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Transcriptome Analysis of the Duodenum, Pancreas, Liver, and Muscle from Diabetic Goto-Kakizaki Rats Fed a Trypsin Inhibitor Derived from Squid Viscera

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Supporting Information

ABSTRACT: Trypsin inhibitors (TIs) have various nutritional effects. However, a detailed mechanism for their effects, especially on the gene expression patterns in various tissues, remains unknown. Here, we used transcriptome techniques and gene ontology (GO) analysis to examine the effects of squid TI (sqTI), a biochemically stable peptide, on diabetic Goto-Kakizaki rats after feeding for 10 weeks. We demonstrated that downregulation of SREBP1c in the liver via duodenal/pancreatic hormones suppresses the blood cholesterol level. Consistently, in GO analysis, the term "cholesterol biosynthetic process" was enriched among downregulated genes. No hypoglycemic or insulinotropic effects were observed, in contrast to the results from our previous studies (single stimulation with the same dose of TI), which can be partly ascribed to the inactive responses of the duodenum and pancreas in this condition.

KEYWORDS: *Trypsin inhibitor, squid, Goto-Kakizaki rat, duodenum, pancreas, liver*

INTRODUCTION

Trypsin inhibitors (TIs) have many nutritional and pharmaceutical effects. The Bowman−Birk inhibitor (BBI) and the Kunitz inhibitor (KI), which are purified from soybeans, are the most well-characterized TIs.[1−3] Pharmaceutical and nutritional functions reported for the BBI and KI include lowering cholesterol levels, anticarcinogenic effects, protective effects against obesity, as well as the potential to act as a therapeutic agent for diabetes and an irritant of the digestive tract.[1−3] There have also been reports about the hypoglycemic effects of TIs, especially of a synthetic TI called camostat. Shimoda et al. reported that oral administration of TI improves glucose tolerance and insulin release and also reduces pathological changes in WBN/Kob rats, which spontaneously develop diabetes mellitus with age.[4] Jia et al. reported that TIs in the diet suppress visceral adipose deposits and the levels of triglycerides, free fatty acids, and cholesterol in the blood of Otsuka Long-Evans Tokushima Fatty (OLETF) rats.[5]

There are many reports about the beneficial effects of plant and synthetic TI, but little is known about the nutritional and pharmaceutical effects of TI extracted from animals. In our previous study, we reported that TI from squid viscera (sqTI) retained its activity after physical treatment with heat (80 °C for 100 min at pH 8) and acid (pH 2 at 37 °C for 180 min), as well as after biochemical treatments with pepsin and chymotrypsin.[6,7] This suggests that sqTI has some advantages with respect to processing, storage, and stability in the digestive tract. We showed that a single application of sqTI had insulinotropic effects in normal Wistar rats and diabetic Goto-Kakisaki (GK) rats.[7]

It is generally accepted that the first reaction to TI stimulation is the secretion of gastrointestinal hormones, such as cholecystokinin (CCK), from the duodenum. CCK subsequently induces the secretion of insulin from the pancreas.[3,8−10] Insulin can act on the cells in the liver, muscle, and fat tissues to facilitate the entry of glucose from the blood, which plays a central role in regulating carbohydrate and fat metabolism.[11,12] Thus, the effects of TIs may involve multiple tissues and hormones; this suggests that hormones may mediate communication and complex inter-relations between tissues by unknown mechanisms.

DNA microarray technology is a powerful tool to simultaneously assess the mRNA expression of thousands of genes and to identify the underlying issues that lead to an event. To date, the only transcriptome analysis of TI administration is our recent study on normal Wistar rats.[13] In the present study, we fed sqTI to diabetic GK rats for 10 weeks and then

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Table 1. Primers Used for qPCR

	forward	reverse
duoderm		
gastrin	TACGGATGGATGGACTTTGG	AGATGGCTGGGCTCTGGAA
gastrokine	GCTCCTGGCTTTGCTTACAC	GTTGATGCTCACCGACTGCT
somatostatin	GACCCCAGACTCCGTCAGTT	GGCATCGTTCTCTGTCTGGTT
cholecystokinin	CACGACCCCTCGCCTCTAA	GGCTGCATTGCACACTCTGA
pancreas		
insulin	TGTGGTTCTCACTTGGTGGA	ATGCTGGTGCAGCACTGAT
glucagon	TCGTGGCTGGATTGTTTGTA	CAATGTTGTTCCGGTTCCTC
liver		
SREBP1c	TGGACTACTAGTGTTGGCCTGCTT	ATCCAGGTCAGCTTGTTTGCGATG
SREBP2	CACCTGTGGAGCAGTCTCAA	TGCCAGAGTGTTGTCCTCAG
internal control		
β-actin	AAGTCCCTCACCCTCCCAAAAG	AAGCAATGCTGTCACCTTCCC

performed DNA microarray analysis of the duodenum, pancreas, liver, and muscle to investigate the genome-wide expression of genes in these tissues. After identifying differentially expressed genes (DEGs), gene ontology (GO) analysis[14] was performed to identify the key biological processes, molecular functions, and cellular components affected by changes in gene expression. We discussed the physiological inter-relationships of these tissues after oral administration of sqTI in diabetic GK rats and compared these results to those from Wistar rats.

