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Absolute Quantification of Tissue Specific Expression of Glucose Transporters in Chickens

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Abstract

The avian glucose transporter (GLUT) family of proteins displays distinct functional patterns compared to mammalian GLUTs. How avian GLUT family members are distributed in different tissues, and how GLUT members are regulated are not well understood. In order to provide data for better understanding of these questions, we determined mRNA expression patterns of GLUTs 1, 2, 3, 6, 8, 9, 10, 12 and HMIT/GLUT13 in chickens using the absolute quantification method of RT-qPCR. Results showed that the most abundant GLUT mRNA was GLUT1 in heart, GLUT2 in liver, small intestine and kidney, and GLUT8 in adipose tissue and pancreas. GLUT9 had the second highest expression in liver, intestine and pancreas, with a lower expression level in kidney. The liver expressed the highest level of GLUT2 (9,740 copies/ng) of total RNA, about 3-5 times that of the highest GLUT mRNA in other tissues. We also examined GLUT1 and GLUT8 expression in response to dietary manipulation and developmental regulation in adipose tissue, an essential tissue for energy balance. Results indicate that GLUT1 was significantly affected by age and diet. GLUT8 was modulated by dietary manipulation, but not age.

Keywords: Avian; Chicken; GLUT; mRNA expression; Adipose; Heart; Intestine; Kidney; Tissue; Absolute quantification; Gene regulation

Abbreviations

ANOVA: Analysis Of Variance; GLUT: Glucose Transporter; HCD: High Caloric Diet; HMIT: H(+)-myo-inositol Transporter; HPD: High Protein Diet; LSD: Least Square Differences; ME: Metabolizable Energy; RSD: Recommended Standard Diet; RT-qPCR: Reverse Transcription Quantitative PCR; SE: Standard Error; SLC: Solute Carrier; WOA: Weeks of Age

Introduction

Glucose transporters (GLUTs) belong to a family of proteins encoded by the *SLC2A* gene family. GLUTs are integral membrane proteins containing about 500 amino acid residues with 12 transmembrane domains. Members of the GLUT protein family mainly facilitate passive transportation of hexose across the cell membrane. Due to the essential role of hexose in energy metabolism and cellular anabolism [1-3], GLUTs have been widely studied [4-15]. In humans, the GLUT family is composed of 14 members, including GLUTs 1-12, HMIT/GLUT13 [16,17] and GLUT14, a duplicon of GLUT3 [18]. All GLUTs are capable of hexose transport under experimental conditions [18]. Physiologically, each GLUT member may have specificity for transporting fructose, urate, ascorbate or myo-inositol [19-22].

In mammals, the expression of GLUTs displays tissue or cell type specificity. GLUT1 is expressed in many cell types, and its principal physiological function is glucose transport, with the ability to transport mannose and galactose. High levels of GLUT1 expression are found in erythrocytes and brain. GLUT2 is the major glucose transporter in hepatocytes and is also expressed in intestine absorptive cells, kidney convoluted tubules and pancreatic β cells. While GLUT3 has been shown to be the primary glucose transporter for neurons [23], GLUT4 is most prominent in skeletal muscle, adipocytes and cardiomyocytes. Also, GLUT4 is the primary transporter for insulin-stimulated glucose uptake, whereas GLUT5 has a high specificity for fructose. The primary role of GLUT5 is to facilitate the absorption of fructose from the lumen of the small intestine. Expression of GLUT5 is also found in fat, muscle, kidney and brain [24]. Recently elucidated GLUT5 structure indicated that it has a global rocker-switch-like transporting mechanism [8]. Less is known about GLUT6 in

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Table 1: Composition of Diets %.

