Original Article

Chlorogenic Acid Maintains Glucose Homeostasis through Modulating the Expression of SGLT-1, GLUT-2, and PLG in Different Intestinal Segments of Sprague-Dawley Rats Fed a High-Fat Diet*

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Abstract

Objective To reveal the effects and related mechanisms of chlorogenic acid (CGA) on intestinal glucose homeostasis.

Methods Forty male Sprague-Dawley rats were randomly and equally divided into four groups: normal chow (NC), high-fat diet (HFD), HFD with low-dose CGA (20 mg/kg, HFD-LC), and HFD with high-dose CGA (90 mg/kg, HFD-HC). The oral glucose tolerance test was performed, and fast serum insulin (FSI) was detected using an enzyme-linked immunosorbent assay. The mRNA expression levels of glucose transporters (*Sglt-1* and *Glut-2*) and proglucagon (*Plg*) in different intestinal segments (the duodenum, jejunum, ileum, and colon) were analyzed using quantitative real-time polymerase chain reaction. SGLT-1 protein and the morphology of epithelial cells in the duodenum and jejunum was localized by using immunofluorescence.

Results At both doses, CGA ameliorated the HFD-induced body weight gain, maintained FSI, and increased postprandial 30-min glucagon-like peptide 1 secretion. High-dose CGA inhibited the HFD-induced elevation in *Sglt-1* expression. Both CGA doses normalized the HFD-induced downregulation of *Glut-2* and elevated the expression of *Plg* in all four intestinal segments.

Conclusion An HFD can cause a glucose metabolism disorder in the rat intestine and affect body glucose homeostasis. CGA can modify intestinal glucose metabolism by regulating the expression of intestinal glucose transporters and *Plg*, thereby controlling the levels of blood glucose and insulin to maintain glucose homeostasis.

Key words: Chlorogenic acid; High-fat diet; Intestine; Glucose homeostasis; SGLT-1; GLUT-2; PLG; GLP-1

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INTRODUCTION

A high-fat diet (HFD) induces a glycometabolic disorder and impairment of insulin sensitivity[1-2]. Maintenance of glucose homeostasis is a crucial component of human physiology and is thus under strict control; failure of these control mechanisms can result in a metabolic syndrome encompassing obesity, hyperglycemia, impaired glucose tolerance, hypertension, and dyslipidemia.

Accumulating studies have demonstrated a potential role for bioactive polyphenols as an adjunct to the treatment of metabolic syndrome[3-4].

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Chlorogenic acid (CGA) is an important group of biologically active dietary phenols, which can be ubiquitously found in coffee, plants, and several types of fruits and vegetables, such as apples, pears, tomatoes, blueberries, potatoes, peanuts, and eggplant[5-6]. CGA is an ester formed from quinic acid and cinnamic acid, with several isomers, and the most common form is 5-caffeoylquinic acid (5-CQA)[7]. CGA is also well known for its beneficial biological properties, such as anti-oxidant, anti-bacterial, and anti-inflammatory effects[8-9].

Recently, CGA was found to have the ability to modify glucose metabolism[10-11]. The intestine is the key site of the maintenance of glucose homeostasis; however, the specific mechanism underlying this process remains unclear. Johnston et al.[12] suggested that CGA might attenuate intestinal glucose absorption rates and shift the site of glucose absorption to more distal parts of the intestine. In the results of Ruan' research suggest that CGA supplementation results in perturbation of energy and amino acid metabolism in rat[13], also can decrease intestinal permeability and increases intestinal expression of tight junction proteins in weaned rats challenged with LPS[14]. We further hypothesized that supplemental CGA would increase insulin secretion[15], modulate glucose transport[16], reduce weight gain[17], and normalize blood glucose levels[18]. Evidence has suggested that sodium glucose transport protein-1 (SGLT-1) as well as glucose transporter-2 (GLUT-2) may play a major role in mediating intestinal glucose transport across the brush-border membrane of enterocytes[19]; both proteins play a key role in the transport of glucose into cells and in maintaining glucose homeostasis.

The aims of this study were to examine whether CGA influences the expression levels of the *Sglt-1*, *Glut-2*, and proglucagon (*Plg*) genes in different intestinal segments of Sprague-Dawley (SD) rats fed an HFD, and the consequent effects on modulating glucose homeostasis. Therefore, we evaluated the effects of CGA on rat weight gain and the results of an oral glucose tolerance test (OGTT) performed in HFD-fed SD rats, as well as its effects on the expression of the *Sglt-1*, *Glut-2*, *Plg*, and glucagon-like peptide 1 (*Glp-1*) genes in different intestinal segments of rats. Moreover, fasting serum insulin (FSI) and postprandial 30-min *Glp*-1 production levels were determined.

