Thyroid hormone regulates the activity and expression of the peptide transporter PEPT1 in Caco-2 cells

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Ashida, Kayoko, Toshiya Katsura, Hideyuki Motoshashi, Hideyuki Saito, and Ken-ichi Inui. Thyroid hormone regulates the activity and expression of the peptide transporter PEPT1 in Caco-2 cells. Am J Physiol Gastrointest Liver Physiol 282: G617–G623, 2002. First published December 12, 2001; 10.1152/ajpgi.00344.2001.—An oligopeptide transporter (PEPT1) in the small intestine plays an important role in the absorption of small peptides and peptide-like drugs. We examined the effect of thyroid hormone 3,5,3′-triiodothyronine (T3) on the activity and expression of PEPT1 in human intestinal Caco-2 cells. Treatment of Caco-2 cells with T3 inhibited [14C]glycylsarcosine uptake in a time- and dose-dependent manner. [14C]Glycylsarcosine uptake was reduced by pretreatment of the cells with 100 nM T3 for 4 days (67% of control value), whereas methyl-d-[U-14C]glucopyranoside and [3H]threonine uptake were not decreased. Kinetic analysis showed that T3 treatment significantly decreased the maximum uptake (Vmax) value for [14C]glycylsarcosine uptake but had no effect on the Km value. Moreover, T3 treatment caused a significant decrease in the amount of PEPT1 mRNA (25% of the control). Western blotting indicated that the amount of PEPT1 protein in the apical membrane was decreased (70% of the control). These findings indicate that T3 treatment inhibits the uptake of [14C]glycylsarcosine by decreasing the transcription and/or stability of PEPT1 mRNA.

intestinal absorption; intestinal oligopeptide transporter; hormonal regulation; gene expression

THE PEPTIDE TRANSPORT SYSTEM in the small intestine has physiological roles in the absorption of small peptides to maintain protein nutrition (7, 13, 19, 20). Moreover, the intestinal peptide transport system mediates the absorption of a broad range of peptide-like drugs, such as β-lactam antibiotics, the anti-cancer agent bestatin, and angiotensin converting enzyme inhibitors, thereby playing an important pharmacological role for oral drug delivery (11, 34, 36). Recently, cDNA encoding an oligopeptide transporter (PEPT1) was isolated from rabbit (4), human (15), rat (25), and mouse (5), and its structural and functional characteristics have been elucidated. In addition, molecular identification of PEPT1 provides a novel opportunity to determine the mechanisms of its regulation. The activity of PEPT1 varies significantly in response to several factors that have been reported to upregulate the activity of PEPT1, such as its own substrates (32, 35), diet (28), insulin (33), α2-adrenergic agonists (1), and the α-receptor ligand (+)-pentazocine (6). For these stimuli, the regulation of PEPT1 activity is considered to be at the level of gene expression, transport function, or protein recruitment to the plasma membrane. On the other hand, it has been reported that cAMP (22) and protein kinase C activation (3) inhibited the activity of PEPT1. These inhibitory effects are likely to be due to posttranscriptional modifications involving phosphorylation/dephosphorylation of PEPT1. Therefore, the mechanism of downregulation of PEPT1 at the level of expression has not been elucidated.

Thyroid hormone is secreted from the thyroid to maintain normal growth and development, normal body temperature, and normal energy levels. Most of its effects appear to be mediated by activation of nuclear receptors that lead to increased formation of mRNA and subsequent protein synthesis (24). Thyroid hormone has prominent effects on gastrointestinal development, structure, and function. Absorption of various nutritional substrates from the small intestine was shown to be altered in response to a thyroid state (14). Because 3,5,3′-triiodothyronine (T3) is known to increase the metabolism of glucose, the effects of T3 on the hexose transport systems in the small intestine (SGLT1, GLUT5) have been studied extensively (16, 17). On the other hand, no information is available about the effects of T3 on the absorption of oligopeptides, which are also important nutritional substrates. To elucidate the effects of T3 on the absorption of oligopeptides, we investigated the activity of PEPT1 by measuring [14C]glycylsarcosine uptake by a human intestinal epithelial cell line Caco-2. Caco-2 cells are known to express PEPT1 and have been used to study the regulation of its activity and expression (1, 3, 22, 32, 33, 35). We report here that T3 decreases the transport activity of PEPT1 by inhibition of the trans-
scription and/or the decrease in the stability of PEPT1 mRNA.

