Research Article

L-Theanine Administration Modulates the Absorption of Dietary Nutrients and Expression of Transporters and Receptors in the Intestinal Mucosa of Rats

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1. Introduction

L-theanine, as a non-protein-forming amino acid (AA), contributes to the umami taste and unique flavor of green tea. Its content in tea leaves is closely related to the quality and price of green tea [1, 2]. L-theanine is beneficial for remedying various nutritional and metabolic diseases in human, including providing antiobesity effects [3, 4], suppressing the body weight increases and fat accumulation [3, 5], and exerting antidiabetic effects [6, 7]. L-theanine is transported through the intestinal brush border membrane mainly via neutral AA systems B, A, ASC, N, and L, based on findings that L-theanine inhibited the absorption of glutamine and large neutral amino acids (AAs, leucine, and tryptophan) into organs [8–10]. Our knowledge data and previous findings also confirmed that most neutral AAs (threonine, valine, methionine, isoleucine, serine, alanine, tyrosine, and leucine) and certain basic AA (lysine) in the serum of L-theanine-administered rats were decreased [8, 11]. These researches indicated that L-theanine could competitively suppress the absorption of AAs.

However, AAs absorption is dependent on the activities of AA transporters located in the brush border membrane of small intestine. Neutral AA transporters, solute carrier family 1, member 5 (SLC1a5) and family 16, member 10 (SLC16a10), are responsible for threonine, serine, alanine, cysteine, glutamine and phenylalanine, tyrosine, and tryptophan transporting, respectively. Basic AA transporters, solute carrier family 7, member 1 (SLC7a1) and member 9 (SLC7a9), are in charge of transporting arginine, lysine, histidine, alanine, serine, cysteine, threonine, asparagine, and glutamine. Acidic AA transporters solute carrier family 1, member 1 (SLC1a1)
and member 2 (SLC1a2) transport glutamate and aspartate. 
It is reported that L-theanine competitively inhibited the 
uptake of glutamate substrate through solute carrier family 
1, member 3 (SLC1a3) and SLC1a2 expressed in cancer cells 
[12, 13]. However, the expression pattern of glutamate 
transporter subtypes in tumor cells is different from normal 
cells. Therefore, it is necessary to investigate the efficacy of 
L-theanine on glutamate transporters in normal tissues. Whether or not the expression of different AA transport 
systems is mediated by L-theanine is unknown yet.

Furthermore, it is reported that the fatty accumulation in 
mice was suppressed by the administration of green tea 
powder [4] and theanine was responsible for this suppressive 
effect [3]. Although serum glucose in rats was not changed, 
the insulin was reduced by oral theanine [14]. These litera-
tures indicate that metabolism of lipid and insulin is regulated 
by L-theanine. In the enterocytes of rats, there are many 
transporters and receptors responses to sugar and fatty acids 
transport, including sodium dependent glucose transporters 
(SGLTs), glucose transporters, G-protein-coupled receptors, 
and fatty acid binding protein 2 (FABP2) [15–21]. Whether 
these transporters and receptors involved in the regulation of 
L-theanine administration on absorption of glucose and lipid 
is unclear. Based on these questions, we measured the nutrient 
content in the blood and mRNA expression of related 
transporters and receptors in small intestine of rats after the 
intragastric administration of L-theanine for two weeks, 
aiming at figuring out the preliminary L-theanine-induced 
regulation mechanism in nutrients absorption in rats.

2. Material and Methods

2.1. Experimental Design. This experiment was conducted 
according to the animal care guidelines of the Animal Care 
Committee, Institute of Subtropical Agriculture, the Chi-
nese Academy of Sciences, Changsha city, Hunan province, 
China (number KYNEAAM-2013-0009). Thirty-four Sprague 
Dawley (SD) rats which are 3 weeks old weighing 74–92.2 g 
were used as experimental animals. The management of SD 
rats and L-theanine administration experiment was the same 
as Li et al. [22]. The animals were individually housed in 
plastic cages under laboratory conditions (25 ± 3°C, 70 ± 
5% relative humidity, good ventilation, and a 12-h light-dark 
cycle) and had free access to food and pure water. After three 
days of adaptation, SD rats were randomly divided into four 
treatment groups. Each group contained eight male rats and 
eight female rats. During fasting (15:00–17:00 h), rats in the 
treatments received gastric intubation of four different doses 
of L-theanine (0, 50, 200, and 400 mg/kg body weight/day), 
respectively. L-theanine was freshly dissolved in 0.9% NaCl 
solution in advance before intubation every day. 1 mL of the 
L-theanine solution was daily administered to each rat for two 
weeks.