MATERIALS AND METHODS

Animals and Experimental Treatments

Forty male SD rats (4 weeks old, 120-140 g) were purchased from Hunan SJA Laboratory Animal Company Limited (HNASLKJ20120229; Changsha, China). The rats were housed in groups of two per cage at 22±1 °C and 40%-60% relative humidity with a 12-h light/dark cycle, and received a chow diet and water *ad libitum.* One week after acclimatizing feeding, the rats were randomly assigned to four groups (*n*=10 per group): the normal control group (NC), high-fat diet group (HFD), HFD with low-dose 5-CQA group (>95% Phytopurify, Chengdu, China; 20 mg/kg; HFD-LC), and HFD with high-dose 5-CQA group (90 mg/kg; HFD-HC). The NC rats were fed a normal chow diet (College of Xiangya Medicine, Central South University, Changsha, China) and the others were fed an HFD (Table 1). The 5-CQA was dissolved in sterile saline, and the CGA intervention groups were individually treated with 5-CQA by gavage needle once a day, whereas the NC and HFD groups were only administered sterile saline. Animal body weight was recorded weekly and food consumption was recorded daily. All experimental protocols were approved by the committee on the Regulation on Management of Experimental Animals (Hunan Province, No. 259, 2012).

OGTT

After 11 weeks of the dietary treatments, an OGTT was performed. The rats were fasted for 12 h, and blood was collected (via the tail vein) before (*t*=0 min) and after (*t*=30, 60, and 120 min) administration of 2.5 g/kg glucose by gavage. The OGTT results were determined with Accu-Chek Advantage II Test Strips (Roche Diagnostics GmbH, Mannheim, Germany). The level of plasma glucose measured at 0 min represents the fasting blood glucose (FBG) level.

Table 1. Composition of the High-fat Diet

Ingredient	Percentage (%)
Normal chow diet	40.2
Egg	19.7
Sucrose	17.5
Soybean oil	2.0
Lard	20.0
Choline bitartrate	0.6

Measurement of FSI

The blood sample (obtained at 0 min) was centrifuged at 3000 ×*g* at 4 °C for 10 min, and then FSI was determined by an enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Rat Insulin ELISA KIT, Bio-Swamp, Shanghai, China) with the RT-6000 enzyme analytical apparatus (Rayto Life and Analytical Sciences Co., Ltd, Shenzhen, China). Insulin resistance was estimated based on homeostatic model assessment-insulin resistance (HOMA-IR) with the following formula: HOMA-IR = FBG (mmol/L) × FSI (mU/L)/22.5[20].

Sample Collection

Twelve weeks after treatment, each rat was deprived of food and water for 12 h, received 2.5 g/kg of glucose by gavage, anesthetized with intraperitoneal injection of sodium pentobarbital (60 mg/kg), and then killed by exsanguination from the ophthalmic artery. Blood was collected via the ocular vein into ethylenediaminetetraacetic acid tubes containing aprotinin for assessment of GLP-1(7-36) levels. Following sacrifice, the duodenum, jejunum, ileum, and colon were removed immediately from the rats of all four groups. The 1-cm front-end segments of the duodenum and jejunum were collected, washed with saline solution, and fixed in 10% formaldehyde. The remaining samples were cleared of food residue by washing with a Sitagliptin-saline mixture (4 °C, 400 mg/L), frozen immediately in liquid nitrogen, and stored at -80 °C.

The GLP-1 (7-36) amide level was measured using an ELISA kit (GLP-1 active ELISA kit, Linco Research, Inc., St Charles, MO, USA) and the RT-6000 enzyme analytical apparatus.

Reverse Transcription-polymerase Chain Reaction (RT-PCR) Analysis of Gene Expression

Total RNA was extracted by the single-step method with Trizol reagent (Invitrogen, Carlsbad, CA, USA). Single-stranded cDNA was synthesized using the ABI 2720 Thermal cycler (Applied Biosystems, Foster City, CA, USA) with the Revert Aid first-strand cDNA synthesis kit (Thermo, Beijing, China). Quantitative real-time PCR assays were performed to determine the expression levels of *Sglt-1* (primers: forward, 5'-GGA CCA GGA TAA AGG ACC GAA-3'; reverse: 5'-AAA TAG GCG TGG CAG AAG ACA-3'), *Glut-2* (primers: forward, 5'-CCA GCA CAT ACG ACA CCA GAC G-3'; reverse, 5'-CCA ACA TGG CTT TGA TCC TTC C-3'), and *Plg* (primers: forward, 5'-CTC CTC GTT TTG AAG TTA CCG C-3'; reverse, 5'-AGC GCA TTT ATG ACA AAG GGT T-3') in different intestinal segments (the duodenum, jejunum, ileum, and colon) using the 7300 real-time PCR system (Applied Biosystems), following the SuperReal PreMix Plus manufacturer protocol (SYBR Green, Tiangen Biotech Co. Ltd., Beijing, China). Reactions were prepared in triplicate and the results were normalized to *β*-actin expression (primers: forward, 5'-AGA TTA CTG CCC TGG CTC CTA G-3'; reverse, 5'-GAC TCA TCG TAC TCC TGC TTG C-3').