MATERIALS AND METHODS

Materials. [14C]Glycylsarcosine (1.78 GBq/mmol) was obtained from Daiichi Pure Chemicals (Ibaraki, Japan). L-[3,5-3H]threonine (485 GBq/mmol) was from Amersham Pharmacia Biotech (Little Chalfont, UK). Methyl-α-D-[U-14C]glucopyranoside (α-methyl-α-D-glucoside; 9.66 GBq/mmol) was from Moravek Biochemical (Brea, CA). T3 was purchased from Nacalai Tesque (Kyoto, Japan). AG-1-X8 anion exchange resin (chloride form; 200–400 mesh) was obtained from Bio-Rad (Hercules, CA). All other chemicals were of the highest purity available.

Cell culture. Caco-2 cells at passage 18 obtained from the American Type Culture Collection (ATCC HTB-37) were maintained by serial passage in plastic culture dishes (Falcon; Becton Dickinson, Lincoln Park, NJ) as described previously (12, 18). For uptake studies, 35-mm plastic dishes were inoculated with 2 × 10⁵ cells in 2 ml of complete culture medium. The medium consisted of DMEM (Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum (Whittaker Bioproducts, Walkersville, MD) and 1% nonessential amino acids (Invitrogen) without antibiotics before use.

To expose the Caco-2 cell monolayers to T3, T3 was added to culture medium containing T3-depleted serum. T3 treatment was applied to postconfluent monolayers. The control cells were incubated with the same concentration of 0.1 M NaOH in each experiment.

Uptake studies by cell monolayers. The uptake of [14C]glycylsarcosine was measured in cells grown on 35-mm plastic dishes as described previously (31). In all experiments, the uptake was measured on day 15. Radioactivity was determined in 5 ml of ACS II (Amersham Pharmacia Biotech) by liquid scintillation counting. The protein contents of cell monolayers solubilized in 1 N NaOH were determined according to the method of Bradford (2) using a Bio-Rad protein assay kit with bovine γ-globulin as the standard.

Competitive PCR. Competitive PCR was performed according to the method of Siebert and Larrick (29) with some modifications. Briefly, aliquots of 1 μg of total cellular RNA, isolated from Caco-2 cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany), were reverse-transcribed in 20 μl of reaction mixtures and diluted to 200 μl. Aliquots of 5 μl of diluted reaction mixtures, in combination with semilogarithmic serial dilution of mimic competitor DNA from 250 to 1 fmol, were amplified by PCR according to the following method: 5 μM human PEPT1 sense primer (5'-CTGGCAATCCCGGACATTTCC-3' corresponding to bases 1803–1826) and human PEPT1 antisense primer (5'-CATCCTTGTCTGGATTTGGCCC-3'; corresponding to bases 2157–2186) in 20 μl were incubated according to the following PCR cycle: an initial denaturation step of 95°C for 3 min followed by 34 cycles of 95°C for 1 min, 65°C for 1 min, and 72°C for 1 min, and a final elongation step of 72°C for 10 min. PCR products were then size-fractionated by 1.5% agarose gel electrophoresis. The amplified cellular fragment (target) was 355 bp and the mimic competitor was 538 bp. The amount of competitor DNA yielding equal molar amounts of product gave that of target human PEPT1 mRNA. The densitometric data were normalized for each batch of RNA by correcting the amount of glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA as an internal control.