2.2. Blood and Tissue Samples Collection. At the end of the 
experiment, SD rats were fasted overnight and anesthetized 
by ether for 4 min, and then blood was collected from the 
jugular vein into tubes without anticoagulant. The blood 
samples were centrifuged at 3500 rpm for 15 min at 4°C, and 
then serum samples were collected and stored at −80°C until 
assay. The whole jejunum and ileum segments were collected 
and rinsed with ice-cold saline (0.9% NaCl wt/vol). Then the 
mucosa were carefully removed, quickly frozen in liquid 
nitrogen, and stored at −80°C prior to subsequent analyses.

2.3. Analysis of Serum. The glucose, total cholesterol, triglyc-
eride (TG), urea, low-density lipoprotein cholesterol (LDL), 
and high-density lipoprotein cholesterol (HDL) were deter-
mined by automatic biochemistry analyzer (Synchron Clini-
cal System CX4 PRO, Beckman Coulter, USA) according 
to the instructions. Insulin was assayed by the ELISA kit 
purchased from Huamei Biotechnology Co., Ltd. (Wuhan, 
Hubei, China). Non-esterified fatty acids (NEFA) were mea-
sured by kit produced by Nanjing Jiancheng Bioengineering 
Institute (Nanjing, Jiangsu, China).

2.4. Real-Time Quantitative PCR. Total RNA was isolated 
from the mucosa of jejunum and ileum using the Trizol 
Reagent (Invitrogen, USA), and cDNA was synthesized using 
the Revert Aid First Strand cDNA synthesis kit (Applied 
Biosystems, Thermo Fisher Scientific, USA). For relative 
quantification of gene expression, the ABI Prism 7900 HT 
Fast Real-Time PCR System (Applied Biosystems, Foster, CA) 
was used. Primers were designed using the Primer 3 plus 
program, and sequences are listed in Table 1. The reaction 
system contained 5 µL SYBR® Premix Ex Taq™ (2x), 0.4 µL 
PCR forward primer (10 µM), 0.4 µL PCR reverse primer 
(10 µM), 0.2 µL ROX reference dye (50x), 1.0 µL cDNA, and 
3 µL sterilized ddH2O. The thermal profile for all reactions 
was 30 s at 95°C, then 40 cycles of denaturation at 95°C for 5 s, 
and annealing at 60°C for 30 s. Each reaction was completed 
with a melting curve analysis to ensure the specificity of the 
reaction. All the samples were analyzed in duplicate, and the 
relative amount of each specific transcript was obtained after 
normalization against the endogenous control β-actin. The 
relative amounts of target genes were quantified according to 
the 2−ΔΔCT method [23].

2.5. Statistical Analysis. Statistical analyses were conducted 
by one-way analysis of variance (ANOVA) using the Mixed 
Proc of SAS (version 8.2, SAS Institute, Cary, NC, USA). 
The main effect tested was the dose of L-theanine. When indicated 
by ANOVA, means were separated using least significant 
differences. Significance was declared at P < 0.05.

3. Results

As shown in Table 2, glucose concentration was decreased by 
400 mg/kg L-theanine administration compared to the con-
trol group (0 mg/kg L-theanine administration) (P < 0.05). 
Insulin concentration was linearly decreased by L-theanine 
administration (P < 0.001). There were no differences (P > 
0.05) in the serum cholesterol, TG, NEFA, and LDL concen-
trations among the L-theanine treatments. Concentrations of 
urea and HDL were decreased by 50 mg/kg L-theanine 
treatment compared to the control group (P < 0.05).

Transcript levels of intestinal AA transporters in the 
intestine of rats are shown in Table 3. Expression of acidic
Table 1: Sequences of primers used for real-time quantitative PCR.