Immunofluorescence

Tissue samples were incubated with an antibody against SGLT-1 (H-85; sc-98974, Santa Cruz Biotechnology, Dallas, TX, USA). Labeling with rabbit SGLT-1 antibody (dilution 1:60-70) was subsequently performed on the same sections with secondary detection by IgG antibody (Hebei Bio-high Technology Deve Co. Ltd, Shijiazhuang, China). Microscopic observations were conducted at 400× magnification and sequential micrography images were obtained.

Statistical Analysis

Data are expressed as mean±SEM. Statistical analysis was performed with SPSS 17.0 [one-way analysis of variance (ANOVA), with least-squares differences]. The differences between the means using Duncan's multiple-range test were considered statistically significant at $P<0.05$.

RESULTS

CGA Reduced the HFD-induced Body Weight Gain

There was no significant difference in the initial body weight among the four experimental groups (Table 2). However, at the end of the experimental period, the final body weight and body weight gain of the HFD-LC group was significantly lower ($P<0.05$) than that of the HFD group, whereas there were no statistically significant differences between the two CGA intervention groups.

CGA Increased FSI Levels in HFD-fed Rats

As shown in Table 3, the blood glucose levels of all four experimental groups were in the normal range in the OGTT. One-way ANOVA showed that there was no significant difference in blood glucose concentration between the NC group and either of

the CGA treatment groups at 60 min. However, compared to the NC group, the blood glucose level of the HFD group was significantly higher at 0 min, 30 min, 60 min, and 120 min (P<0.01, P<0.01, P<0.05, and P<0.01, respectively); that of the HFD-LC group was significantly higher at 30 min (P<0.05); and that of the HFD-HC group was significantly higher at 0 min and 30 min (P<0.05 and P<0.01, respectively). By contrast, compared to the HFD group, the blood glucose level of the HFD-LC group was significantly lower at 0 and 120 min (P<0.05, P<0.01, respectively), and that of the HFD-HC group was significantly lower at 120 min (P<0.05). The FSI in the HFD group was increased by 13.5% (P<0.05) compared to that of the NC group. The serum GLP-1 levels in both CGA groups were respectively increased by 42.7% and 35.3% compared with that of the HFD group, and were even slightly increased compared with that of the NC group. These results demonstrated that CGA supplementation could stabilize FSI levels.

CGA Prevented the HFD-induced Elevation of Sglt-1 Expression in Different Intestinal Segments

As illustrated in Figure 1, compared with the NC group, the HFD significantly enhanced the expression of *Sglt-1* mRNA in both the jejunum and ilium by 44% (P<0.01). There was no significant difference in *Sglt-1* mRNA expression in the four intestinal segments between the HFD-LC and HFD groups. The HFD-HC reduced *Sglt-1* expression in the jejunum by 24.2% and in the ileum by 26.4% compared

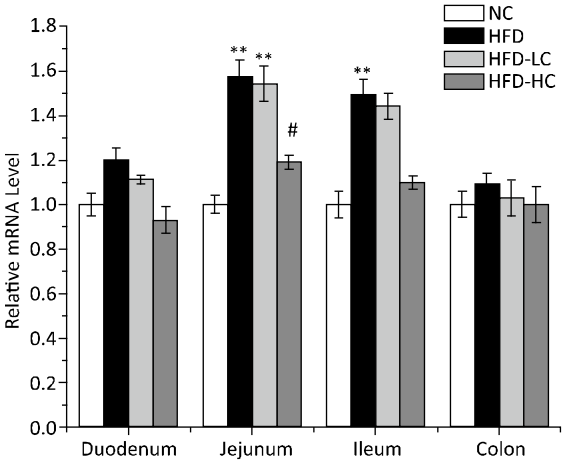

Figure 1. Effect of CGA on *Sglt-1* mRNA expression on duodenum, jejunum, ileum, and colon in different groups of rats. Rats were treated with CGA (20 mg/kg or 90 mg/kg) for 12 weeks. All the data are expressed as mean±SEM, n=10 per group. $^{**}P$<0.01 *vs.* NC group; $^{\#}P$<0.05 *vs.* HFD group.