Dietary Treatments	RSD	HCD	HCD	HPD
Experiment	1, 2	1	2	2
Feed Ingredients				
Corn, yellow # 2 (8% CP)	57.75	44.13	44.13	44
Soybean meal (48% CP)	34	40.5	40.2	40
Alfalfa meal (17% CP)	1	1	1	1
Poultry blended fat (8158 kcal ME/kg)	3.4	10.6	10.6	3.4
Sucrose	0	0	1.22	8.75
Dicalcium Phosphate (18% P, 22% Ca)	2.05	1.9	1	1
Limestone Flour (38% Ca)	1	1.05	1.05	1.05
Salt	0.42	0.42	0.42	0.42
Vitamin-mineral Pre-mix[1]	0.25	0.25	0.25	0.25
D, L-Methionine (98%)[2]	0.13	0.15	0.13	0.13
Calculated Analyses				
Metabolizable Energy, kcal/kg	3,054	3,343	3,382	3,078
Crude Protein, %	21.11	23.14	23	23.02
Calcium, %	0.96	0.96		
Total Phosphorus, %	0.75	0.73		
Available Phosphorus, %	0.51	0.49		
Methionine, %	0.45	0.49		
Methionine + Cystine, %	0.8	0.85		
Lysine, %	1.15	1.31		
Crude fat, %	5.62	11.65	13.16	5.95

[1]Provided per kg of diet: retinyl acetate, 3,500 IU; cholecalciferol, 1,000 ICU; DL-α-tocopheryl acetate, 4.5 IU; menadione sodium bisulfite complex, 2.8mg; vitamin B12, 5.0mg; riboflavin, 2.5mg; pantothenic acid, 4.0mg; niacin, 15mg; choline, 172mg; folic acid, 230mg; ethoxyquin, 56.7mg; manganese, 65mg; iodine, 1mg; iron, 54.8mg; copper, 6mg; zinc, 55mg; selenium, 0.3mg.
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mammals. Known cells that express GLUT6 include granulosa cells and endometrial cancer cells [25,26]. In previous reports, knockdown of GLUT6 expression significantly reduced glucose uptake, suggesting that GLUT6 transports glucose [26]. GLUT7 expression has been demonstrated in small intestine and granulosa cells [25,27], and its glucose and fructose transport activity was shown using the *Xenopus* oocyte system. Much attention has been paid to GLUT8. Expression of GLUT8 has been found in many tissues and cell types, including testis, cerebellum, liver, brown adipose tissue, spleen and lung. Early studies suggested that GLUT8 is localized only in an intracellular compartment (see review [17]). Recently, surface-localization of GLUT8, and its clear role in hepatic fructose transport, have been demonstrated [28]. GLUT8 also mediates intestinal ascorbate uptake [29]. GLUT9 is expressed in the liver, kidney, intestine and leukocytes, and its primary role is urate transport, with no fructose transport activity [22,30]. GLUT10 was reportedly most abundant in smooth muscle-rich tissues, such as arteries [31]. Its expression was also found in other tissues. The role of GLUT10 in hexose transport is questionable, but evidence suggests that it is an intracellular ascorbic acid transporter [32]. GLUT11 has been detected in heart and skeletal muscle [33]. GLUT12 is insulin independent and membrane-bound [34]. When ectopically expressed in frog oocytes, human GLUT12 displayed an ability to facilitate glucose transport [35]. HMIT/GLUT13 is primarily expressed in the human and rat brain, including neurons and astrocytes. Contradictory reports exist regarding its role as an H(+)-myo-inositol co-transporter [36,37]. Overall, it can be seen that expression of mammalian GLUT family members displays clear tissue and cell type specificity and substrate selectivity.

The avian GLUT family displays distinct functional organizations. Early studies identified and characterized several GLUTs in chickens [6,38-42] and a few other avians [43,44], determined the expression patterns, distribution and some functional characteristics of avian GLUT1-3 and alluded to the possibility that chickens lack GLUT4 expression. Further analysis of the genome and mRNA expression confirmed that the chicken lacks the GLUT4 ortholog, which is responsible for the reduced responsiveness of serum glucose levels to insulin stimulation in chickens [40,45], though continuous infusion of insulin could result in hypoglycemia [7]. Characterization of other GLUTs, such as GLUT5 [41], GLUT8 [1] and GLUT12 [46,47], was also reported by several groups. Several studies have examined the regulation of GLUT expression under insulin stimulation [48], dietary manipulation [49] and genetic selection [50]. Most of these studies were focused on a few glucose transporters, especially GLUT1, 2, 3 and 5 [51] under various nutritional and physiological conditions [52-54]. Few studies examined other GLUTs in chicken and other avian species, such as pigeons and ducks [55,56]. Phylogenetic analysis of avian GLUT family members also revealed that chickens lack GLUT7, and preserved several GLUT members that were lost in the mammalian lineage [57].