EXPERIMENTAL SECTION

Animals. Male GK rats (7 weeks old) were purchased from Japan SLC (Hamamatsu, Japan). They were housed at 2 rats per cage in an air-conditioned room (room temperature, 23 ± 1 °C; humidity, 30 ± 10%) under a 12 h dark/12 h light cycle (lights on at 7:00 am), with free access to tap water and a MF diet (Oriental Yeast, Tokyo, Japan). Experiments commenced when the rats reached the age of 11 weeks after a 4 week adaptation period. We confirmed that they were diabetic at the age of 7 weeks: the blood glucose level at 60 min after oral administration of D-glucose (2 g/kg of body weight) was approximately 4 times higher than that in the initial phase.[6,7] All animal experiments were performed according to the Guidelines Concerning Animal Experiments at Hokkaido University.

Preparation of sqTI. The sqTI was prepared according to a previously reported method.[6,7] Briefly, frozen squid (*Todarodes pacificus*) viscera were minced, suspended in an equal volume of distilled water, and then stirred at 5 °C for 2 h. The slurry was centrifuged (20000*g* for 10 min). The pH of the supernatant was adjusted to 5.0 using 1 M HCl, and the supernatant was recentrifuged (20000*g* for 10 min). The pH of the supernatant was then adjusted to 7.0 using 0.5 M NaOH, after which the supernatant was recentrifuged (20000*g* for 10 min). The supernatant obtained was used as the sqTI fraction. A total of 1 unit of porcine trypsin activity is defined as the amount of enzyme causing an increase in absorbance of 1.0/min at 247 nm using *p*-toluenesulfonyl-L-arginine methyl ester (TAME) as the substrate.[15] A total of 1 unit of sqTI activity is defined as the amount of inhibitor that decreases the activity of 1 mg of trypsin by 50%. Although the crude fraction of sqTI contained several other proteins, these proteins are digested or denatured by the time they reach the duodenum, because they are exposed to acidic conditions (pH 2.0) and pepsin digestion in the stomach as well as chymotrypsin digestion in the duodenum. In contrast, sqTI is insensitive to these conditions *in vitro*,[6,7] indicating that sqTI is the sole component of the crude TI solution that is active in the duodenum.

Oral Administration of sqTI. The rats in the experimental group (n = 8; 11 weeks old) were orally administered the sqTI fraction (2.4 IU, 120 mg of protein) every morning (at 10:00 am) using a probe for 10 weeks. In the control group (n = 8; 11 weeks old), distilled water was orally administered instead of sqTI. At the end of the treatment period, the rats (n = 8 for each group) were sacrificed after overnight food deprivation under pentobarbital anesthesia and their organs and blood were collected for the below-described study.

Blood Sample Assays. The following parameters were determined using a Hitachi 7180 autoanalyzer: total protein using total protein HRII, albumin using albumin HRII, albumin/globulin ratio (A/G ratio) calculated as albumin/(total protein − albumin), total bilirubin using E-HR, LD using L-type Wako LDH, triglyceride using L-type Wako TG-H, total cholesterol using L-type Wako CHO (Wako Pure Chemical Industries, Osaka, Japan), glucose using Merckoquant Glucose Test (Merck KGaA, Darmstadt, Germany), free fatty acids using (NEFA-SS, Eiken Chemical, Tokyo, Japan), urea nitrogen using Determiner L UN (Kyowa Medex, Tokyo, Japan), creatine using Accuras Auto CRE (Shino-test, Tokyo, Japan), AST using Liquitech AST, ALT using Liquitech ALT, and ALP using Liquitech ALP (Roche Diagnostics K.K., Tokyo, Japan).

Oral Glucose Tolerance Test. An oral glucose tolerance test was performed after the experiment (20 weeks). GK rats were fasted for 16 h and then administered glucose (2 mg/g of body weight) and sqTI (2.4 IU) orally using a probe. Distilled water was orally administered instead of sqTI in the control group. Blood was periodically sampled (0, 15, 30, 60, and 120 min thereafter) to measure glucose and insulin levels using the Glucocard Diameter (Arkray, Tokyo, Japan) and insulin enzyme-linked immunosorbent assay kits (Morinaga Institute of Biological Science, Inc., Yokohama, Japan), respectively.