Table 2. Effect of CGA on Physiological and Metabolic Variables of Sprague-Dawley Rats on a High-fat Diet (HFD)

Variables	NC	HFD	HFD-LC	HFD-HC
Initial body weight (g)	130.45±1.54	129.57±1.87	130.94±1.96	131.03±1.63
Final body weight (g)	503.85±8.03	522.72±7.51*	469.60±13.09$^{\#}$	489.98±16.18$^{*\#}$
Body weight gain (g)	369.98±5.88	391.32±5.56*	349.08±11.21$^{\#}$	337.76±10.27$^{*\#\#}$
Food intake [g (day rat)$^{-1}$]	26.79±0.50	24.18±0.91	23.11±0.87	24.98±0.72
Energy intake [kJ (day rat)$^{-1}$]	0.18±0.01	0.25±0.02**	0.24±0.01**	0.25±0.03**
Epididymal fat (g kg^{-1})	8.54±095	19.32±0.35**	14.94±0.83$^{**\#}$	12.41±1.12$^{*\#\#}$
Perirenal fat (g kg^{-1})	10.81±0.49	21.71±0.02**	17.89±0.26$^{*\#}$	14.91±0.56$^{*\#\#}$
Visceral adipose (g kg^{-1})	18.72±0.46	40.02±1.05**	29.06±0.18$^{*\#}$	26.01±0.73$^{*\#\#}$

***Note*.** Data represent the mean±SEM (n=10), $^{*}P$<0.05, $^{**}P$<0.01 *vs.* the NC group; $^{\#}P$<0.05, $^{\#\#}P$<0.01 *vs.* the HFD group.

Table 3. Effect of CGA Treatment on OGTT Results, Fasting Serum Insulin (FSI), and GLP-1 Levels of Sprague-Dawley Rats on a High-fat Ddiet (HFD)

Group	Blood Glucose (mmol/L)				FSI (mU/L)	HOMA-IR	Serum GLP-1 (pmol/L)
	0 min	30 min	60 min	120 min			
NC	3.8±0.1	5.8±0.1	5.9±0.1	5.0±0.1	14.1±0.54	2.4±0.62	11.9±0.13
HFD	5.3±0.1**	7.2±0.2**	6.4±0.1*	6.0±0.1**	16.0±0.52*	3.8±0.5*	9.53±0.22*
HFD-LC	3.9±0.2$^{\#}$	6.6±0.3*	6.2±0.2	5.2±0.1$^{\#\#}$	15.5±0.52$^{\#}$	2.7±0.1$^{\#}$	13.6±0.21$^{\#\#}$
HFD-HD	3.8±0.2*	6.7±0.2**	6.2±0.1	4.9±0.1$^{\#\#}$	14.8±0.49$^{\#\#}$	2.5±0.59$^{\#}$	12.9±0.11$^{\#\#}$

***Note*.** $^{*}P$<0.05, $^{**}P$<0.01 *vs.* NC group; $^{\#}P$<0.05, $^{\#\#}P$<0.01 *vs.* HFD group.

with the HFD group (P<0.05); reduction in expression levels were also observed in the duodenum and colon, by 16% and 8%, respectively, but the differences were not statistically significant. With respect to the effect of CGA dose, the expression of *Sglt-1* in the jejunum of the HFD-LC group was significantly higher than that of the HFD-HC group (P<0.05).

CGA Prevented the HFD-induced Reduction in Glut-2 in Different Intestinal Segments

As shown in Figure 2, *Glut-2* expression was reduced in all three HFD-fed groups compared to the NC groups. The expression of *Glut-2* mRNA in the HFD group was significantly lower (P<0.05) than that of the NC group in the duodenum, jejunum, and ileum, whereas the difference was significant between the HFD-LC group and NC group in the jejunum and ileum only (P<0.05). In both the duodenum and jejunum, the high dose of CGA (HFD-HC) significantly enhanced the expression of *Glut-2* mRNA compared with the HFD group (P<0.05).

CGA Upregulated the mRNA Expression of Plg in Different Intestinal Segment of HFD-fed SD Rats

As summarized in Figure 3, the expression of *Plg* mRNA increased in the four intestinal segments in all treatment groups compared to the NC group, although there was no significant difference in the duodenum. In particular, compared with the NC group, expression of *Plg* in the HFD-LC group increased significantly in the jejunum, ileum, and colon (P<0.01, P<0.05, and P<0.01, respectively), and the expression in the HFD-HC group was significantly up-regulated in the jejunum (P<0.05). Moreover, compared with the HFD group, the low-dose CGA treatment significantly up-regulated the expression of *Plg* mRNA in the jejunum and ileum (P<0.05); however, there was no dose-dependent effect.

