Expression of the chicken peptide transporter 1 and the peroxisome proliferator-activated receptor α following feed restriction and subsequent refeeding

S. L. Madsen and E. A. Wong

Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg 24061

ABSTRACT The peptide transporter 1 (PepT1) transports di- and tripeptides from the lumen of the small intestine into the enterocyte. Expression of this transporter is affected by numerous factors, including feed restriction. During a fasting state, PepT1 is thought to be regulated by peroxisome proliferator-activated receptor α (PPARα). The objective of this study was to evaluate the effects of a feed restriction–refeeding regimen on expression of chicken PepT1 and PPARα. Ten-day-old broiler chicks were placed on a 24-h feed restriction with 6 birds sampled before and after the restriction. Following feed restriction, the remaining birds were divided into 3 groups: continuously fasted, refed–food withdrawn, and refed ad libitum. The duodenum, jejunum, and ileum were sampled 1, 2, 3, 5, and 7 h post feed restriction. Expression of PepT1 and PPARα increased almost 2-fold post feed restriction (P < 0.002). A significant group × time interaction was observed for PPARα, with the continuously fasted group showing a peak at 29 h postrestriction (P = 0.002). A group × segment interaction was found for both PepT1 (P = 0.002) and PPARα (P = 0.01); within the continuously fasted group, PepT1 expression was greatest in the jejunum (P < 0.001) and ileum (P = 0.01) when compared with the duodenum. No difference was observed between the jejunum and ileum. The PPARα expression was greatest in the jejunum (P = 0.03) when compared with the duodenum, with no difference between the jejunum and ileum or between the duodenum and ileum. The increase in PepT1 expression during a time of reduced feed intake suggests the importance of having transporters ready to scavenge any available luminal nutrients. The concurrent increase in PPARα suggests a possible regulatory role for this receptor in the regulation of PepT1 during feed restriction.

Key words: broiler, peptide transporter 1, peroxisome proliferator-activated receptor α, feed restriction, gene expression

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INTRODUCTION

Protein digestion in the chicken is achieved through the secretion of proteolytic enzymes from the proventriculus, duodenum, and pancreas and results in the formation of small peptides and free amino acids. Absorption of peptides and amino acids occurs in the small intestine and is mediated through transporter proteins, which are expressed in the apical membranes of enterocytes. Peptide transporter 1 (PepT1) is an H+-dependent peptide transporter and is a member of the proton-coupled oligopeptide transporter superfamily (Botka et al., 2000). Peptide transporter 1 is capable of transporting most di- and tripeptides (Daniel, 2004) as well as several peptidomimetic drugs such as the β-lactam antibiotics, angiotensin-converting enzyme inhibitors for treating hypertension, antivirals (e.g., valacyclovir), and anticancer agents (e.g., bestatin; Meredith and Price, 2006).

The distribution of PepT1 in tissues is quite broad across species. Peptide transporter 1 is widely expressed in the rat small intestine, with some expression seen in the kidney because of its role in reabsorbing proteins from the glomerular filtrate (Meredith and Boyd, 2000). In ruminants such as dairy cattle and sheep, PepT1 is also expressed in the omasum and the rumen (Chen et al., 1999). In avians, specifically the chicken, the distribution of PepT1 is greatest in the small intestine and lower in the kidney and ceca (Chen et al., 1999, 2002). Within the small intestine, the duodenum has been shown to have the highest expression of PepT1, followed by the jejunum and ileum, respectively (Chen et al., 1999, 2002; Gilbert et al., 2007). However, in chickens selected for high and low juvenile BW, PepT1 expression was highest in the ileum of low...
weight birds, which may be an adaptation to maximize nutrient absorption in times when nutrient availability is low (Mott et al., 2008).

