d and plasma Na in chicks fed different Na intakes

<table>
<thead>
<tr>
<th>[Na] feed, %</th>
<th>0.05</th>
<th>0.14</th>
<th>0.32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>139.3 ± 6.02(^b)</td>
<td>319.2 ± 14.53(^a)</td>
<td>296.8 ± 10.24(^a)</td>
</tr>
<tr>
<td>Intestinal weight, g</td>
<td>9.94 ± 0.47(^a)</td>
<td>20.95 ± 0.97(^a)</td>
<td>20.43 ± 0.82(^a)</td>
</tr>
<tr>
<td>Intestine/body weight, %</td>
<td>7.12 ± 0.39(^a)</td>
<td>6.56 ± 0.37(^a)</td>
<td>6.8 ± 0.32(^a)</td>
</tr>
<tr>
<td>Feed intake, g</td>
<td>198 ± 8(^b)</td>
<td>345 ± 18(^a)</td>
<td>339 ± 23(^a)</td>
</tr>
<tr>
<td>Feed efficiency, %</td>
<td>47.9 ± 1.1(^b)</td>
<td>72.1 ± 1.2(^a)</td>
<td>67.5 ± 1.2(^a)</td>
</tr>
<tr>
<td>[Na] plasma, mmol</td>
<td>139.3 ± 6.02(^b)</td>
<td>153 ± 5(^a)</td>
<td>162 ± 4</td>
</tr>
</tbody>
</table>

\(^a\)^Values within rows not followed by the same superscript differ significantly (P < 0.05).
concentration but was also probably accompanied by greater Na excretion by the kidney, which is an energy-dependent process possibly contributing to the lower feed efficiency. High dietary Na did not change the mRNA expression of the α-subunit or the number of pump units. Thus, increased Na⁺ consumption influenced the intestinal kinetic behavior possibly reducing absorption by decreasing the affinity of the Na⁺-K⁺-ATPase.

In a previous study of expression and activity of the chick intestinal Na-glucose transporter, we reported that nutritional status can alter expression and affinity of the transporter (Gal-Garber et al., 2000). These may be complementary processes regulating intestinal uptake. Other possible regulatory pathways acting when Na consumption was increased may be due to a short-term increase in enterocyte cytoplasmatic Na concentration, which is the first signal leading to enhanced activity of the surface Na pump in one kind of aldosterone-mediated short-term regulation (Bertorello and Katz, 1993). Hormones, such as corticosteroids, in particular aldosterone, have a significant role in long-term adaptation to Na intake changes (Therien and Blostein, 2000) and probably play an important role in Na regulation in the HS birds. It has been reported that long- and short-term regulation mechanisms generally affect de novo Na⁺-K⁺-ATPase synthesis and degradation, kinetic behavior of the enzyme, and translocation of Na pumps between the plasma membrane and the intracellular stores in the kidney (Therien and Blostein, 2000). Thus the expression, turnover, and affinity of the Na⁺-K⁺ pump in different tissues including the colon are influenced by hormones, including aldosterone, thyroid hormones, and insulin (Fuller and Verity, 1990; Ewart and Klip, 1995; Therien and Blostein, 2000). Thus in this study it is possible that central signals induced reduction in feed intake and contributed to feedback influencing expression and turnover of the mRNA of the pump in the small intestine.

In summary, dietary Na influenced intestinal transport and uptake altering expression of the basolateral mRNA of Na⁺-K⁺-ATPase and its affinity for Na. Na⁺-K⁺-ATPase activity decreased in parallel to growth in chicks fed diets with low Na concentrations. This study, thus confirms that determination of the activity of the basolateral Na⁺-K⁺-ATPase is a useful measure of intestinal Na-dependent transport, and, together with determination of expression of the mRNA of Na⁺-K⁺-ATPase can provide clearer understanding of changes occurring in intestinal transport due to dietary perturbations.


SAAMI. 1997. SAAMII, a Program for Kinetic Analysis. Version 1.1, Seattle, WA.