Despite these studies on avian glucose transporters, much remains to be learned. The expression of many avian GLUT members has not been examined. No studies have examined avian GLUT transcripts to determine their abundance relative to each other. In addition, the regulation of avian GLUT members is not well understood. To provide information for better understanding avian GLUT members and their expression regulation, we studied the tissue distribution of chicken GLUTs 1, 2, 3, 6, 8, 9, 10, 12 and HMIT/GLUT13 using the absolute quantification method of RT-qPCR. We also examined the expression of GLUT1 and 8 mRNA in adipose tissue in response to dietary caloric manipulation and developmental regulation. Here we report this study.

Materials and Methods

Animals and dietary treatment

The use and care of animals were approved by Tennessee State University's Animal Care and Use Committee. The design and management of experimental birds for experiment 1 [from hatch to 8 weeks of age (WOA)] were described in our previous publication [58]. For clarity, the dietary treatment is briefly described here. For the 2, 4, 6 and 8 WOA tests, newly-hatched broiler chickens (113 males and 113 females, commercial generation) were purchased from Ideal Poultry (Texas, USA) and reared at Tennessee State University in the Frank A. Young Poultry Research Farm. Birds of each sex were randomly assigned to two dietary treatment groups, recommended standard diet (RSD) or high caloric diet (HCD). There were six replicates for each treatment of each sex. The diets were corn and soy based (Table 1). RSD contained 21% crude protein, 5.6% crude fat and 3,054 kcal/kg of metabolizable energy (ME), formulated per National Research Council recommendations for broiler chickens. HCD contained 23% crude protein, 11.65% crude fat and 3,343kcal/kg of ME. HCD was formulated based on experimental rations designed to induce obesity in growing broiler chickens. Five males were used for GLUT mRNA expression analysis for each age and treatment.

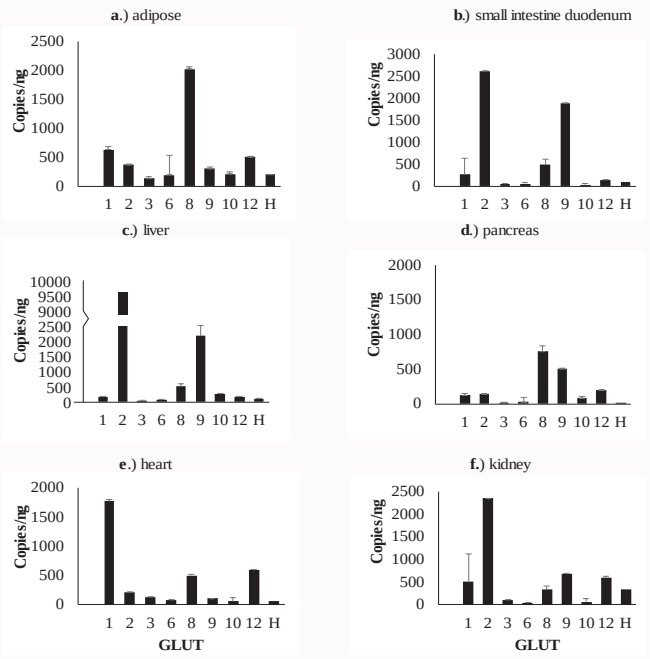

Figure 1: Levels of GLUT mRNA expression in RSD, male chicken tissues at 4 WOA. H = HMIT/GLUT13. Each sample was measured in duplicate (mean ± SE, n = 4).

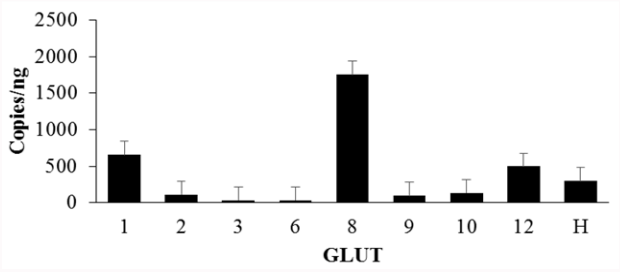

Figure 2: Levels of GLUT mRNA expression in chicken adipose tissue at 8 WOA (mean ± SE, n = 4, duplicated determination).