The expression of PepT1 can be regulated by diet, development, hormones, pharmacological agents, and pathological states (Rubio-Aliaga and Daniel, 2008). Peptide transporter 1 expression in rats increases 2-fold in response to feeding a high protein diet (Erickson et al., 1995). Conversely, a decrease in expression was noted in rats fed a protein-free diet (Shiraga et al., 1999). In chickens, levels of dietary CP similarly affect PepT1 gene expression (Chen et al., 2005). Chickens fed a 12% CP diet decreased PepT1 mRNA, whereas chickens fed 18 or 24% CP diets increased PepT1 mRNA. Protein quality, or the combination of amino acids required for maximal growth and efficient protein synthesis, also has an effect on transporter expression. Gilbert et al. (2008) demonstrated the importance of protein quality by feeding a high quality protein source (soybean meal) and a lower quality protein source (corn gluten meal) to Aviagen Line A and B chicks (Aviagen, Huntsville, AL). In this study, PepT1 mRNA levels were greater in those chicks fed the soybean meal diet at a quantity restricted to that consumed by chicks fed the corn gluten meal.

In fasting rats, PepT1 expression increased along with a concomitant increase in the expression of the peroxisome proliferator-activated receptor α (PPARα), a transcription factor that plays a role in the adaptive response to fasting by activating the transcription of nutritionally important genes, such as the β-oxidation pathways in the mitochondria and peroxisomes (Shimakura et al., 2006). Activation of PPARα occurs when dietary fatty acids or synthetic ligands cross the cell membrane and bind to PPARα in the nuclear membrane, causing the receptor to heterodimerize with the retinoid X receptor (Miyata et al., 1994). Once bound, the PPAR–retinoid X receptor complex then binds to PPAR response elements in the promoter region of target genes. During times of fasting, an increase in circulating levels of fatty acids causes activation of PPARα, which is thought to activate PepT1. Shimakura et al. (2006) observed an increase in PepT1 gene expression following oral administration of the synthetic PPARα ligand WY-14643. The role of PPARα was confirmed by fasting PPARα-null and wild-type mice. In the PPARα knockout mice, the response of PepT1 to fasting was completely abolished, whereas it was increased in the wild type (Shimakura et al., 2006). The authors concluded that PPARα plays a critical role in the induction of PepT1 during a fasting state. Hirai et al. (2007), however, showed that in mice treated with 2 different PPARα agonists, including WY-14643, an up-regulation in the expression of 494 genes occurred, but PepT1 was not one of those upregulated genes. The authors concluded that the amount of WY-14643 used by Shimakura et al. (2006) was possibly too large and activated PepT1 through an off-target effect. Given the important role that PepT1 plays in the absorption of dietary amino acids, a better understanding of the mechanism that regulates expression of this transporter is important to the development of poultry diets. Thus, the objective of this study was to investigate the effects of feed restriction on the expression of PepT1 and PPARα in broiler chicks.

**MATERIALS AND METHODS**

**Birds and Tissue Collection**

Ninety 3-d-old Aviagen Line B broilers were housed in batteries of cages (5–6 birds/cage), exposed to 24-h light, and given free access to feed and water. On d 10, the birds were feed restricted for 24 h (feed withdrawn). One group of 6 birds was sampled before feed restriction and one group of 6 birds was sampled after feed restriction to determine the effect of feed restriction on PepT1 and PPARα expression. After feed restriction the remaining birds were divided into 3 treatments: refed 1 h–food withdrawn (RFW), refed ad libitum (RA), and continuously feed restricted (CF). Five groups were refed for 1 h and then feed was withdrawn (RFW). One group of 6 birds was sampled 1 h after refeeding and the other 4 groups were sampled 2, 3, 5, and 7 h after refeeding. After the 24-h feed restriction, 4 groups were refed ad libitum (RA) and sampled 2, 3, 5, and 7 h after refeeding. An additional 5 groups were continually feed restricted throughout the sampling period (CF). These groups were sampled at 1, 2, 3, 5, and 7 h after the end of the 24-h feed restriction. Birds were killed by cervical dislocation and the small intestine was separated into the duodenum, jejunum, and ileum. Segments were rinsed in ice-cold PBS and minced with a razor blade. Single aliquots of 20 to 30 mg of tissue were placed in microfuge tubes and frozen on dry ice and stored at −80°C. Total RNA was extracted from each sample using the RNeasy Kit (Qiagen, Valencia, CA) according to the animal tissue protocol using a homogenizer. All animal procedures for this experiment were approved by the Institutional Animal Care and Use Committee at Virginia Tech.