Polyclonal antibodies against human PEPT1 and Western blot analysis. Polyclonal antibodies were raised in rabbits against a synthetic peptide (RFRHRSKAFPKREHWLDW) corresponding to positions 247–264 of human PEPT1 (15). The peptide was synthesized with cysteine at its NH₂ terminus and conjugated to keyhole limpet hemocyanin. The apical membrane fraction was purified from Caco-2 cells according to the method of Inui et al. (10) with some modifications. Briefly, Caco-2 cells were homogenized in buffer A consisting of (in mM) 250 sucrose, 10 Tris, 0.5 EGTA, and 0.5 phenylmethylsulfonyl fluoride; pH 7.5. Magnesium chloride was added to a final concentration of 10 mM, and the mixture was allowed to stand for 15 min (step 1). The suspension was centrifuged at 1,000 g for 10 min, and the resulting supernatant was centrifuged at 27,000 g for 30 min (step 2). The pellet from high-speed centrifugation was resuspended in buffer A. Steps 1 and 2 were repeated on this homogenate, and the resulting pellet was resuspended in buffer B (250 mM sucrose, 10 mM Tris, and 0.5 mM phenylmethylsulfonyl fluoride; pH 7.5) by repeated passage through a 27-gauge needle. The protein concentration of the membrane suspension was measured using a Bio-Rad protein assay kit. The membrane fractions were solubilized in lysis buffer (2% SDS, 125 mM Tris, and 20% glycerol). Samples were separated by 8.5% SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Immobilon-P; Millipore, Bedford, MA) by semidyrect electrophotoblot. Blots were blocked with 5% nonfat dry milk in Tris-buffered saline (TBS; 20 mM Tris, 137 mM

Fig. 1. Dose-dependent effect of T3 on [14C]glycylsarcosine uptake by Caco-2 cells. The cells were treated with various concentrations of T3 (0.1 nM–3 μM) for 3 days after reaching confluence. After treatment, the monolayers were rinsed once with incubation medium (pH 7.4) and then incubated for 10 min at 37°C with [14C]glycylsarcosine (20 μM) in incubation medium (pH 6.0). Thereafter, the radioactivity of the solubilized cells was determined. Each column represents the mean ± SE of 9 monolayers from 3 separate experiments. * P < 0.05, ** P < 0.01, significantly different from control.
NaCl, pH 7.5) with 0.1% Tween 20 (TBS-T) for 3 h at room temperature. The blots were washed in TBS-T and then incubated with the affinity-purified anti-PEPT1 antibody (1:50 dilution) and left overnight at 4°C. The blots were washed three times with TBS-T, and the bound antibody was detected on X-ray film by enhanced chemiluminescence with a horseradish peroxidase-conjugated anti-rabbit IgG antibody (Amersham Pharmacia Biotech).

**Statistical analysis.** Data were analyzed statistically by nonpaired t-test or one-way ANOVA followed by Schef"e’s test when multiple comparisons were needed. Probability values < 0.05 were considered significant.

**RESULTS**

**Effect of T₃ pretreatment concentration and time on [¹⁴C]glycylsarcosine uptake in Caco-2 cells.** To investigate the effect of T₃ on [¹⁴C]glycylsarcosine transport in Caco-2 cells, we used serum treated with anion exchange resin to deplete thyroid hormone. Supplemental culture medium with thyroid hormone-depleted serum for 3 days had no effect on [¹⁴C]glycylsarcosine uptake compared with medium containing untreated serum (data not shown). Therefore, in the present study we used thyroid hormone-depleted serum during treatment of Caco-2 cells with T₃. Figure 1 shows the effect of various concentrations of T₃ (0.1 nM to 1 μM) on [¹⁴C]glycylsarcosine uptake by Caco-2 cells. H⁺-coupled [¹⁴C]glycylsarcosine transport was decreased by T₃ pretreatment for 3 days in a concentration-dependent manner.

Time dependence of the effect of T₃ was examined by treating the cells with 100 nM T₃ for 1–7 days. As shown in Fig. 2, the uptake of [¹⁴C]glycylsarcosine was decreased in a time-dependent manner for 3 days. The maximal decrease in [¹⁴C]glycylsarcosine uptake was observed after 3–4 days pretreatment with T₃. Subsequent experiments were carried out by pretreating the cells with 100 nM T₃ for 4 days.

**Specificity of T₃-induced inhibition of [¹⁴C]glycylsarcosine uptake.** We then examined the effect of T₃ pretreatment on α-methyl-D-glucoside and [³H]threonine uptake by Caco-2 cells to determine whether the effect of T₃ was specific to peptide transport. As shown in Fig. 3, α-methyl-D-glucoside and [³H]threonine uptake were not diminished by T₃ treatment, indicating that the inhibitory effect of T₃ on [¹⁴C]glycylsarcosine uptake appeared to be specific.