A follow up study (experiment 2) was done to gauge the effect of high caloric versus high protein level on GLUT expression at 4 WOA. A tissue panel characterization of GLUT expression patterns was generated using all male chickens at 4 WOA from RSD group. For these studies, newly-hatched broiler chickens (15 males and 15 females, commercial generation) were donated courtesy of Aviagen (Alabama, USA) and reared at Tennessee State University in the Frank A. Young Poultry Research Farm. Birds of each sex were assigned at random to three dietary treatment groups, RSD, HCD, or high protein diet (HPD). Each treatment group included five males and five females. Diets were corn, soy and sugar based (Table 1). RSD contained 21% crude protein, 5.6% crude fat and 3,054kcal/kg ME. HCD contained 23% crude protein, 13.16% crude fat, 1.2% sucrose and 3,382 ME. HPD contained 23% crude protein, 5.95% crude fat, 8.75% sucrose and 3,078 ME.

All experimental broilers were wing-banded and randomly assigned to broiler batteries with heat lamps for warmth. Chickens were fed rationed diets and were weighed weekly until date of sacrifice. Feed and water were provided at free choice. For the first study where chickens at 2, 4, 6 and 8 WOA were tested, one chicken from each replicate was sacrificed at the indicated ages. All remaining birds were sacrificed at 8 WOA. Samples of abdominal fat pad and around the gizzard were collected, weighed and placed in liquid nitrogen for direct freeze, then transferred to -80°C until RNA isolation. For 4 WOA tests, all birds were sacrificed at 4 WOA. Samples of abdominal fat pad, small intestinal (duodenum), liver, pancreas, heart and kidney were collected, and samples were placed in liquid nitrogen for direct freeze and then transferred to -80°C until RNA isolation.

RNA isolation and RT-qPCR

Abdominal adipose RNA was extracted with RNeasy Lipid Tissue Midi kit (Qiagen). RNA from duodenum, liver, pancreas and kidney was extracted with RNeasy Mini kit (Qiagen). For tissues rich in RNases, 10µl β-mercaptoethanol was added to 1ml Buffer RLT to prevent RNA degradation from occurring. Heart RNA was extracted with RNeasy Fibrous Tissue Midi kit (Qiagen). All protocols were strictly followed. RNA concentrations were measured with NanoDrop spectrophotometer (Thermofisher). Samples from 8 WOA were analyzed with Experion RNA StdSens analysis kit. PCR primers were designed using Primer Express 2.0 (Applied Biosystems). Table 2 is a list of primers used for RT-qPCR analysis of gene expression in this study. All RT-qPCR tests were conducted using Quantitect SYBR Green RT-PCR kit (Qiagen). Reaction was done in 20µl containing 25ng total RNA and 0.4µM of each primer. Thermal cycles contained one cycle of preincubation at 50°C for 10min and 95°C for 15min, 45 cycles of amplification (95°C for 15s and 60°C for 60s). Primers were validated by melting curve analysis, standard curve and no template control reactions.

Preparation of reference standards

For determination of absolute copy number, reference standards were generated with the same primer sets from Table 2. RT-PCR products for GLUTs 2, 3, 6, 9, 10, 12 and HMIT/GLUT13 (H) were separated with agarose gel, recovered from the gel, then measured with NanoDrop and serial diluted with carrier DNA from salmon testes to maintain a constant nucleic acid concentration (25ng/µl) during the copy number standard dilution. Templates for GLUT1 and GLUT8 were insufficient from gel recovery of RT-qPCR product, so GLUT1 and GLUT8 PCR products were cloned. GLUT1 and GLUT8 copy number standards were plasmids with GLUT1 and GLUT8 inserts. Plasmids were diluted the same way as purified PCR products for GLUT standards. The copy number standards were used as template during RT-qPCR running in parallel with unknown samples. For all standards, including constructs, the copy numbers were calculated using the formula for molecular weight of double stranded DNA.

