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Metabolic fate of an antihyperuricemic peptide, Tyr-Leu-Asp-Asn-Tyr (YLDNY), upon ingestion in rats

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Abstract

Tyr-Leu-Asp-Asn-Tyr (YLDNY) is an orally active antihyperuricemic peptide. However, its metabolic fate after oral administration and mechanism of action remain unclear. YLDNY resisted pepsin digestion, and intact YLDNY, with smaller amounts of peptide fragments, remained in the stomach after administration to rats. In contrast, YLDNY was completely degraded by exopeptidase digestion and was not detected in the luminal contents of the anterior parts of the small intestine. Only a small quantity of tetrapeptide (Tyr-Leu-Asp-Asn (YLDN)) was detected in the luminal contents of the anterior parts of the small intestine, although there was no significant increase after administration. None of the intact or modified peptides increased in portal and abdominal bloods, stomach and liver extracts. However, hepatic xanthine levels increased significantly 2 h after YLDNY administration compared to amino acid mixture administration, suggesting hepatic xanthine oxidase activity suppression. This discrepancy implies the involvement of an alternative mechanism of stomach or gut-liver signaling.

Keywords: Hyperuricemia; Peptide; Bioavailability; Xanthine oxidase; Metabolic fate.

1. Introduction

Hyperuricemia is rapidly becoming a global public health concern (Liu et al., 2024). It induces gout with severe pain and inflammation and is recognized as a risk factor for chronic kidney disease and cardiovascular complications (Dalbeth et al., 2019). Current pharmacological strategies for treating hyperuricemia are primarily classified into two categories: targeting uric acid synthesis and excretion (Zhang et al., 2022). Allopurinol and febuxostat reduce uric acid production by inhibiting xanthine oxidase, a time-limiting enzyme involved in uric acid synthesis. Benzbromarone and rasburicase reduce blood uric acid levels by enhancing uric acid excretion into urine by suppressing renal reabsorption through the inhibition of urate transporter 1 (URAT1) and by converting uric acid into allantoin, respectively. However, the use of these drugs is often limited by their side effects, including skin rashes, kidney toxicity, gastrointestinal symptoms, liver dysfunction, hepatotoxicity, and urolithiasis (Zhang et al., 2022). These safety concerns have prompted growing interest in alternative approaches with fewer side effects. Natural compounds present in foods with a long history of consumption are generally considered safe. Some food ingredients exhibit antihyperuricemic activity (Zhang et al., 2022). Among them, peptides can be obtained from underutilized proteins, such as fish byproducts (Murota et al., 2014; Wang et al., 2025), walnuts (Li et al., 2018), which provides enough efficacy to prevent hyperuricemia.

Based on *in vitro* xanthine oxidase inhibitory assays, molecular docking simulations, and *ex vivo* analyses, food-derived bioactive peptides have been shown to exert antihyperuricemic activity either by reducing uric acid synthesis through xanthine oxidase

inhibition or by promoting uric acid excretion, for example, by suppressing URAT1-mediated renal reabsorption (Murota et al., 2014; Liu et al., 2020; Mehmood et al., 2024). Food-derived peptides have attracted increasing attention as a promising strategy for dietary management of hyperuricemia.

Tyr-Leu-Asp-Asn-Tyr (YLDNY), a pentapeptide, was identified from an alcalase digest of shark cartilage water extract using *in vivo* activity-guided fractionation based on its antihyperuricemic activity upon oral administration to oxonate-induced hyperuricemic rats; however, the metabolic fate of YLDNY remains to be determined (Murota et al., 2014).

Most oligopeptides in food protein hydrolysates, including pentapeptides, such as YLDNY, resist digestion by endoproteinases, such as pepsin and trypsin, but can be degraded by exopeptidases (Chen et al., 2019; Sri Wijanarti et al., 2024). In contrast, some sequence-specific peptides, such as Pro-Gly (Shigemura et al., 2012) and Pro-Hyp (Iwai et al., 2005); modified peptides, such as diketopiperazines (DKPs); aspartyl peptides containing β-peptide bonds or D-aspartyl residues; and γ-glutamyl peptides present in fermented foods resist exopeptidase digestion (Nagao et al., 2024; Rahmadian et al., 2025). Iwasaki et al. (2024) reported that DKPs are generated during digestion and absorption processes after the ingestion of collagen hydrolysate. Therefore, it can be assumed that YLDNY is degraded into some fragment peptides and further converted to modified peptides, such as aspartyl β-peptides and DKPs, during digestion and absorption, some of which may exert biological activity in the body.