**Reverse Transcription and Real-Time PCR**

Following extraction, the RNA concentration was determined in duplicate at 260/280 nm using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE). Quality of the RNA was examined by agarose gel electrophoresis. Complementary DNA was synthesized using the cDNA Archive Kit (Applied Biosystems, Carlsbad, CA) according to the manufacturer’s instructions. Each reverse transcription reaction included 10 μL of sample RNA diluted to 0.2 μg/μL and 10 μL of 2× RT master mix. The reverse transcription reaction was run at 25°C for 10 min followed by 37°C for 120 min.

Quantitative real-time PCR was performed using a 7300 Real-Time PCR instrument (Applied Biosys-
tions). Each reaction was conducted in a 96-well plate and included 2 µL of cDNA diluted 1:30, 12.5 µL of 2× SYBR Green Master Mix (Applied Biosystems), 0.5 µL each of the forward primer (5 µM) and reverse primer (5 µM), and 9.5 µL of nuclease-free water. The real-time PCR reaction was run at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The genes that were evaluated include chicken PepT1, PPARα, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The PCR was performed using the following primers for PepT1, PPARα, and GAPDH: PepT1 forward primer: 5′ CCCCTGAGGAGGAT- CACTGTT 3′; PepT1 reverse primer: 3′ CAAAAGAG- CAGCAGCAACGA 5′ (Gilbert et al., 2007); PPARα forward primer: 5′ GGTCCAGGATCTGATGGATG- CCCTGAGGAGGAT- CACTGTT 3′; PPARα reverse primer: 3′ GGAGCTGTGCAGCAGCAACGA 5′; GAPDH forward primer: 5′ GCCGTCCTCTCTGGCAAAG 3′; GAPDH reverse primer: 3′ TGTAACCATGTGATTCA 5′ (Gilbert et al., 2007).

Primers were designed using the Primer Express software (Applied Biosystems) and synthesized by MWG-Biotech Inc. (Huntsville, AL). Duplicate quantitative real-time PCR reactions were run. The 96-well plates were analyzed using the auto function of the software program for the 7300 Real-Time PCR instrument, which provided the ability to analyze 10 or more plates at a time. Average gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

**Statistical Analysis**

All data were analyzed using PROC MIXED of SAS (SAS Institute, Cary, NC). For the feeding trial, the model included the main effects of time, group, segment, and all 2-way interactions. The 3-way interactions were removed from the model because they were determined to be nonsignificant ($P > 0.05$). Significant effects were further evaluated with Tukey’s test for pairwise comparisons.

**RESULTS**

The transcription factor PPARα is thought to play a key role in fasting-induced intestinal PepT1 expression. Ten-day-old chicks were feed restricted for 24 h and then divided into 3 groups: CF, RFW, and RA. The 24-h feed restriction increased both PepT1 and PPARα mRNA abundance (Figure 1). Peptide transporter 1 exhibited an almost 2-fold increase after feed restriction compared with baseline ($P = 0.01$). Expression of PPARα showed a similar 2-fold increase after feed restriction ($P < 0.0001$).

Expression of PepT1 and PPARα mRNA in the CF, RFW, and RA groups is summarized in Table 1 with separate rows for main effects of segment, time, and group, and appropriate 2-way interactions. The main effect of time included all 3 intestinal segments for the 5 time points examined. The CF group demonstrated 2-fold or greater expression for both PepT1 and PPARα ($P < 0.0001$) than the RFW and RA groups (Table 1). A significant group × segment interaction was observed for both genes. Peptide transporter 1 expression was greatest in the jejunum and ileum relative to the duodenum of the CF group ($P = 0.002$; Figure 2). Again no difference in expression was found between the duodenum, jejunum, and ileum for the RFW and RA groups. The PPARα expression exhibited a similar group × segment interaction as seen with PepT1. Within the CF group, PPARα expression was greatest in the jejunum when compared with the duodenum, with no difference in expression between the jejunum and ileum or between the duodenum and ileum ($P = 0.01$; Figure 3). Again no difference in expression was found between the duodenum, jejunum, and ileum for the RFW and RA groups. The time course for expression of PPARα mRNA is shown in Figure 4. Following refeeding, expression of PPARα mRNA declined in the RFW and RA groups. A significant group × time interaction was found. Compared with the RFW and RA groups, the CF group had peak expression at 5 h post feed restriction. Though not significant ($P = 0.20$), the group × time interaction for PepT1 showed a similar trend, with the CF group peaking in expression at 5 h post feed restriction (data not shown).