PCR Cloning, Ligation and Transformation

RT-PCR products of GLUT1 and GLUT8, amplified with primers from Table 2, were cloned using StrataClone Ultra Blunt PCR Cloning kit (Agilent Technologies). Reaction was done in 25µl containing 0.5µl template DNA, 0.5µl of each primer and 10µl dNTP. Thermal cycles contained 1 cycle preincubation at 95°C for 2min, 30 cycles of amplification (95°C for 20s, 58°C for 20s and 72°C for 15s) and 1 cycle at 72°C for 3min, then held at 4°C. Amplification of DNA and RT-qPCR results were confirmed using agarose gel electrophoresis. The PCR products were ligated to blunt vector arms using StrataClone Ultra Blunt PCR Cloning kit (Agilent Technologies). Briefly, 3µl StrataClone Blunt Cloning Buffer, 2µl PCR product and 1µl StrataClone Blunt Vector Mix were mixed, then incubated at room temperature for 5min. An aliquot (1µl) of the cloning reaction mixture was added to a thawed tube of competent cells and gently mixed by pipetting. Competent cells were then incubated on ice

for 20min, heat-shocked at 42°C for 45s, then incubated on ice for 2min. Transformed competent cells were allowed to recover for 1h at 37°C with gentle agitation after adding 250µl of pre-warmed LB broth. Recovered cells were then spread onto agar plates containing ampicillin together with 40µl 2% X-gal for blue-white color screening. Plates were incubated overnight at 37°C. White colonies were chosen for plasmid DNA preparation. Colonies used for copy number reference were sequenced, and concentrations were measured with NanoDrop.

Data analysis

ANOVA and Fisher LSD post hoc tests were used to determine statistical significance for RT-qPCR data. Bars labeled without a common script were significantly different from one another. *T*-test was used to compare mean bodyweight between groups.

Results

Tissue specific expression of GLUTs at 4 WOA

Since our knowledge about chicken tissue specificity of GLUTs is limited, we characterized the tissue specificity of GLUT expression at 4 WOA. Levels of GLUT mRNA were examined in tissue panels containing abdominal adipose tissue, duodenum, liver, pancreas, heart and kidney. The tissue panel was constructed on the same four male individuals from RSD group (n=4), with mean bodyweight 1,217 ± 67g. Each sample was measured in duplicate with RT-qPCR. To compare among mRNA levels of different GLUTs in the same tissue, we determined the absolute copy number per nanogram of total RNA for GLUTs 1, 2, 3, 6, 8, 9, 10, 12 and H.

Figure 1 depicts levels of GLUT mRNA expression in RSD male, 4 WOA chickens. GLUT8 had the highest mRNA expression, having 2,028copies/ng on average, followed by GLUT1, 12, respectively having 629 and 502 copies/ng. Other GLUTs examined, including GLUT2, 3, 6, 9, 10, H were expressed at lower levels, ranging from 129-368 copies/ng. In small intestinal duodenum, GLUT2 was the highest expressed GLUT at transcription level (2,618copies/ng), followed by GLUT9 (1,890copies/ng). GLUTs 8 and 1 had moderate expression at 479 and 267 copies/ng, respectively. GLUT3, 6, 10, 12, H expression levels were low (32-144 copies/ng) in the duodenum. In liver, GLUT2 also had the highest mRNA expression (9,740copies/ng), followed by GLUT9 (2,192copies/ng). GLUT8, 10 were expressed moderately at 510 and 257 copies/ng, respectively. GLUT1, 3, 6, 12, H were expressed at much lower levels (55-177 copies/ng). In pancreas, GLUT8 had the highest mRNA expression, averaging 759copies/ng, followed by GLUT9 and 12 with 508 and 198 copies/ng respectively. GLUT1 and 2 had moderate expression levels with respectively 123 and 142 copies/ng. GLUT3, 6, 10, H were only minimally expressed in pancreas (ranging from 11-85 copies/ng). In the heart, all GLUTs tested showed some mRNA expression. GLUT1 had the highest mRNA expression in heart with 1,770copies/ng, followed by GLUT12, 8 (with 595 and 502 copies/ng, respectively). GLUTs 2, 3 and 9 were moderately expressed at 209, 135 and 106 copies/ng, respectively. GLUT6, 10, H were the lowest expressed, ranging from 56-72 copies/ng, respectively. In kidney, GLUT2 had the highest mRNA expression, averaging 2,350copies/ng, followed by GLUT9 (691copies/ng), GLUT12 (591copies/ng) and GLUT1 (514copies/ng). GLUT8, H had moderate expression levels, with respectively 328 and 333 copies/ng. GLUT3, 6, 10 were least expressed in kidney, ranging from 41-106 copies/ng.