Therefore, the objective of the present study was to examine the metabolic fate of YLDNY in rats after administration, including not only intact YLDNY but also its fragment peptides, and their potentially modified forms. Consequently, intact YLDNY was observed in the luminal contents of the stomach but was completely degraded in the upper small intestine. No increase in YLDNY-derived peptides was observed in the bloods and liver, where uric acid is synthesized (Chandel 2021). These findings suggest that the antihyperuricemia activity of YLDNY may be mediated by a pathway other than the direct transport of peptides to the liver via the bloodstream.

2. Materials and methods

2.1. Reagents

Porcine pepsin and pancreatin were obtained from Nacalai Tesque (Kyoto, Japan). Leucine aminopeptidase from porcine kidneys and uric acid were purchased from Merck (Darmstadt, Germany). 6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AccQ) was purchased from Toronto Research Chemicals (Toronto, ON, Canada). 9-Fluorenylmethoxycarboxynyl (Fmoc) amino acid derivatives, Fmoc amino acid-bound resins (Wang resin and Alko resin), and 1H-benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) were purchased from Watanabe Chemicals (Hiroshima, Japan). 1-Hydroxybenzotriazole (HOBt) was purchased from Peptide Institute (Osaka, Japan). *N, N*-dimethylformamide (DMF), 4-methylmorpholine (NMM), piperidine (PIP), *t*-butyl methyl ether (MTBE), and trifluoroacetic acid (TFA) were purchased from Fujifilm Wako Pure Chemical (Osaka, Japan). Acetonitrile (HPLC grade), phosphate-buffered saline (PBS), L-tyrosine, L-leucine, L-aspartic acid, and L-asparagine monohydrate were purchased from Nacalai Tesque (Kyoto, Japan). Hypoxanthine and xanthine were obtained from Kojin (Tokyo, Japan). All the other reagents were of analytical grade or higher.

2.2. Synthesis of peptides

Four isomers of Asp residue (L-α Asp, L-β Asp, D-α Asp, and D-β Asp residues) in YLDNY and its fragment peptides, except for the oligopeptides with aspartyl residue at the carboxyl terminus, were synthesized using the method described previously (Ejima et al., 2019). These peptides were synthesized by the Fmoc strategy using a PSSM-8 solid-phase peptide synthesizer (Shimadzu, Kyoto, Japan). Solid-phase synthesis reactions were performed using PyBOP, HOBt, DMF, NMM, and PIP, according to the manufacturer's protocols. To release the synthetic peptides from the resin, after washing the resin with 1 mL of methanol (five times) and MTBE (two times), 1 mL of TFA was added to the resin coupled with the peptides and allowed to stand for 2 h. Released peptides in TFA were flushed into a glass tube. TFA was removed by flowing nitrogen gas. The residue was solubilized in 1 mL PBS. If necessary, the pH of the solution was adjusted to 7.0 by adding 0.1 M sodium hydroxide to increase the solubility of peptides and precipitate chemicals used for synthesis.

Synthetic YLDNY with L-α Asp was first purified by reversed-phase high performance liquid chromatography (RP-HPLC) using a Cosmosil 5C18MS-II column (10 mm inner diameter (I.D.) × 250 mm, Nacalai Tesque) for animal experiment. A binary linear gradient was performed using 0.1% formic acid (solvent A) and 0.1% formic acid in 80% acetonitrile (solvent B) at a flow rate of 2 mL/min. The gradient program was as follows: 0−30 min, 0−100% B; 30−35 min, 100% B; 35−45 min, 100−0% B; 45−50 min, 0% B. The fractions included YLDNY were combined and freeze dried and dissolved into PBS. This fraction was subjected to second RP-HPLC purification using same column. The gradient program was as follows: 0−40 min, 0−40% B; 40−40.01 min, 40−100% B; 40.01−50 min, 100% B; 50−50.01 min, 100−0% B; 50.01−60 min, 0% B. After purification, the YLDNY fraction was freeze dried and stored in a desiccator until further use. The purity of YLDNY was confirmed by mass spectrometer as shown in Figure S1.