RNA Extraction. RNA was extracted from the duodenum, pancreas, liver, and muscle using the SV Total RNA Isolation System (Promega, Carlsbad, CA), following the instructions of the manufacturer. RNA quantity, purity, and concentration were determined using a spectrophotometer and agarose gel electrophoresis. For all samples, mRNA quality was assessed by electrophoresis of total RNA, followed by staining with ethidium bromide. A 2:1 ratio of the 28S to the 18S rRNA bands was considered the criteria for intact RNA. This method relies on the assumption that rRNA quality and quantity reflect that of the mRNA population. Total RNA was used for microarray analysis and qPCR as described below.

DNA Microarray Analysis. To ensure the reproducibility of DNA microarray experiments, the dye-swap design analysis was performed using the two-color system following the Two-Color Microarray Gene Expression Analysis protocol (Agilent Technologies, Santa Clara, CA). Total RNA [500 ng; 62.5 ng from each rat (n = 8)] was reverse-transcribed. Using the Low RNA Input Linear Amplification Kit Plus, Two Color (Agilent Technologies), 3′- or 5′-cyanine-labeled cRNA was synthesized and then hybridized to Whole Rat Genome Microarray (4 × 44K) chips (Agilent Technologies). Scanning was performed using a GenePix 4000B scanner (Agilent Technologies), and image and statistical analyses of primary spot intensities were performed using Agilent's Feature Extraction Software.

Gene Ontology and Pathway Analysis. For gene ontology (GO) analysis,[14] we used the Database for Annotation, Visualization, and Integrated Discovery (DAVID; david.abcc.ncifcrf.gov) and

Cytoscape 2.6.3 (www.cytoscape.org/) to analyze the lists of differentially expressed genes (DEGs), which were up- or down-regulated more than 2-fold after filtration with Feature Extraction as described above. The functional annotation of DEGs with GO terms was performed according to statistical significance ($p < 0.01$). No significant annotated data were obtained for some DEGs (GO terms for biological process, molecular function, and cellular component for the upregulated genes in the liver and muscle, molecular function for the downregulated genes in the liver and muscle, and cellular component for the upregulated genes in the pancreas).

Quantitative Real-Time PCR (qPCR). qPCR analysis was performed using Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) in an ABI PRISM 7000 Sequence Detection system (Applied Biosystems). The primers used are listed below. The analysis was performed using the following optimized assay conditions: 10 min at 94 °C, followed by 15 s at 94 °C, and 1 min at 60 °C for 40 cycles. Amplifications were performed in duplicate or triplicate wells. Melting curve analysis was performed after each run to confirm the specificity of the primers used. The primers used for each gene were summarized in Table 1. In all data shown, the ratio of the relative expression of each gene was normalized against that of β-actin. To validate the data shown in this study, we adopted internal controls in addition to β-actin (e.g., GADPH and 16S rRNA) for some experiments and obtained results similar to those presented here.

Statistical Methods. All experiments were performed using multiple independent samples ($n = 8$ for each group). Differences were compared using the Mann−Whitney test and Student's t test.

RESULTS

Physiological Effects of Treatment with sqTI. Examination of blood samples showed that the number of leukocytes and cholesterol level were significantly suppressed in the sqTI-treated GK rats (Table 2). After oral administration of glucose, we measured the blood levels of glucose and insulin. However, no significant difference was observed between the groups at each time point (0, 15, 30, 60, and 120 min thereafter). There were no significant differences in growth, food intake, or tissue weight (see Supplementary File 1 of the Supporting Information).

Transcriptome and qPCR Analysis of Each Tissue. *Duodenum.* After filtration by Feature Extraction, 12 449 of ~44 000 spots were judged as positive. Among them, 201 and 233 were upregulated (>2.0) or downregulated (<0.5) by sqTI treatment, respectively (see Tables 1 and 2 in Supplementary File 2 of the Supporting Information). These were designated DEGs. In the analysis of upregulated DEGs, GO terms enriched in the biological process category were "negative regulation of transcription from RNA polymerase II promoter", "regulation of gene-specific transcription from RNA polymerase II promoter", and "positive regulation of gene-specific transcription" at the deepest hierarchy (Figure 1A). In the analysis of downregulated genes, GO terms enriched in the biological process category included "response to peptide hormone stimulus", "response to sucrose stimulus", "response to food", "response to nutrient", "fatty acid catabolic process", "fatty acid oxidation", "cholesterol homeostasis", "regulation of fatty acid metabolic process", and "regulation of cholesterol transport" (Figure 1B). The results of the GO analysis are summarized in Supplementary File 3 of the Supporting Information. Gastrin and gastrokine, both of which are gastrointestinal hormones, were highly upregulated in sqTI-treated GK rats, with expression levels increased about 3-fold in the qPCR analysis (Figure 2). Contrary to our expectations, CCK was not in the DEG list validated by qPCR. We also tested the mRNA expression level of somatostatin, a gastrointestinal hormone, in qPCR. It was highly upregulated in sqTI-treated GK rats (Figure 2).