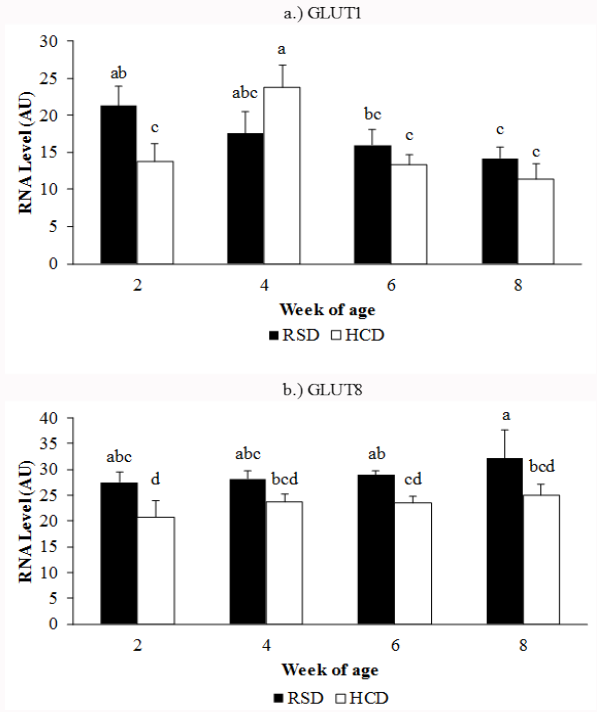

Figure 3: Levels of mRNA expression in adipose tissue during early development (mean + SE, n = 5). ANOVA and Fisher LSD post hoc tests were used to determine statistical significance (duplicated determination). Bars labeled without a common script were significantly different from one another. (a) GLUT1 displayed a significant difference in age ($p < 0.008$) and age: diet interaction ($p < 0.03$). RSD had higher expression levels than HCD in GLUT1 at 2, 6 and 8 WOA, with a significant difference at 2 WOA ($p < 0.02$). (b) GLUT8 exhibited a significant difference between dietary groups only ($p < 0.001$), not in age or age: diet interactions. The RSD groups in GLUT8 displayed significantly higher mRNA expression across all age groups except at 4 WOA. Expression of mRNA at 4 WOA was higher among RSD group; however, the differences were not statistically significant.

GLUT mRNA expression in adipose tissue at 8 WOA

GLUT mRNA expression was also evaluated in chicken adipose tissue at 8 WOA (Figure 2). To compare mRNA expression, we determined the absolute copy number of total RNA for GLUTs 1, 2, 3, 6, 8, 9, 10, 12 and HMIT/GLUT13 (H). GLUT8 was highest expressed (1,756copies/ng), followed by GLUT1 (656copies/ng).

Regulation of GLUT1 and GLUT8 by dietary factors at 2, 4, 6 and 8 WOA

Adipose tissue expressed the highest level of GLUT8, followed by GLUT1. Figure 3 shows the effect of RSD versus HCD on GLUT1 and GLUT8 mRNA expression levels across 2, 4, 6 and 8 WOA in adipose tissue. Expression of GLUT1 and GLUT8 were examined with RT-qPCR. In chicken adipose tissue, GLUT1 mRNA expression displayed a significant difference in age ($p < 0.008$) and age: diet interaction ($p < 0.03$). RSD exhibited higher mRNA expression than HCD in GLUT1 at 2, 6 and 8 WOA, with a significant difference at 2 WOA ($p < 0.02$, Figure 3a). GLUT8 displayed a significant difference among dietary groups ($p < 0.001$), but was not affected by age or age: diet interaction. The RSD groups displayed significantly higher GLUT8 mRNA expression than HCD across all ages except at 4 WOA, where the trend was still clearly the same (Figure 3b).