CGA Affected SGLT-1 Immunofluorescence and the Morphology of Epithelial Cells in the Duodenum and Jejunum

The positive area of immunofluorescence for SGLT-1 is illustrated in Figure 4; and there was no substantial difference among the four groups in the duodenum. The expression of SGLT-1 in the high-dose CGA was markedly reduced in the jejunum compared to that of the HFD group, whereas there was no similar effect observed with treatment of the low dose of CGA. These data were consistent with the results from RT-PCR. Figures 5 and 6 show the microscopic images of the immunofluorescence of SGLT-1 in the sections of the duodenum and jejunum under 400× magnification. With respect to the physical appearance, the intestinal epithelial cells were arranged neatly and the structures were tight

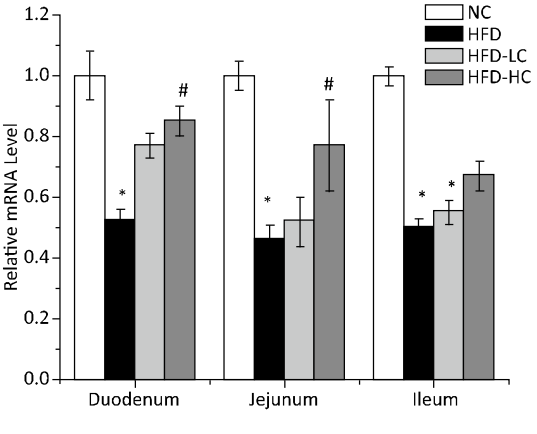

Figure 2. Effect of CGA on *Glut-2* mRNA expression in duodenum, jejunum, and ileum in different groups of rats. Rats were treated with CGA (20 mg/kg or 90 mg/kg) for 12 weeks. All the data are expressed as mean±SEM, n=10 per group. $^{*}P$<0.05 *vs.* NC group; $^{\#}P$<0.05 *vs.* HFD group.

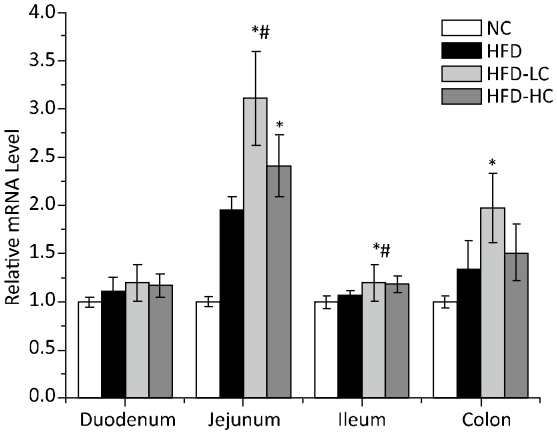

Figure 3. Effect of CGA on *Plg* mRNA expression in duodenum, jejunum, ileum, and colon in different groups of rats. Rats were treated with CGA (20 mg/kg or 90 mg/kg) for 12 weeks. All the data are expressed as mean±SEM, n=10 per group. $^{*}P$<0.05 *vs.* NC group; $^{\#}P$<0.05 *vs.* HFD group.

and orderly in the NC group, whereas in the HFD group, the intestinal epithelial cells appeared oval and were arranged loosely and disorderly; moreover, the number of goblet cells was increased in the HFD group compared to the NC group. The structures of the intestinal epithelial cells in the CGA intervention group were similar to those of the NC group, especially for the low-dose CGA group.

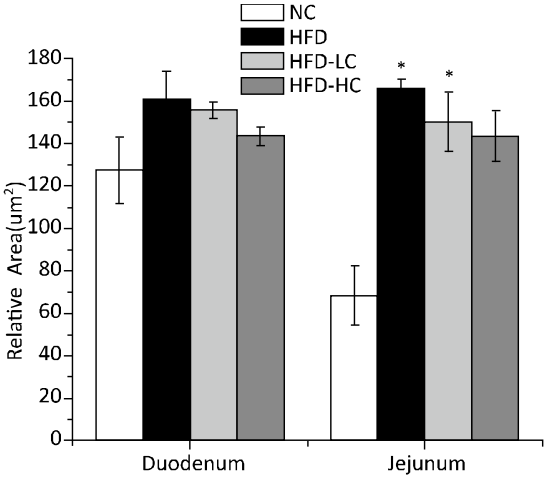

Figure 4. Effect of CGA on the SGLT-1-positive area as seen by immunofluorescence in the duodenum and jejunum in different groups of rats. Rats were treated with CGA (20 mg/kg or 90 mg/kg) for 12 weeks. All the data are expressed as mean±SEM, *n*=10 per group. *P<0.05 *vs.* NC group.