Table 2. Results of the Blood Test at the End of the Experiment[a]

	control	sqTI fed
total protein (g/dL)	6.23 ± 0.03	6.20 ± 0.05
albumin (g/dL)	4.11 ± 0.03	4.03 ± 0.05
A/G ratio	1.96 ± 0.05	1.89 ± 0.05
total bilirubin (mg/dL)	0.09 ± 0.00	0.08 ± 0.00
glucose (mg/dL)	303.25 ± 10.77	293.5 ± 13.26
triglyceride (mg/dL)	34.88 ± 1.94	34.12 ± 1.32
free fatty acids (μEQ/L)	728.63 ± 80.70	684.38 ± 70.87
total cholesterol (mg/dL)	80.50 ± 1.07	74.13 ± 2.29[b]
urea nitrogen (mg/dL)	18.78 ± 0.78	17.80 ± 0.59
creatine (mg/dL)	0.280 ± 0.04	0.30 ± 0.00
sodium (mEQ/L)	142.63 ± 0.26	141.88 ± 0.48
chloride (mEQ/L)	103.13 ± 0.35	103.13 ± 0.59
potassium (mEQ/L)	3.45 ± 0.08	3.43 ± 0.06
calcium (mEQ/L)	9.69 ± 0.08	9.48 ± 0.06
AST (IU/L)	79.50 ± 11.63	72.00 ± 2.96
ALT (IU/L)	49.50 ± 10.23	37.75 ± 1.49
LD (IU/L)	245.50 ± 65.13	311.00 ± 38.23
ALP (IU/L)	395.25 ± 17.71	398.88 ± 20.10
reticulocyte (mL^{-1})	32.25 ± 1.39	34.25 ± 1.09
platelet ($\times 10^3$ mm^3)	70.80 ± 1.68	66.65 ± 6.09
SEG (%)	20.38 ± 2.51	22.50 ± 2.46
EOSIN (%)	2.13 ± 0.55	2.88 ± 0.55
MONO (%)	0.13 ± 0.12	0.50 ± 0.18
LYMPH (%)	77.38 ± 2.82	74.13 ± 2.80
Hb (g/dL)	16.03 ± 0.14	15.48 ± 0.23
RBC (cells/μL)	856.63 ± 7.83	836.88 ± 12.50
WBC (cells/μL)	7362.50 ± 434.28	6150.00 ± 254.30[b]
HT (%)	47.34 ± 0.40	96.48 ± 8.70
MCV (%)	55.50 ± 0.19	55.63 ± 0.50
MCH (pg)	18.75 ± 0.16	18.63 ± 0.26
MCHC (%)	33.88 ± 0.12	33.38 ± 0.05

[a]Values are means ± standard error (SE). [b]$p < 0.05$.

Pancreas. After filtration, 740 spots were judged as positive; 77 (>2.0) and 27 (<0.5) were identified as DEGs, respectively (see Tables 3 and 4 in Supplementary File 2 of the Supporting Information). GO analysis of the downregulated DEGs assigned eight genes to "response to organic substance" and six genes to "response to hormone stimulus". The genes allocated in this analysis are shown in Supplementary File 4 of the Supporting Information. qPCR confirmed that both insulin and glucagon were significantly downregulated in TI-treated GK rats, although neither gene was judged as a DEG in the filtration (Figure 3).

Liver. After filtration, 9560 spots were judged as positive; 17 (>2.0) and 24 (<0.5) were identified as DEGs (see Tables 5 and 6 in Supplementary File 2 of the Supporting Information). In the GO analysis of biological processes for the down-regulated DEGs, the assigned terms included the "cholesterol biosynthetic process" (Figure 4A and Supplementary File 5 of the Supporting Information). The genes categorized in this GO term were mevalonate (diphospho) decarboxylase, isopentenyl-diphosphate δ-isomerase, and hydroxysteroid (17-β) dehydrogenase 7 (see Supplementary File 5 of the Supporting Information). Figure 4B shows the fold change in the expression levels of genes related to the cholesterol synthetic pathway. Five genes, including 3-hydroxy-3-methylglutaryl-