Levels of mRNA expression by treatment in chicken adipose tissue at 4 WOA

Since the HCD group from the study above contained higher levels

of crude protein and fat, it was not clear whether the downregulation of GLUT8 in adipose tissue was caused by high protein level or by high fat content. Therefore, we conducted a second experiment to examine whether the downregulation was due to high protein level or high caloric level in the diet. Chickens for this study were raised to 4 WOA on RSD, HPD or HCD. RSD was the same as our previous experiment and consisted of a higher concentration of carbohydrates from corn. HPD consisted of a higher protein content with the same level of carbohydrates. HCD contained the same level of protein as HPD, with higher fat and lower carbohydrate content. Male and female chickens were selected from each of the three groups, RSD, HPD and HCD, with bodyweights in the median range. Mean RSD bodyweight was 1,175g (n=6). Mean HPD bodyweight was 1,359g (n=6). Mean HCD bodyweight was 1,355g (n=7). *T*-tests showed there were no significant differences between bodyweights among the dietary treatment groups (RSD: HPD, $p = 0.08$; RSD: HCD, $p = 0.18$ and HPD: HCD, $p = 0.97$).

Figure 4 depicts statistical analysis of GLUT8 mRNA expression levels across RSD, HPD and HCD dietary treatment groups at 4 WOA. Compared with RSD group, the HPD group expressed the same level of GLUT8 mRNA, while the HCD group expressed a significantly lower level of GLUT8 mRNA ($p < 0.02$), suggesting that fat repressed GLUT8 mRNA expression.

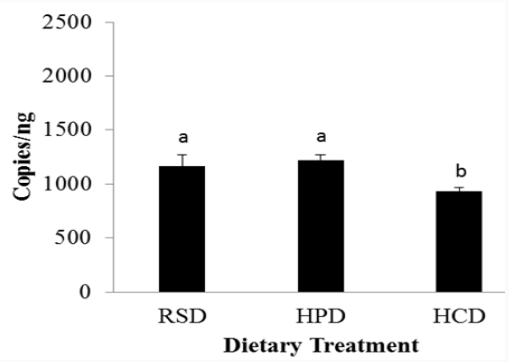

Figure 4: Statistical analysis of GLUT8 mRNA expression across RSD, HPD and HCD at 4 WOA (RSD and HPD, n = 6. HCD, n = 7). ANOVA and Fisher LSD post hoc tests were used to examine statistical significance (duplicated determination). Lower case scripts mark significant difference in mRNA levels (mean ± SE) grouped by diet, where bars without a common script were significantly different from one another. HCD significantly lowered GLUT8 mRNA expression versus RSD and HPD ($p < 0.02$).

Discussion

Absolute transcript abundance of chicken glucose transporters

Analysis of mRNA levels is often done using the relative quantification RT-qPCR method. The relative quantification method permits comparisons of mRNA levels of the same gene across different conditions or treatments. However, such method could not determine the absolute expression levels of different GLUTs. Thus, the relative quantification method is not appropriate for comparison of mRNA levels of two different genes. Since most of the GLUTs are expressed in any given tissue, it would be of interest to compare the abundance across different GLUTs, which could provide insight into the functional significance of these GLUTs in each tissue. The absolute quantification method employed in our study allowed us to examine and compare the mRNA expressions of various GLUTs in absolute values. The profile of GLUT expression revealed in our study was different from the report by Kono et al. [1], who used the relative method, and the expression was normalized to GAPDH. Kono et al. reported high GLUT1 expression in adipose tissue, but in our study, adipose GLUT1 mRNA level was rather low compared with adipose GLUT8 and heart GLUT1 mRNA level.

The liver is a metabolic organ playing a central role in energy metabolism, glucose homeostasis and fatty acid synthesis. These processes require high glucose transporting capacity. The mRNA expression of glucose transporters is well aligned with these roles of the liver. The liver expressed far higher levels of GLUTs than any other organ we have examined. GLUT2 mRNA is most abundant in chicken liver, 3-5 times higher than the most abundant GLUTs in any other tissues. The second highest expressed GLUT in the liver was GLUT9, which was expressed at a level comparable to the most abundant GLUT members in other tissues. In chicken small intestine, glucose is absorbed from the diet and then released to the blood stream. We found that the most abundant glucose transporter mRNA was GLUT2 in chicken small intestine. This is similar to findings in mammals, where GLUT2 and GLUT5 are most abundant in the small intestine [17]. GLUT2 mediates the exit of monosaccharides from enterocytes lining the small intestine. All other GLUTs examined were expressed at much lower levels in the small intestine. Adipose tissue also synthesizes fatty acid, but at a much lower rate. The level of glucose transporter mRNA expression seems to be consistent with this notion. In our study, we found that in adipose tissue GLUT8 mRNA was most abundant. All other GLUTs were expressed at much lower levels in the adipose tissue. In general, chicken pancreas expressed low levels of GLUT mRNA.