| Gene   | GenBank accession | Primer                             | Length (bp) |
|--------|-------------------|------------------------------------|-------------|
| SLC7a1 | NM_013111.3       | Forward-CCTTCATCATCAGCTGGAAC       | 100         |
|        |                   | Reverse-GGTTGCGCTATCGCTGTC         |             |
| SLC1a5 | NM_175758.3       | Forward-GGAGAAATGGACTGTTGCTG      | 107         |
|        |                   | Reverse-CCCCAGAAGGCTCTGCAAT        |             |
| SLC1a2 | NM_001035233.1    | Forward-GGAGAAATGGACTGTTGCTG      | 101         |
|        |                   | Reverse-CCCCAGAAGGCTCTGCAAT        |             |
| SLC1a1 | NM_013032.3       | Forward-GGAGAAATGGACTGTTGCTG      | 106         |
|        |                   | Reverse-CCCCAGAAGGCTCTGCAAT        |             |
| SLC16a10| NM_138831.1      | Forward-GGAGAAATGGACTGTTGCTG      | 115         |
|        |                   | Reverse-CCCCAGAAGGCTCTGCAAT        |             |
| SLC7a9 | NM_053929.1       | Forward-GGAGAAATGGACTGTTGCTG      | 117         |
|        |                   | Reverse-CCCCAGAAGGCTCTGCAAT        |             |
| SGLT1  | NM_013032.3       | Forward-GGAGAAATGGACTGTTGCTG      | 122         |
|        |                   | Reverse-CCCCAGAAGGCTCTGCAAT        |             |
| SGLT3  | NM_00106383       | Forward-GGAGAAATGGACTGTTGCTG      | 101         |
|        |                   | Reverse-CCCCAGAAGGCTCTGCAAT        |             |
| GLUT2  | NM_012879         | Forward-GGAGAAATGGACTGTTGCTG      | 113         |
|        |                   | Reverse-CCCCAGAAGGCTCTGCAAT        |             |
| GLUT5  | NM_031741         | Forward-GGAGAAATGGACTGTTGCTG      | 108         |
|        |                   | Reverse-CCCCAGAAGGCTCTGCAAT        |             |
| FATP   | NM_053580.2       | Forward-GGAGAAATGGACTGTTGCTG      | 120         |
|        |                   | Reverse-CCCCAGAAGGCTCTGCAAT        |             |
| GPR43  | NM_001005877      | Forward-GGAGAAATGGACTGTTGCTG      | 113         |
|        |                   | Reverse-CCCCAGAAGGCTCTGCAAT        |             |
| GPR120 | NM_001047088.1    | Forward-GGAGAAATGGACTGTTGCTG      | 119         |
|        |                   | Reverse-CCCCAGAAGGCTCTGCAAT        |             |
| FABP2  | NM_013068.1       | Forward-GGAGAAATGGACTGTTGCTG      | 109         |
|        |                   | Reverse-CCCCAGAAGGCTCTGCAAT        |             |
| β-actin| NM_031144.3       | Forward-GGAGAAATGGACTGTTGCTG      | 165         |
|        |                   | Reverse-CCCCAGAAGGCTCTGCAAT        |             |

Table 2: Effects of L-theanine administration on average daily gain and biochemical parameters in serum of rats.

| Item               | Treatments (mg/kg BW⋅d) | P value          |
|--------------------|-------------------------|------------------|
|                    | 0            | 50             | 200            | 400            | Linear   | Quadratic |
| Average daily gain, g/d | 5.24 ± 0.17b | 6.01 ± 0.17a | 6.15 ± 0.17a | 5.98 ± 0.17a | 0.038    | <0.01     |
| Glucose, mM        | 5.65 ± 0.29a   | 5.31 ± 0.29ab  | 5.74 ± 0.29a   | 4.77 ± 0.30b   | NS       | NS        |
| Insulin, uIU/mL    | 43.2 ± 2.18a   | 41.7 ± 2.18a   | 26.3 ± 2.26b   | 19.0 ± 3.08c   | <0.001   | NS        |
| Cholesterol, mM    | 2.23 ± 0.09    | 2.08 ± 0.09    | 2.13 ± 0.09    | 2.05 ± 0.09    | NS       | NS        |
| Triglyceride, mM   | 1.23 ± 0.07    | 1.21 ± 0.07    | 1.02 ± 0.07    | 1.16 ± 0.07    | NS       | NS        |
| NEFA, mM           | 1.27 ± 0.13    | 1.35 ± 0.10    | 1.45 ± 0.11    | 1.31 ± 0.10    | NS       | NS        |
| Urea, mM           | 5.42 ± 0.16a   | 4.85 ± 0.16b   | 5.70 ± 0.16a   | 5.38 ± 0.17a   | NS       | NS        |
| LDL, mM            | 0.339 ± 0.02   | 0.341 ± 0.02   | 0.354 ± 0.02   | 0.347 ± 0.02   | NS       | NS        |
| HDL, mM            | 1.74 ± 0.06a   | 1.52 ± 0.06b   | 1.60 ± 0.06ab  | 1.60 ± 0.06ab  | NS       | NS        |

BW: body weight, NEFA: non-esterified fatty acids, LDL: low-density lipoprotein, HDL: high-density lipoprotein, and NS: not significant. a-c Means within a row not bearing a common superscript letter differ (P < 0.05). Data were reported as mean ± SE. Data of averaged daily gain were cited by Tong et al. (2016).