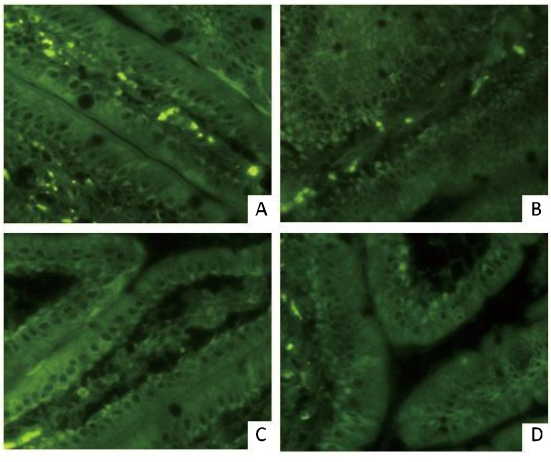

Figure 5. Effect of CGA on the SGLT-1 immunofluorescence sections in the duodenum under 400× magnification. A: NC group (× 400), B: HFD group (× 400), C: HFD-LC group, (× 400), D: HFD-HC group (× 400).

DISCUSSION

Fruits and coffee are the major sources of chlorogenic acid in human diet[21]. To define the dose of CGA to the experimental rats, it was crucial to confirm that the dose was akin to daily human intake. Coffee is a major source of chlorogenic acid in human diet, daily intake in coffee drinkers is 0.25-1 g[22-23], this corresponded to approximately 3.57 mg/kg and 14.29 mg/kg for a 70 kg man; the dose for Sprague-Dawley rats is generally five to six times than that for humans[24-25]; 20 mg/kg and 90 mg/kg were chosen for the low and high dose of CGA in this study.

At the beginning of the study, there was no significant difference in the body weight among the four groups of rats. However, at the end of the study, the central obesity of the CGA-treated group was lower than that of the HFD group, with a significant effect observed for the low-dose CGA group. A previous study demonstrated that CGA did not influence the daily food intake of the animals[26], and exerted effects on visceral fat and body weight by promoting the metabolism and utilization of fat *in vivo*[27], whereas other investigators suggested that CGA inhibited preadipocyte population growth, which provides a potential mechanism for its effects on obesity reduction[28].

The results of the OGTT and FSI and GLP-1 level assessments showed that CGA could regulate the concentration of blood glucose rapidly and effectively

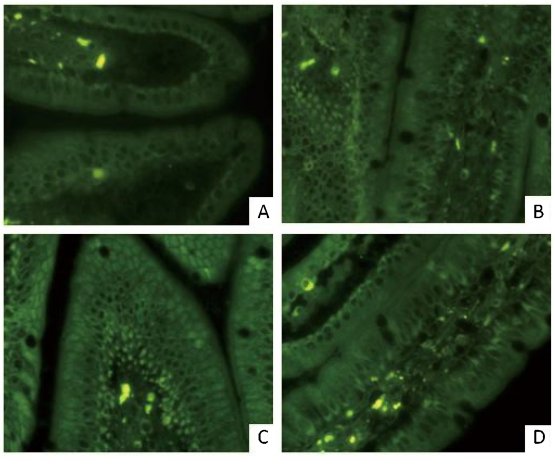

Figure 6. Effect of CGA on the SGLT-1 immunofluorescence sections in the jejunum under 400× magnification. A: NC group (× 400), B: HFD group (× 400), C: HFD-LC group (× 400), D: HFD-HC group (× 400).

to ultimately modify the balance of glucose metabolism. These results are consistent with previous findings that CGA could effectively adjust the fasting and postprandial blood glucose levels[29]. Accordingly, utilization of glucose as an energy substrate could decrease the amount of glucose in circulation due to the hypoglycemic effect of CGA. Our data also showed that the HFD resulted in an earlier peak of the OGTT compared to that of the NC group.

Insulin is a hormone that is produced by islet beta cells under the stimulation of endogenous or exogenous substances, and plays the role of hypoglycemic action in the body. In this experiment, the insulin level of the HFD group was slightly lower than that of the NC group, suggesting that long-term exposure to the HFD reduced the sensitivity of islet beta cells. The insulin levels of the CGA intervention groups were intermediate between those of the HFD and NC groups. Specifically, the insulin level of the HFD-HC group was similar to that of the NC group, but was significantly higher than that of the HFD group. This result confirmed that CGA could regulate insulin secretion. One potential mechanism to explain this result might be that CGA increases the sensitivity of islet beta cells in a high blood glucose environment, thereby maintaining insulin secretion and regulating blood glucose levels.