GLUT9 mRNA levels were high in liver and intestine. The physiological function of GLUT9 is well established as a urate transporter. A much lower level of GLUT9 mRNA was found in

Table 2: Primers Used for RT-qPCR Analysis of Gene Expression.

Accession Number	GLUT	Forward	Reverse	Amplicon Size (bp)
NM_205209.1	1	CCATCCTCATCGCAATCGT	GTGGAGTAGTAGAAAACCGCATTGA	72
NM_207178.1	2	GAAGGTGGAGGAGGCCAAA	TTTCATCGGGTCAVAGTTTCC	60
NM_205511.1	3	TGTTGCTGCCATTGGATCTC	CTGGATGATCTTCTCAGGAGCAT	72
XM_004945945	6	ACCTGCAGAGCCTCAACAACA	GGCAAAGCCAAAGCTGAAGT	79
AB083371	8	GACCATACGTGGACCATGAATG	GCTTAGTCCCTCCTCCTCAACA	67
XM_004936166	9	GTGGCCTTGTGGGTGCTATT	ACAGTGTACATTTCCGTCCAAAGA	65
XM_417383.4	10	AACTGGGCGGCCAATTTAC	CGAGAAACCAATGGCATCAAT	65
XM_419733.3	12	AGCAGGCTGTGGAACATTTACTTT	CATAACCCATTAGCAGTCCACTTACA	70
XM_001232939	HMIT	TGGGCAGCTTAGCAGGTACTG	GGCGAGACCTGAGCTGACA	75

chicken kidney, which appears strange at first glance. However, there are several urate transporters including URAT1 and GLUT9. Other urate transporters may be expressed at higher levels in the chicken kidney.

The data obtained from this study clearly indicate that each tissue has one or more predominant GLUT paralogs. Other paralogs are often expressed, but at a much lower abundance. In the tissues we have examined, GLUT6, 10 and HMIT were always expressed at very low levels. It is not clear whether their proteins are also expressed at low levels. At present, the physiological functions of GLUT6 and 10 are not established. They may be expressed at high levels in specific cell types in these tissues. The RT-qPCR data were consistent with RNA-seq data for GLUT mRNA in general, where GLUT8 was the highest expressed, followed by GLUT1 and GLUT3 mRNA. Other GLUTs were in low abundance (our unpublished data).

Dietary regulation of chicken GLUT mRNA

In our first study, chickens were raised to 8 WOA with RSD or HCD. RSD contained more carbohydrates and lower fat content than HCD. RSD contained 5.62% crude fat, 21% crude protein with a higher percentage of carbohydrates. HCD contained 11.65% crude fat, 23% of crude protein with a lower concentration of carbohydrates. In this study, GLUT8 was affected by diet across all ages of growth. RSD group chickens displayed higher levels of GLUT8 mRNA expression. GLUT8 mRNA expression was significantly lowered across HCD treatment groups. Since the HCD also contained a higher level of crude protein and fat, it was not clear whether the repression of GLUT8 mRNA was caused by high protein level or by high fat content. Therefore, we conducted a second experiment, in which chickens were raised to 4 WOA with RSD, HPD or HCD. RSD was the same as our first experiment. HPD consisted of 23% crude protein, 8.75% sucrose from table sugar and 5.95% crude fat. HCD contained 13.16% crude fat and 1.22% sucrose. Results showed that GLUT8 mRNA level was not affected in the HPD chickens, when compared with that in RSD chickens. Expression of GLUT8 mRNA was significantly lowered in the HCD group ($P < 0.02$), confirming the results from our first study. It can be concluded that high fat diet repressed GLUT8 mRNA, or high carbohydrate diet induced GLUT8 mRNA. This observation is consistent with the notion that GLUT8 plays a role in monosaccharide transport in adipose tissue. However, the exact mechanism by which GLUT8 is regulated by dietary fat or carbohydrate requires further investigation.

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