**Kinetic analysis of [¹⁴C]glycylsarcosine uptake.** To determine the effect of T₃ on the kinetics of [¹⁴C]glycylsarcosine uptake, the concentration dependence of [¹⁴C]glycylsarcosine uptake was examined in Caco-2 cells pretreated with or without T₃. Figure 4 shows the initial uptake of [¹⁴C]glycylsarcosine as a function of the substrate concentration. Specific uptake was calculated by subtracting the nonspecific uptake, which was estimated in the presence of excess unlabeled dipeptide, from total uptake. Although the osmolarity of the incubation medium without excess unlabeled dipeptide...
Fig. 4. Concentration dependence of \(^{14}\text{C}\)glycylsarcosine uptake by Caco-2 cells treated without or with T3. The cells were treated without (A) or with (B) 100 nM T3 for 4 days after reaching confluence. Cell monolayers were incubated with incubation medium containing various concentrations of \(^{14}\text{C}\)glycylsarcosine (pH 6.0) in the absence (○) or presence (●) of 50 mM unlabeled glycylleucine for 10 min at 37°C. Thereafter, radioactivity of solubilized cells was determined. Inset, Eadie-Hofstee plots of \(^{14}\text{C}\)glycylsarcosine uptake after correction for the nonsaturable component. Each point represents the mean ± SE of three monolayers from a typical experiment. Error bars not shown are smaller than the symbol. V, uptake rate (nmol·mg protein\(^{-1}\)·min\(^{-1}\)); S, glycylsarcosine concentration (mM).

was not adjusted, we confirmed that hyperosmotic solution has little effect on PEPT1 activity using incubation medium including 50 mM mannitol. The \(^{14}\text{C}\)glycylsarcosine uptake values in the presence or absence of 50 mM mannitol were 197.0 ± 2.0 and 183.9 ± 4.7 pmol·mg protein\(^{-1}\)·min\(^{-1}\), respectively. Kinetic parameters were calculated according to the Michaelis-Menten equation using nonlinear least-squares regression analysis. The Michaelis constants (\(K_m\)) in the cells treated without and with T3 were 0.87 ± 0.01 and 1.02 ± 0.26 mM (mean ± SE of three separate experiments, \(P = 0.567\)), respectively, and the maximum uptake rates (\(V_{\text{max}}\)) in the cells treated without and with T3 were 9.90 ± 0.84 and 5.22 ± 1.06 nmol·mg protein\(^{-1}\)·min\(^{-1}\) (mean ± SE of three separate experiments, \(P < 0.05\)), respectively. Thus treatment of the cells with T3 significantly decreased the maximum velocity value for \(^{14}\text{C}\)glycylsarcosine uptake, whereas the apparent \(K_m\) did not change significantly.

**Human PEPT1 mRNA expression.** Decrease in the \(V_{\text{max}}\) value for \(^{14}\text{C}\)glycylsarcosine uptake by T3 treatment suggested decreased expression of the peptide transporter PEPT1 in Caco-2 cells. Therefore, we then examined the expression of PEPT1 mRNA by competitive PCR in Caco-2 cells pretreated with T3. In a preliminary experiment, PEPT1 was amplified from cDNA in the presence of serial dilutions of the PEPT1 competitor to find the most appropriate amount of competitor (data not shown). Quantitative investigation of PEPT1 mRNA was then carried out using total RNA isolated from Caco-2 cells pretreated with or without T3. Typical results of competitive PCR analysis are shown in Fig. 5A. Densitometric quantification indicated that treatment with 100 nM T3 for 4 days led to a decrease in PEPT1 mRNA content (25% of the control, \(P < 0.01\)) (Fig. 5B). Therefore, it was suggested that the decrease in \(^{14}\text{C}\)glycylsarcosine transport activity was due to the decreased transcription of PEPT1 mRNA. The amount of GAPDH mRNA used as an internal standard was not changed significantly (\(P = 0.752\)) by this treatment (data not shown).

**Human PEPT1 protein expression.** To investigate the effect of T3 on the expression of PEPT1 protein, apical membranes were purified from Caco-2 cells and were subjected to immunoblot analysis. A primary band of ~80 kDa was detected using affinity-purified anti-PEPT1 antibodies, which disappeared when the antibody was preabsorbed to the synthetic antigen peptide (Fig. 6A). Densitometric quantification indicated that expression of PEPT1 protein was decreased in cells pretreated with T3 (Fig. 6, B and C, \(P < 0.05\)). An additional band below PEPT1 was also detected. This band may correspond to a degradation product of the PEPT1 protein.