**DISCUSSION**

In this study, the expression levels of PepT1 and PPARα mRNA in broiler chicks showed an almost 2-fold increase following a 24-h feed restriction. Expression levels of these 2 genes remained high within the CF group. These findings are in agreement with previo

![Figure 1](https://academic.oup.com/ps/article-abstract/90/10/2295/1578887/figure1)
ous studies that examined the effect of feed restriction in rats. Thamotharan et al. (1999) found that PepT1 mRNA in rats increased 3-fold after just 1 d of fasting. Rats subjected to a 4-d fast showed a 179% increase in the expression of PepT1 mRNA (Ihara et al., 2000). Reduced feed intake and total parenteral nutrition (TPN) also affect PepT1 expression levels. Ihara et al. (2000) fed rats at 50% of the intake for controls for 10 d and treated rats with TPN for 10 d and found a 164 and 161% increase, respectively, in PepT1 expression. The increase in PepT1 expression during a time of reduced feed intake suggests the importance of having transporters to take up any available nutrients. Reduced feed intake and starvation situations, however, change the mucosal lining of the small intestine, where nutrient absorption occurs. Delayed access to feed for 36 h posthatch resulted in depressed villus height and decreased crypt depth in addition to decreased growth in all 3 intestinal segments in chicks (Uni et al., 1998). Silva et al. (2007) fed male broiler chicks at 30% of ad libitum intake from 7 to 14 d and found a decrease in the surface area of the tip of the enterocyte in the small intestine on d 14. In their study where rats were either feed restricted or TPN treated, Ihara et al. (2000) provided evidence for indirect activation of PepT1 when they demonstrated an increase in the transcription factors SP1 and CDX2 in response to fasting.

Figure 2. Relative peptide transporter 1 (PepT1) expression: segment × group interaction. A segment × group interaction was found for PepT1 gene expression in feed restricted/refed broiler chicks (n = 6). Relative gene expression (2−ΔΔCt) ± SEM was calculated using the ΔΔCt method with glyceraldehyde 3-phosphate dehydrogenase as the endogenous control and the average cycle threshold value for the duodenum before feed restriction as the calibrator. The letters a and b indicate a significant difference (P < 0.002). The data represent the means of the 5 time points after feed restriction. CF = continuously fasted; RFW = refed–food withdrawn; RA = refed ad libitum. Duo = duodenum; Jej = jejunum; Ile = ileum.

Table 1. Relative expression of peptide transporter 1 (PepT1) and peroxisome proliferator-activated receptor α (PPARα) following feed restriction-refeeding

<table>
<thead>
<tr>
<th>Item</th>
<th>PepT1</th>
<th>PPARα</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Segment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>1.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.55</td>
</tr>
<tr>
<td>Jejunum</td>
<td>1.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.66</td>
</tr>
<tr>
<td>Ileum</td>
<td>1.29&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.45</td>
</tr>
<tr>
<td><strong>SEM</strong></td>
<td>0.10</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>0.02</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>Time after feed restriction</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>1.29</td>
<td>1.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 h</td>
<td>1.34</td>
<td>1.27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3 h</td>
<td>1.31</td>
<td>1.39&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>5 h</td>
<td>1.48</td>
<td>2.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>7 h</td>
<td>1.06</td>
<td>1.76&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td><strong>SEM</strong></td>
<td>0.16</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>0.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Group</strong>&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>2.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.51&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>RFW</td>
<td>0.92&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.06&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>RA</td>
<td>0.84&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>1.10&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>0.12</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Interaction</strong>&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time × segment</td>
<td>0.35</td>
<td>0.77</td>
</tr>
<tr>
<td>Group × time</td>
<td>0.20</td>
<td>0.002</td>
</tr>
<tr>
<td>Segment × group</td>
<td>0.002</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Within a column, means without a common superscript within segment, group, and time differ significantly.
<sup>1</sup>Relative gene expression (2−ΔΔCt) ± SEM was calculated using the ΔΔCt method with glyceraldehyde 3-phosphate dehydrogenase as the endogenous control and the average cycle threshold value for the duodenum samples before feed restriction as the calibrator.
<sup>2</sup>The main effect of time included all 3 intestinal segments for the 3 groups.
<sup>3</sup>The main effect of group included all 3 intestinal segments for the 5 time points. CF = continuously fasted; RFW = refed–food withdrawn; RA = refed ad libitum.
<sup>4</sup>P-values given.