AA transporter SLC1a1 was upregulated in the jejunum and ileum (Quadratic, P < 0.001), while jejunal SLC1a2 transcript was linearly decreased (P < 0.001) with the increasing doses of L-theanine but increased by L-theanine treatments in the ileum (Quadratic, P < 0.05). Expression of neutral AA transporter SLC1a5 was increased by doses of L-theanine (jejunum, Linear, P < 0.001; ileum, Linear and Quadratic, P < 0.001); therein the maximal values both occurred in the 400 mg/kg L-theanine treatment. Another neutral AA transporter SLC16a10 expression in the jejunum and ileum was upregulated by doses of L-theanine (Quadratic, P < 0.001). Basic AA transporters SLC7a1 (jejunum, Linear, P <
unchanged by L-theanine treatments (linear, \( P < 0.05 \)) by high doses of L-theanine treatments compared to the 50 mg/kg L-theanine. Ileal GLUT2 expression was upregulated (linear, \( P < 0.01 \)) by increasing doses of L-theanine. Comparing with the 50 mg/kg L-theanine treatment, jejunal GLUT2 expression was suppressed (linear, \( P < 0.05 \)) by 200 mg/kg L-theanine. Ileal GLUT2 expression was upregulated (linear, \( P < 0.01 \)) by 50 mg/kg L-theanine and then inhibited (linear, \( P < 0.05 \)) by high doses of L-theanine treatments compared to 50 mg/kg L-theanine. Jejunal GLUT5 expression was inhibited (linear, \( P < 0.05 \)) by high doses of L-theanine treatment compared to the control group, while its expression in the ileum was linearly decreased (linear, \( P < 0.01 \)) by increasing doses of L-theanine.

The mRNA abundance of the fatty acid transporters and receptors in the intestine of rats is shown in Table 5. Jejunal FATP expression was decreased by L-theanine treatments (linear, \( P < 0.01 \)), while its expression levels in the treatments of 50 and 400 mg/kg L-theanine were lower (linear, \( P < 0.01 \)) than that of control group and 200 mg/kg L-theanine treatment. Ileal FATP expression was not affected by L-theanine treatments (linear, \( P > 0.05 \)). Jejunal GPR43 expression was unchanged by L-theanine treatments (linear, \( P > 0.05 \)). However, its expression in the ileum of 50 mg/kg L-theanine treatment was decreased (linear, \( P < 0.05 \)) compared with control group and 200 mg/kg L-theanine treatment. GPR120 (jejenum, linear and quadratic, \( P < 0.001 \); ileum, linear, \( P < 0.001 \)) and FABP2 (jejenum, linear, \( P < 0.001 \); ileum, linear and quadratic, \( P < 0.001 \)) expression levels were both suppressed by L-theanine treatments.

### 4. Discussion

To the best of our knowledge, this experiment is a new attempt to investigate the link between serum nutrients and the expression of nutrient-associated transporters and receptors in the small intestine of L-theanine-administered rats. In this study, the declines of glucose, insulin, and urea in the serum were observed by L-theanine administration, indicating that L-theanine could inhibit the absorption of glucose, nitrogen, and secretion of insulin. Our results are partly in line with the data of Yamada et al. (2008) which observed reduced insulin level with unchanged glucose concentration in the serum of rats administrated by 4 g/kg oral L-theanine. These results are inconsistent with the findings of Zheng et al. (2004) which discovered that TG and NEFA levels in the serum of mice were decreased by 0.03% L-theanine administration. This discrepancy appears to be due to the dosage of L-theanine ingested, method of administration, and experimental period.

The upregulating effects of L-theanine are reflected in the AA transporters expression at the mRNA level in small intestine in this study, except SLC1a2. This finding can partly explain the increased AAs concentrations in rat serum after L-theanine ingestion [11], including acidic acid (aspartic acid and glutamic acid), neutral acid (glutamine), and basic acid (histidine) (see Supplemental Table 1 in [11]; see Supplementary Material available online at [https://doi.org/10.1155/2017/9747256]), indicating that L-theanine promotes the AAs absorption in rat small intestine. The opposing effect of L-theanine on jejunal SLC1a2 expression was observed, reflecting that asparagine absorption in
the jejunum might be blocked by L-theanine. Although direct evidences about the regulatory mechanism of AA transporters transcription by L-theanine are lacking, previous literatures showed that activating transcription factor 4 (ATF4) could transcriptionally upregulate SLC7a1 [24] and regulatory factor X proteins (RFXs) induced mRNA of SLC1a5 [25]. After MatInspector online analysis [26], we find that there are ATF4 binding sites in the promoter regions of SLC7a1 (between nucleotides +10 and +18) and SLC7a9 (between nucleotides −155 and −146) genes and RFXs located in SLC1a5 (between nucleotides −239 and −86) promoter sequence. Additionally, elements for E-box binding factors (EBOX) and CAMP-responsive element binding proteins (CREB) binding are identified in the promoter sequences of SLC1a5, SLC7a1, and SLC7a9 genes. Therefore, we speculated that L-theanine, as an amino acid, changed SLC7a1, SLC1a5, SLC7a1, and SLC7a9 mRNA transcription via acting with ATF4, RFX, EBOX, and CREB proteins.