Fig. 5. Effect of T3 on contents of PEPT1 mRNA in Caco-2 cells. Cells were treated with or without 100 nM T3 for 4 days after reaching confluence. After treatment, total cellular RNA was extracted and human PEPT1 mRNA was quantified by the competitive PCR method as described in MATERIALS AND METHODS. A: typical results of agarose gel electrophoresis of the PCR products from T3-treated cells. Lanes 1-6: 1, 2.5, 10, 25, 100, and 250 pmol of PEPT1 mimic competitor cDNA. B: densitometric quantification of PEPT1 mRNA, corrected for loading using the amount of GAPDH mRNA as an internal control. Each column represents the mean ± SE of 6 monolayers. ** \(P < 0.01\), significantly different from control.
PEPT1 or other proteins detected by anti-PEPT1 antibodies. Because the identity of the smaller bands are not known, we did not include the smaller bands in the quantitative analysis.

**DISCUSSION**

In the present study, we showed that the [14C]glycylsarcosine uptake by Caco-2 cells was inhibited by T3 treatment. The effect of T3 on [14C]glycylsarcosine uptake appeared to be specific to PEPT1 and to be different from those on glucose and amino acid transport, because α-methyl-d-glucoside and [3H]threonine uptake were not decreased by T3 treatment. Previously, Matosin-Matekalo et al. (16) showed that T3 treatment stimulated [1H]methyl-D-glucoside uptake via the Na+/H+ glucose cotransporter (SGLT1) in Caco-2 cells. Their observations were inconsistent with the present results. Although reasons for this discrepancy are not clear, differences in the results of these two studies may have been due to the characteristics of Caco-2 cells and/or experimental conditions used. They used a clone, Caco-2/TC7, showing markedly higher levels of SGLT1 mRNA expression than parental Caco-2 cells. Their observations were inconsistent with the present results. Although reasons for this discrepancy are not clear, differences in the results of these two studies may have been due to the characteristics of Caco-2 cells and/or experimental conditions used. They used a clone, Caco-2/TC7, showing markedly higher levels of SGLT1 mRNA expression than parental Caco-2 cells. In addition, they treated the cells with 1 μM T3 for 20 days, whereas we treated with 0.1 μM T3 for 4 days. These concentrations were higher than the physiological concentration of T3 (~2.3 nM). However, we supposed the condition of hyperthyroidism and the concentration of T3 used was adequate.

Earlier investigations suggested that the activity of PEPT1 varies in response to several factors. The regulatory effects of substrate and dietary factors have been widely investigated (28, 32, 35). It was shown that the presence of dipeptide in the culture medium stimulated the uptake of dipeptide by Caco-2 cells (32, 35). The mechanism of this stimulation by dipeptide appeared to be increased levels of PEPT1 protein and mRNA. Recently, Shiraga et al. (28) identified the promoter region of the rat PEPT1 gene and showed the presence of an amino acid-responsive element in the promoter region. They demonstrated that upregulation of dipeptide transport activity by dietary protein was caused by transcriptional activation of the PEPT1 gene by selected amino acids and dipeptides in the diet. Hormonal regulation of PEPT1 in Caco-2 cells, for example by insulin, has also been studied (33). Insulin stimulated dipeptide uptake by Caco-2 cells by increasing the amount of PEPT1 in the plasma membrane. The mechanism of this effect appeared to be increased translocation of PEPT1 to the plasma membrane from a preformed cytoplasmic pool. In addition, α2-adrenergic agonists stimulated oligopeptide transport in Caco-2 cells by increasing translocation to the apical membrane of preformed cytoplasmic transporter molecules (1). Moreover, peptide transport in the small intestine was relatively resistant to starvation, protein-caloric malnutrition, and intestinal damage (9, 30). It has been reported that the resistance of peptide
transporter to tissue damage may be due to a relative increase in PEPT1 synthesis (30) and that PEPT1 expression was enhanced in the rat jejunum under various conditions of malnutrition (9). In contrast, it has been reported that cAMP (22) and protein kinase C activation (3) inhibited the activity of PEPT1 in Caco-2 cells. Because human PEPT1 possesses sites for phosphorylation by protein kinase C, it is likely that PEPT1 is regulated by posttranslational modifications involving phosphorylation/dephosphorylation. A change in the phosphorylation state of the transport protein could result in the inhibition of its activity. In the present study, we demonstrated that T₃ treatment inhibited the activity of PEPT1 and decreased the Vₘₐₓ value for [¹⁴C]glycylsarcosine uptake without changing the Kₘ value. In addition, the expression levels of PEPT1 mRNA and protein in Caco-2 cells were decreased by T₃ treatment. It was reported that treatment with thyroid hormone increased the expression of Na⁺/K⁺-ATPase α₁-protein and did not change the expression of SGLT1 protein in Caco-2 cells (16). In addition, the uptake of α-methyl-d-glucoside and threonine was not decreased by T₃ treatment (Fig. 3), suggesting that the expression of these transporters was not decreased. Therefore, it seems likely that T₃ treatment does not lead to a general decrease in protein expression. This is the first study showing the hormonal downregulation of PEPT1 expression. During the preparation of this manuscript, it was reported that expression of PEPT1 was decreased by epidermal growth factor treatment in Caco-2 cells (23).