Kojima et al.[30] reported that *Sglt-1* mRNA was expressed mainly in the SD rat small intestine (duodenum, jejunum, and ileum), but not in the colon, as determined by northern blot analysis. With respect to the localization of SGLT-1 in the intestine, Lenzen et al.[31] found that SGLT-1 expression was typically higher in duodenum and proximal jejunum, and decreased towards the ileum in control rats. Our results generally corresponded with these previous studies, with the exception that weak expression of SGLT-1 was detected in the colon in our study; this slight difference might have resulted from the difference in the equipment used and/or methods of analysis, or due to the influence of age, and the small intestine sampling location.

Accumulating evidence indicates that dietary polyphenols influence glucose metabolism. Most of the dietary carbohydrate is digested to monosaccharides in the upper gastrointestinal tract; these are then absorbed into the circulation. CGA has been highlighted for its inhibitory effects on intestinal glucose uptake and hepatic glucose 6-phosphatase activity, and some extracts of plants rich in CGA have been found to inhibit SGLT-1-mediated glucose transport[32-33]; however, the underlying mechanism of these effects remains uncertain. The results of the present study indicate that the high dose of CGA inhibited the upregulated expression of *Sglt-1* in different intestinal segments induced by the HFD. However, the expression of SGLT-1 did not differ significantly between the HFD-LC group and HFD group, indicating that the low dose of CGA is not sufficient to prevent the induction of *Sglt-1* expression.

Until recently, the mechanism underlying the inhibitory effect of polyphenols on glucose uptake has been controversial. Some studies indicated that tea polyphenols could act as antagonist-like molecules to inhibit the transport activity of SGLT-1 in a competitive manner[34], with a possible influence on sodium-dependent glucose transportation. However, no similar study has been reported with respect to CGA. The present results demonstrated that the high dose of CGA inhibited *Sglt-1* mRNA expression, and the results of SGLT-1 immunofluorescence in the duodenum and jejunum demonstrated that CGA would cause the total rate of sodium-dependent glucose transportation to decline, thus maintaining a steady state of postprandial blood glucose. A previous *in vitro* study showed that treatment with a high dose of CGA (1 mmol/L) could inhibit sodium-dependent glucose transportation in the intestine, whereas no such inhibitory effect was observed when Caco-2 cells were treated with 100 μmol/L CGA[35]. In the present study, high-dose CGA did not alter SGLT-1 expression levels from those of the control group; hence, we speculated that the effect of high-dose CGA on glucose transportation inhibition is due to the suppression of the HFD-induced up-regulated of SGLT-1. In other words, CGA has the ability to maintain normal glucose-uptake rates in spite of a HFD.

GLUT-2 provides a glucose entry pathway to fulfill the metabolic needs of enterocytes in the intestinal glucose absorption metabolism system. The present study results showed that the expression of *Glut-2* was significantly decreased in the HFD group compared to that of the NC group by 48%, 52%, and 50% in the duodenum, jejunum, and ileum respectively; on the other hand, this downregulation was reversed with CGA intervention at different concentrations, especially at a high dose, in which the *Glut-2* expression levels in the duodenum and jejunum were similar to those of the control rats.

Therefore, the present results collectively

indicate that CGA intervention could decrease the amount of luminal glucose transported into the enterocytes by SGLT-1 and enhance glucose transportation out of the cell via GLUT-2 under HFD conditions. Our previous study showed that CGA increased GLUT-4 expression in the skeletal muscle of female Kunming mice fed a high-fat emulsion[36]. Therefore, we deduced that CGA is likely to maintain the rate of glucose transport out of enterocytes, increase peripheral tissue glucose uptake, and decrease liver glucose production. Accordingly, we suggest that CGA regulates rat postprandial blood glucose levels through stimulation of glucose utilization by peripheral tissues under the HFD condition. As an antihyperglycemic drug used in the treatment of type 2 diabetes, metformin (dimethylbiguanide) was reported to increase the expression of the hexose transporter gene, which could in turn increase hexose uptake at the brush-border membrane[37]. Moreover, metformin was also demonstrated to enhance the accumulation of GLUT-2 in the apical membrane[38]. Together, these findings suggest that GLUT-2 would transport glucose from the circulation to the lumen. Thus, it would be interesting to investigate whether CGA functions by a similar mechanism to metformin.

In a previous study, the expression of GLUT-2 was also found to be up-regulated in the intestine of streptozotocin-induced diabetic rats in the brush-border membrane, but was down-regulated in the basolateral membrane; an HFD also induced higher expression of GLUT-2 in the apical enterocytes of these rats[39]. In the present study, SD rats fed an HFD showed significantly decreased levels of GLUT-2 expression in different intestinal segments, indicating that the process for glucose transport out of the enterocytes was inhibited. Although the HFD resulted in down-regulation of GLUT-2, CGA intervention reversed the suppression so that the regular transport of glucose was ensured.