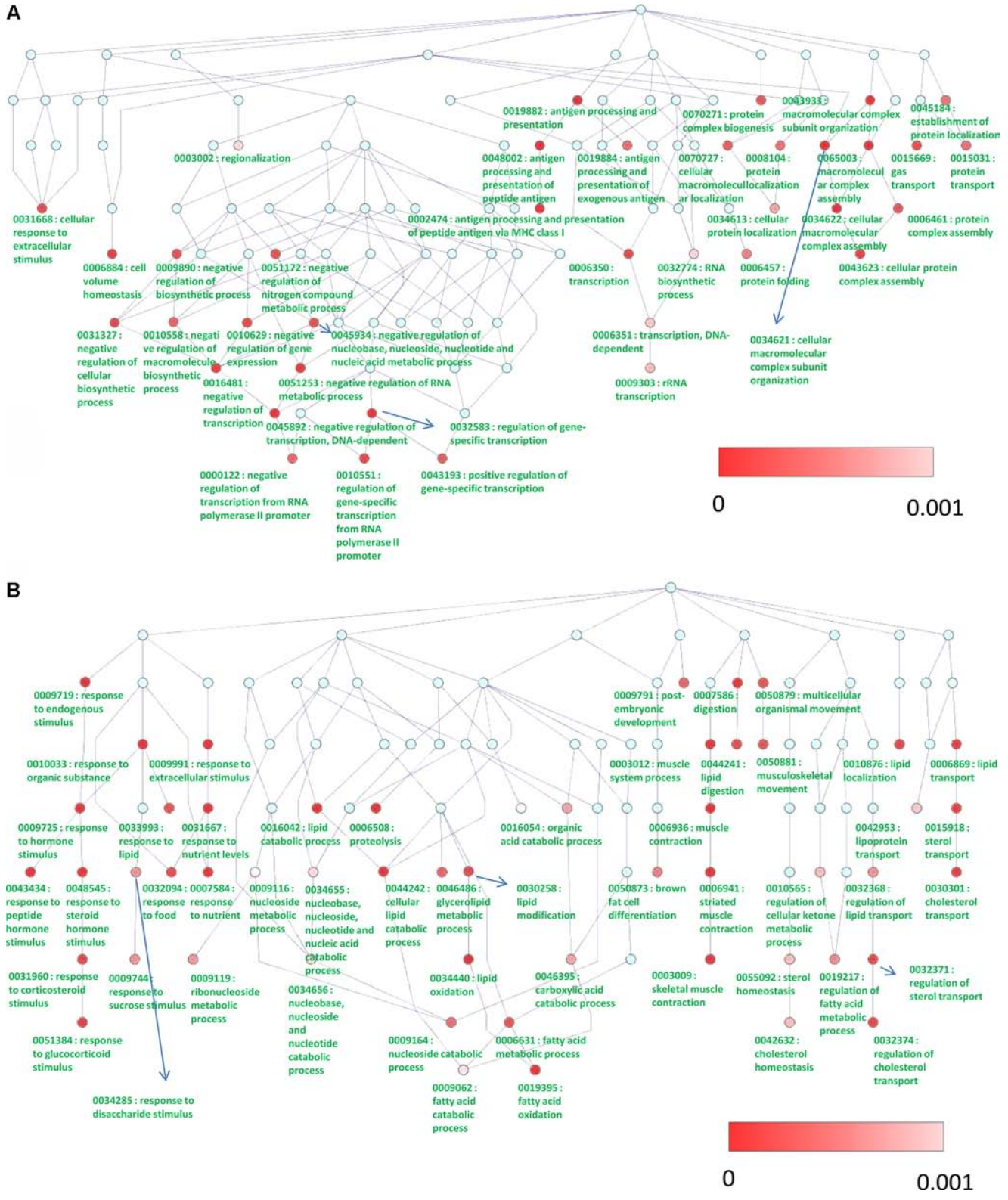

Figure 1. (A) GO analysis of upregulated DEGs in the duodenum for the category of the biological process. Significantly enriched GO terms ($p < 0.001$) found in the top 201 upregulated DEGs. (B) GO analysis of downregulated DEGs in the duodenum for the category of the biological process. Significantly enriched GO terms ($p < 0.001$) found in the top 233 downregulated DEGs. The color of the node and the bar indicate the p value in Functional Annotation Clustering in DAVID. Arrows were used when there was no space for the GO term.

coenzyme A synthase 1, mevalonate (diphospho) decarboxylase, squalene epoxidase, sterol-C4-methyl oxidase-like, and 7-dehydrocholesterol reductase, were downregulated. We also used qPCR to test the expression levels of SREBP1c and SREBP2, the key transcription factors regulating cholesterol metabolism. Both were significantly downregulated (Figure 5).