Glucose transporting from the intestinal lumen to the blood mainly depends on Na⁺-glucose cotransporter SGLT1, which absorbs glucose and galactose and the passive glucose transporter GLUT2, which acts as a glucose sensor [27–30]. SGLT3 is also a glucose sensor in cholinergic neurons neighboring enterocytes and induces membrane currents upon Na⁺-glucose binding [27]. GLUT5 is primarily in charge of fructose absorption into the cytosol. Although decreases in SGLT1 and GLUT2 mRNA abundance in the intestine of rats receiving 200 mg/kg L-theanine, in which glucose absorption was declined, were not observed in this study, we found that intestinal SGLT3 and ileal GLUT5 transcripts in L-theanine-ingested rats were decreased in a dose-dependent manner. These results indicated that rats intestinal GLUT2 was less impressible than GLUT5 to L-theanine administration at the transcriptional level, and SGLT3 and GLUT5 genes rather than SGLTI and GLUT2 play a role in intestinal glucose absorption of L-theanine-ingested rats. It is reported that period circadian clock 1 (PER1) exerted an indirect suppressive effect on rat SGLT1 promoter [31] and hepatic nuclear factors (HNF) regulated SGLT1 and GLUT2 promoter activities [32, 33]. By analyzing [26], we also identified HNF-element located in SGLT3 and peroxisome proliferator-activated receptor (PPARG) element encompassed in GLUT5 promoter regions. Therefore, we predicted that L-theanine may target transcription factors (PER1, HNF, and PPARG)
and further inhibit the expression of glucose transporters mRNA.

It is reported that GPR43 binds short-chain fatty acids, whereas GPR120 responds to medium and long chain fatty acids [34, 35]. FABP2 also displays high-affinity binding for long chain fatty acids and is believed to be involved with uptake and trafficking of lipids in the intestine [21]. In the present study, GPR120 and FABP2 transcripts in jejunum and ileum were decreased by L-theanine. Jejunal FABP mRNA was also suppressed by 50 mg/kg and 400 mg/kg L-theanine. However, triglyceride and cholesterol contents in the serum of L-theanine-treated rats were not affected (Table 2). These results state that the intestinal uptake of dietary fatty acids might have been inhibited by L-theanine. Further research is needed to explore the regulatory mechanism of L-theanine on intestinal uptake of dietary lipids.

In summary, L-theanine administration had decreased serum glucose probably by inhibiting intestinal SGLT3 and GLUT5 mRNA expression in rats. Dietary fatty acids uptake might be suppressed by downregulating GPR120 and FABP2 transcripts in the intestine of rats. Meanwhile, intestinal transporters responding to AAs absorption were upregulated by L-theanine administration. Our data provide theoretical basis for further investigation of L-theanine and nutrients interaction.

Abbreviations

AA: Amino acid
ATF4: Activating transcription factor 4
cDNA: Complementary DNA
CREB: cAMP-responsive element binding proteins
ddH₂O: Distilled water
EBOX: E-box binding factors
ELISA: Enzyme-linked immunosorbent assay
FABP2: Fatty acid binding protein 2
FATP: Fatty acid transport protein
GLUT2: Glucose transporter protein, member 2
GLUT5: Glucose transporter protein, member 5
GPR43: G-protein-coupled receptor 43
GPR120: G-protein-coupled receptor 120
HLDL: High-density lipoprotein cholesterol
HNF: Hepatic nuclear factors
LDL: Low-density lipoprotein cholesterol
mRNA: Messenger RNA
NEFA: Non-esterified fatty acids
PER1: Period circadian clock 1
PPARG: Peroxisome proliferator-activated receptor
RXFs: Regulatory factor X proteins
SLC1a1: Solute carrier family 1, member 1
SLC1a2: Solute carrier family 1, member 2
SLC1a5: Solute carrier family 1, member 5
SLC1a10: Solute carrier family 16, member 10
SLC7a1: Solute carrier family 7, member 1
SLC7a9: Solute carrier family 7, member 9
SGLT1: Sodium dependent glucose transporter 1
SGLT3: Sodium dependent glucose transporter 3
SD: Sprague Dawley
TG: Triglyceride.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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