GLP-1 is one of the active peptides that is encoded by *Plg*. In a high blood glucose environment, GLP-1 can promote the secretion of insulin and restrain glucagon release, and thus plays a significant role in maintaining a blood sugar balance[40-41]. In the present study, the HFD increased the expression of *Plg* in the intestine, which was likely due to strengthening of intestinal glucose metabolism to adapt to the metabolic stress from the eutrophic conditions. The expression of *Plg* was further up-regulated in rats fed the HFD that received CGA intervention. This effect of CGA on improving *Plg* expression indicates that it could facilitate GLP-1 release by the intestinal L-cells; this may explain why drinking coffee rich in CGA or eating extracts of CGA-rich plants could increase the release of GLP-1[15,42]. CGA might also influence the production of incretin hormones. Indeed, McCarty et al.[43] proposed that coffee increased the production of the incretin hormone GLP-1, possibly owing to an inhibitory effect of CGA (the chief polyphenol in coffee) on glucose absorption. A corollary of this hypothesis is that administration of supplemental CGA with meals (without caffeine) should lower the glycemic index of the meal while promoting GLP-1 production and reducing the risk for diabetes. Our experimental results support these hypotheses, as CGA upregulated the expression of GLP-1.

The expression of *Plg* in the HFD-LC group was higher than that in the HFD-HC group of SD rats, with a particularly significant difference in the jejunum and colon. These findings might be related to the influence of the dose of CGA on the glucose transporter expression. Similarly, the HFD-induced elevated expression of *Sglt-1* was inhibited by the high dose of CGA; in other words, there was less glucose transported into the intestinal epithelial cells, and high-dose CGA could accelerate glucose transportation from the intestinal epithelial cells into the blood circulation, thus resulting in relatively less accumulation of glucose in the intestinal epithelial cells. Secretion of the intestinal proglucagon-derived peptides is under the regulatory control of a wide variety of intestinal endocrine and neurocrine peptides, as well as nutrients (fats) and neurotransmitters[44]; indeed, the expression of *Plg* was reduced in the high-dose CGA intervention group. An *in vitro* study suggested that GLP-1 secretion was impeded by the inhibitory effect of phloridzin on sodium-dependent glucose transportation[45], and demonstrated the relationship between sodium-dependent glucose transportation and GLP-1 secretion from another point of view.

Low-dose CGA treatment did not have a repressive effect on HFD-induced *Sglt-1* expression. This suggests that the transport capacity of GLUT-2 under low-dose CGA intervention was weaker than that under the high dose of CGA intervention, leading to increased glucose accumulation in the intestinal epithelial cells, which could in turn stimulate the expression of *Plg*. Given the patterns of expression in these two transporters, it appears that the HFD group had the highest accumulation of glucose in the intestinal epithelial cells, but PLG

expression was lowest in the different intestinal segments. Together, this suggests that *Plg* responds to metabolic stress more quickly under eutrophic conditions with CGA intervention.

The *Plg* gene encodes GLP-1 and glucagon. GLP-1 can promote the secretion of insulin and restrain glucagon release. GLP-1 secreted by the intestinal L-cells in response to nutrient ingestion and is responsible for up to 70% of the insulin response after a meal[46]. GLP-1 could suppress appetite and food intake, so that GLP-1 receptor knockout mice do not become obese[47]. Glucagon produced by islet α-cells play opposite roles in the glucose homeostasis mechanism. Indeed, a recent study demonstrated that *Plg* expression was activated in a cell type-specific manner[48].

Glucose production has been shown to depend on the primary trophic factor was glucose, and insulin was produced by pancreas of experimental rats in a short time to decrease blood glucose, while the GLP-1 could enhance insulin secretion. Since glucose stimulation could increase the expression of *Plg*, we speculated that CGA might regulate the posttranslational processing activity of the enzymes encoded by *Plg* to influence the transcription products. Previous work has shown that GLP-1 could increase the activity of the homeobox transcription factor IDX-1 (also known as PDX-1)[49-50], which in turn stimulated the transcription of glucokinase and GLUT-2[51-52]. Therefore, the general mechanism of the absorption and transportation of glucose is complex, involving the interaction of many factors.

In conclusion, CGA can maintain glucose homeostasis through modulating the mRNA expression levels of intestine glucose transporters and *Plg*. These data could be used to explain the mechanism underlying the effects of CGA on improving the insulin resistance induced by an HFD.

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