Thyroid hormone is responsible for optimal growth, development, function, and maintenance of all body tissues. In the small intestine, the processes of enterocyte growth and differentiation are altered by various developmental, dietary, and hormonal factors (8). Thyroid hormone is among the most potent regulators of intestinal epithelial growth and differentiation. For example, T₃ regulates the developmental changes in the activity of several brush-border enzymes such as alkaline phosphatase and lactase (8). Developmental expression of PEPT1 in the rat small intestine has also been reported (21, 27). Shen et al. (27) showed that expression levels of PEPT1 mRNA and protein were maximal 3–5 days after birth in the duodenum, jejunum, and ileum, and then declined rapidly. They suggested that this change in PEPT1 expression might be related to an adaptive response to changes in the diet, from high-protein milk to an adult diet containing more carbohydrate than protein. However, it was reported that the serum concentration of thyroid hormone rises from postnatal day 5 to 15 (8) at the same time that expression levels of PEPT1 decline (27). Therefore, it seems likely that thyroid hormone regulates the expression of PEPT1 during development. Further studies are required to determine the precise mechanism of developmental regulation of PEPT1 expression.

Mechanisms of action of thyroid hormone are quite diverse. Although thyroid hormone may exert its effects via a number of cellular loci, its major effect appears to be on transcriptional regulation of target genes. Thyroid hormone enters the cells and proceeds to the nucleus, where it binds to the thyroid hormone receptor. The formation of ligand-bound thyroid hormone receptor complexes specifically interacting with thyroid hormone-responsive elements located in regulatory regions of target genes is presumably a necessary first step for activation or suppression of target genes (37). In the present study, we demonstrated that T₃ treatment markedly decreased PEPT1 mRNA levels (Fig. 5). It is likely that the inhibition of [¹⁴C]glycylsarcosine transport by T₃ is due to the inhibitory effect of T₃ on the transcription of PEPT1 mRNA. However, it is not yet known whether the inhibition of transcription of PEPT1 mRNA is due to a direct effect of T₃ on PEPT1 mRNA. Recently, promoter analyses of mouse and rat PEPT1 were reported (5, 28). These analyses revealed the putative nucleotide sequence upstream of the translation start site and the corresponding transcription factor. Thyroid hormone-responsive elements were not found in the promoter regions of rat and mouse PEPT1 genes, although these may be differences among rat, mouse, and human homologues. We have examined the effect of thyroid hormone on PEPT1 activity and expression in hyperthyroid rats and found that treatment of thyroid hormone decreased the activity of PEPT1 in rat small intestine (unpublished observations by K. Ashida, T. Katsura, H. Saito, and K. Inui). Although the precise mechanism of the effect of T₃ on PEPT1 expression remains to be determined, it is possible that T₃ may act indirectly with PEPT1 mRNA in rats and humans. In conclusion, we demonstrated that the activity of PEPT1 is inhibited by T₃ and that the inhibitory effect of T₃ is due to inhibition of the transcription and/or the decrease in the stability of PEPT1 mRNA. These results may have important implications for protein nutrition as well as for drug absorption in thyrotoxicosis.

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