Muscle. After filtration, 5957 spots were judged as positive; 12 (>2.0) and 40 (<0.5) were identified as DEGs. The DEGs

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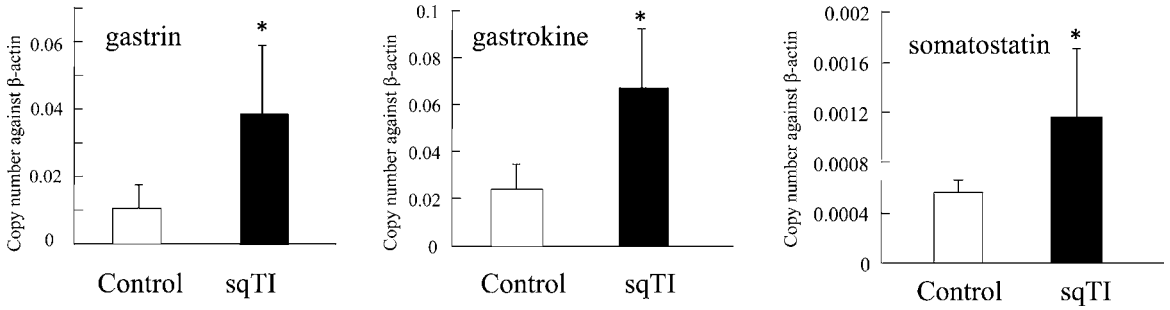

Figure 2. qPCR analysis of gastrin, gastrokine, and somatostatin mRNAs in the duodenum. The vertical axis indicates the relative expression ratio of each gene normalized against β-actin. Data are the mean ± standard error (SE) values for eight observations. (*) $p < 0.05$ compared to control mice by the Mann−Whitney test.

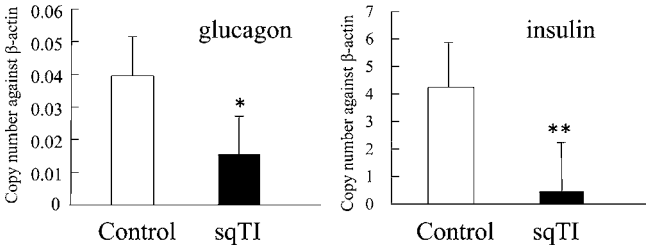

Figure 3. qPCR analysis of glucagon and insulin mRNA in the pancreas. The vertical axis indicates the ratio of the relative expression of each gene normalized against β-actin. Data are the mean ± SE values for eight observations. (**) $p < 0.01$ and (*) $p < 0.05$ compared to control mice by Student's t test.

are shown in Tables 7 and 8 in Supplementary File 2 of the Supporting Information, and the results of the GO analysis are shown in Supplementary File 5 of the Supporting Information.

DISCUSSION

The present study shows that the effects of sqTI in GK rats are distinct from the effects of sqTI in Wistar rats. In our previous study of Wistar rats, we confirmed that the duodenum actively participates in the insulinotropic effects of sqTI stimulation.[13] In the GO analysis of the upregulated DEGs in the duodenum of Wistar rats, terms consistent with TI stimulation, such as "cell differentiation", "tissue development", "response to nutrient", "regulation of transport", "regulation of secretion", and others, were enriched.[13] In contrast, in GK rats, terms such as "negative regulation of biosynthetic process", "negative regulation of nitrogen compound metabolic process", and others were enriched (Figure 1A), indicating that the duodenum does not actively respond to sqTI stimulation. In Venn diagram analyses using up- or downregulated DEGs in the duodenum of Wistar and GK rats, no genes were found at the intersection of the two data sets. In addition, CCK, a potent regulator of TI stimulation, was significantly enhanced in qPCR analysis of Wistar rats,[13] while no enhancement was observed in GK rats (data not shown). Our two studies show that sqTI can affect the expression profile of the duodenum differently in the two rat models. Because the duodenum is the first physiological stage of the gastrointestinal system,[8−10] where TI stimulation first occurs, physiological differences between GK and Wistar rats in other tissues might be attributed to the differences in their duodenal response profiles. Although the diabetic phenotype of GK rats is due to the dysfunction of the mitochondria,[16,17] no such indication was found in the GO analysis of the duodenum.