The increases seen in PepT1 expression as a result of fasting may be mediated by PPARα. In PPARα-null mice, the fasting-induced increase in PepT1 expression was abolished; however, in wild-type mice significant increases were found in PepT1 and PPARα expression following a 48-h fast (Shimakura et al., 2006). In this study, similar results were obtained following a 24-h fast, suggesting that PepT1 is either directly regulated by PPARα or indirectly regulated via transcription factors that are recruited by PPARα. Shimakura et al. (2006) provided evidence for indirect activation of PepT1 when they demonstrated an increase in the transcription factors SP1 and CDX2 in response to fasting.

Consistent with previous feed restriction studies, the CF group had the greatest expression of both PepT1 and PPARα. Within the CF group, expression of both genes was significantly greater in the jejunum compared with the duodenum. Naruhashi et al. (2002) and Howard et al. (2004) demonstrated that starved rats had increased PepT1 expression, with the distal small intestine having the greatest levels. By measuring ce-

PepT1 may help compensate for the loss of functionality of the enterocyte, and thus the mucosa.
fadroxil transport with a Ussing chamber. Naruhashi et al. (2002) demonstrated that PepT1 activity levels showed excellent correlation with PepT1 mRNA expression levels.

An interesting time × group interaction was observed regarding PPARα gene expression in the CF group. Following the initial 24-h feed restriction, PPARα expression remained high (compared with the RFW and RA groups) in the CF group for the next 7 h. However, PPARα peaked at 29 h following the initial feed restriction. Why PPARα expression would peak after 29 h of feed restriction, and not earlier, could be the result of either the depletion of glucose and switch to fat utilization or fasting-related stress. In the case of the former, the carbohydrate stores in the body can supply the necessary energy to allow for normal bodily function for about half a day. Once those stores have been exhausted, the body switches to fat utilization. As fat is increasingly mobilized, the triglycerides that were stored in the adipose tissue are broken down to free fatty acids (FFA) and glycerol. During a starvation situation, FFA increase in the blood in preparation for transport to the liver to be converted to glucose. As stated earlier, FFA have been shown to be natural activators of PPARα. At the 29 h post feed restriction time point, the peak in PPARα expression could reflect the increase in blood FFA following the natural shift from metabolism of carbohydrates to metabolism of fat. However, a discussion on feed restriction or fasting is not complete without some reference to fasting stress. For any animal, fasting is an incredibly stressful situation that is not without harmful effects. Cortisol, a glucocorticoid, is increased in times of stress and promotes the mobilization of FFA from adipose tissue (Brindley et al., 1993). This cortisol-mediated shift from glucose to fat utilization requires several hours to become fully functional (Guyton and Hall, 1996) and could explain why PPARα took 29 h to peak following the beginning of the initial feed restriction. Though this peak at 29 h in PPARα expression was significant, a nonsignificant peak in PepT1 expression was also observed at this time, suggesting that PepT1 was slowly increasing following the induction of PPARα and may have increased more had the experiment been conducted longer.

In conclusion, feed restriction was found to increase PepT1 mRNA levels in birds along with a concomitant increase in PPARα. These results further support the idea that PPARα plays an important role in regulating PepT1 expression during feed restriction.

ACKNOWLEDGMENTS

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REFERENCES