Further, in the GO analysis of the downregulated DEGs in the pancreas, which is affected by gastrointestinal hormones from the duodenum, "response to organic substance" and "response to hormone stimulus" were enriched (see Supplementary File 4 of the Supporting Information), indicating that the pancreas also does not respond to stimulation with sqTI. Considering that a single dose of sqTI has clear insulinotropic and hypoglycemic effects in GK rats,[6,7] 10 weeks of feeding could "desensitize" the duodenum and pancreas to long-term stimulation and subsequent effects. Thus, inactive responses of the duodenum and pancreas could be a main reason for the absence of insulinotropic and hypoglycemic effects in the present study. Another reason might be the period of feeding. Jia et al. reported that camostat (synthetic TI) feeding in OLETF rats for 70 weeks had clear hypoglycemic and insulinotropic effects.[5] However, in the early stages of their experiment (up to 44−52 weeks), ingestion of TI had adverse effects on the rats. In the present study, we tested the effects of sqTI on GK rats after 10 weeks of feeding, and we cannot rule out the possibility that our results follow the same pattern described in the study by Jia et al.

The blood level of insulin during the glucose tolerance test was consistent with the expression level of insulin in qPCR analysis. This may, in part, be attributed to the desensitization of the pancreas, but the upregulation of somatostatin may have suppressed the expression of insulin, because Redmon et al. reported that somatostatin inhibits insulin gene transcription.[18] The profile of somatostatin expression may be due to negative feedback: enhancement of somatostatin by gastric acid suppresses the secretion of gastrin to modulate the acid secretory response.[19] This is supported by the data in the present study showing the upregulation of gastrin (Figure 2).

The cholesterol-lowering effect of sqTI in the blood was supported by the downregulation of genes in the cholesterol biosynthetic pathway in the liver and the suppression of SREBP1c,[20] a key regulator of these genes, which was confirmed by qPCR analysis. Shimomura et al. reported that SREBP1c was downregulated in the livers from insulin-depleted diabetic rats subjected to streptozotocin treatment,[21] indicating that the downregulation of SREBP1c (Figure 5) can, at the least in part, be attributed to insulin deficiency in the present study. However, the prediction was solely based on the gene expression profile, and it requires an integrative physiological approach for validation. Several negative inducers of SREBP1c have been identified, such as protein kinase C, AMP kinase, and leptin.[22−24] However, after filtration of the microarray data, no data about these genes were available in this study.

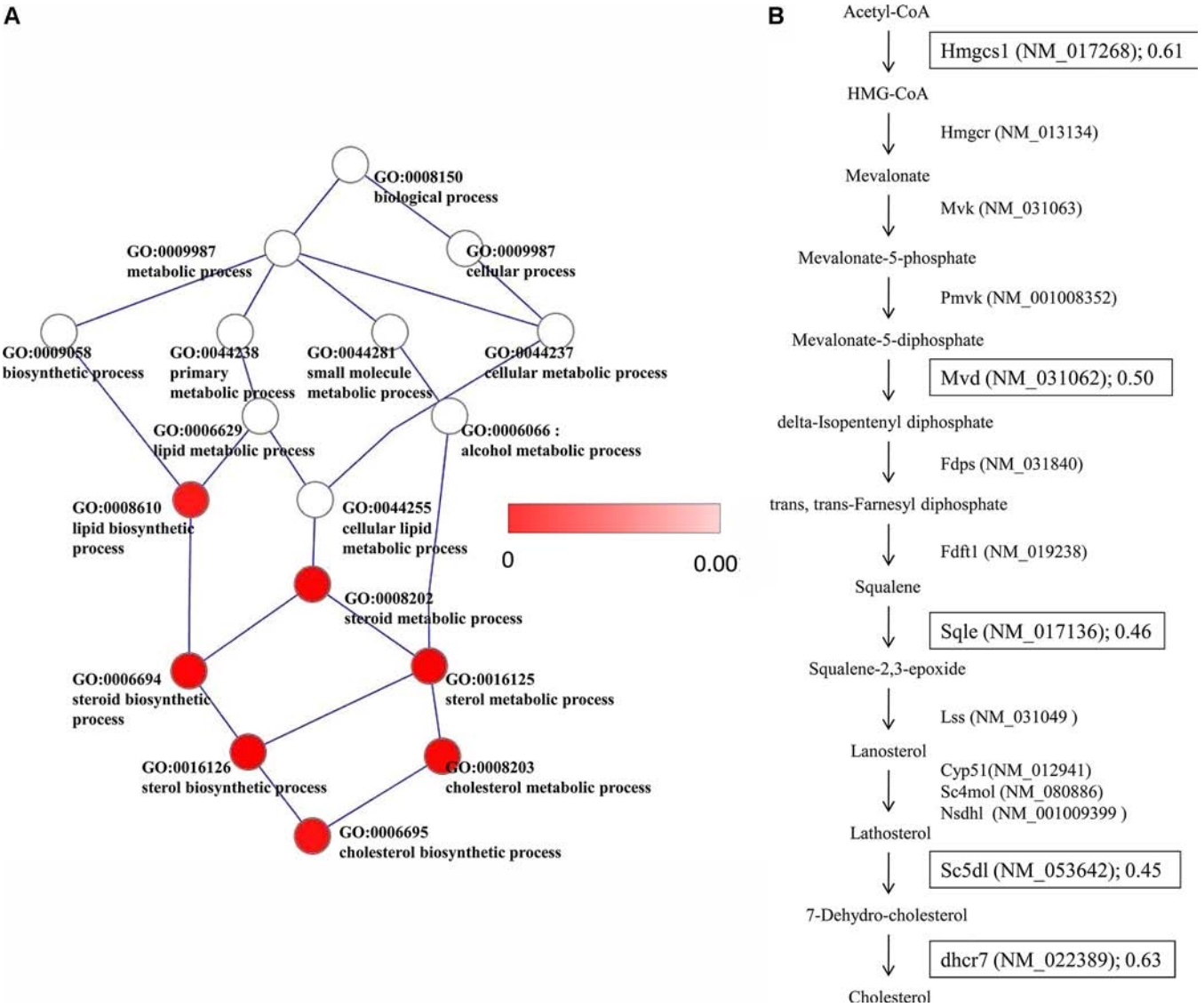

Figure 4. (A) Gene ontology analysis for the downregulated DEGs in the liver. Significantly enriched GO terms ($p < 0.01$) found in the top 25 downregulated DEGs. The color of the node and the bar indicate the p value in Functional Annotation Clustering in DAVID. (B) Fold change of the expression level of genes related to the cholesterol synthetic pathway. The boxed enzymes are encoded by genes that surpassed filtration. Fold changes (sqTI-treated rat versus control rat) are noted in the boxes. Hmgcs1, 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1; Mvd, mevalonate (diphospho) decarboxylase; Sqle, squalene epoxidase; Sc5dl, sterol-C4-methyl oxidase-like; and Dhcr7, 7-dehydrocholesterol reductase.

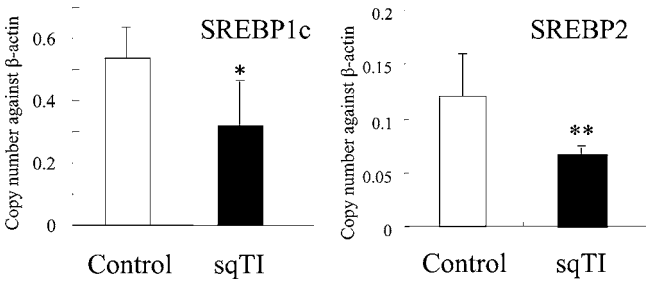

Figure 5. qPCR analysis of SREBP1c and 2 mRNAs in the liver. The vertical axis indicates the ratio of the relative expression of each gene normalized against β-actin. Data are the mean ± SE values for eight observations. (**) $p < 0.01$ and (*) $p < 0.05$ compared to control mice by Student's t test.

The proximate composition of the sqTI fraction (on a dry weight basis) was 72.0% protein, 4.6% lipid, 8.4% carbohydrate, and 15% mineral. These data indicated the possible effects of other components on the duodenum response, mainly the effects of mineral. However, there is little information describing the duodenal, hypoglycemic, and insulinotropic effects of the minerals derived from squids. In the present study, we adopted the crude fraction for commercial use, but we should purify the sqTI peptide for the direct comparison to TI from other sources.

In conclusion, sqTI can induce insulinotropic effects in normal Wistar rats, but the results cannot be directly applied to disease model animals, such as GK rats. This may be attributed to a desensitized tissue response. However, we cannot exclude the possibility that the distinct results obtained with GK rats in the present study were due to experimental conditions, such as sqTI dose, duration of treatment, or purification procedure.

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■ ASSOCIATED CONTENT

Supporting Information

Body and organ weights after the experiment (Supplementary File 1), DEGs and their fold-change values for the duodenum, pancreas, liver, and muscle (Supplementary File 2), GO terms assigned in DAVID using DEGs in the duodenum (Supplementary File 3), GO terms assigned in DAVID using DEGs in the pancreas (Supplementary File 4), GO terms assigned in DAVID using DEGs in the liver (Supplementary File 5), and GO terms assigned in DAVID using DEGs in muscle (Supplementary File 6). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

A/G ratio, albumin/globulin ratio; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate amino transferase; CCK, cholecystokinin; DAVID, Database for Annotation, Visualization, and Integrated Discovery; DEGs, differentially expressed genes; GK rat, Goto-Kakizaki rat; GO, gene ontology; LD, lactate dehydrogenase; qPCR, quantitative real-time PCR; RBCs, red blood cells; TI, trypsin inhibitor; sqTI, squid trypsin inhibitor; SREBP, sterol regulatory element-binding protein; WBCs, white blood cells

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