YLDNY fragment peptides consisted of no Tyr residue (namely LDN, DN, and LD) and their isomers were purified twice as described above. The second purification was performed using a smaller column (4.6 mm I.D. × 250 mm) at a flow rate of 1 mL/min under the same gradient condition and was used as a standard for a liquid chromatography-electrospray ionization tandem mass spectrometer (LC-MS/MS) using an LCMS 8040 (Shimadzu, Kyoto, Japan). YLDNY (for LC-MS/MS standard) and its fragment peptides consisting of Tyr residues were first purified via RP-HPLC using the large column with same gradient condition as mentioned above. In second purification, the small column was used with a different binary linear gradient using 0.1% formic acid or 200 mM ammonium acetate (pH 6.0) (solvent A) and 80% acetonitrile containing 0.1% formic acid or 100% acetonitrile (solvent B). The gradient program was as follows: 0−50 min, 0−50% B; 50−55 min, 50−60% B; 55−60 min, 60−75% B; 60−65 min, 75−100% B; 65−75 min, 100% B; 75−75.01 min, 100−0% B; 75.01−85 min, 0% B. In all cases, columns were maintained at 40 °C. Peptide elution was monitored by measuring absorbance at 214 and 254 nm.

Peptide purity was evaluated by mass spectrometry. The concentrations of the purified peptides were evaluated by amino acid analysis following HCl hydrolysis, as previously described (Sri Wijanarti et al., 2024).

2.3. In vitro digestion

YLDNY was successively digested with pepsin and mixture of

pancreatin and leucine aminopeptidase using the method described by Chen et al. (2019) with slight modifications. YLDNY (2 mg) was dissolved in 0.9 mL of 10 mM HCl. Aliquot (25 µL) was collected and diluted twice with distilled water and used as non-digested YLDNY. Remaining YLDNY solution (0.875 mL) was mixed with 0.1 mL of pepsin solution (2 mg pepsin in 2 mL of 10 mM HCl) and incubated at 37 °C for 180 min. An aliquot (100 µL) was collected and passed through an ultrafilter (Amicon Ultra 0.5 mL, 10,000 NMWL, Merck, Darmastadt, Germany) at 10,000 × *g* at 5 °C for 10 min to remove pepsin from the digest. The filtrate was used as pepsin digest of YLDNY. The remaining pepsin digest was neutralized by adding 0.45 mL of 20 mM sodium hydroxide to adjust the pH to 7.5 and further digested with pancreatin and leucine aminopeptidase by mixing with 0.45 mL of pancreatin solution (2 mg pancreatin in 2 mL PBS) and 20 µL of leucine aminopeptidase (cytosol) at 37 °C for 180 min. Then, the aliquot (200 µL) was collected and passed through the same ultrafilter to terminate digestion. The filtrate was used as the pancreatin and leucine aminopeptidase digest of YLDNY for subsequent experiments.

2.4. Animal experiment

Freeze dried YLDNY (75 mg) was suspended in approximately 5 mL distilled water. To adjust the pH to 7.0, 0.1 M NaOH solution was added and then distilled water was added to make up the volume to 6 mL, resulting in a concentration of 12.5 mg/mL. An amino acid mixture was prepared by mixing the constituent amino acids in YLDNY at equal molar concentrations. Tyrosine (52.8 mg), leucine (19.1 mg), aspartic acid (19.4 mg), and asparagine monohydrate (21 mg) were suspended in distilled water (approximately 5 mL), and pH was adjusted to 7.0 and finally make up to 8 mL. All animals were treated according to the guidelines provided by the National Institutes of Health (NIH) for animal experiments. All the experimental procedures were approved by the Animal Care Committee of the Louis Pasteur Center for Medical Research (Kyoto, Japan; approval number 20222). Five-week-old male Wistar/ST rats (120-140 g body weight) were purchased from Japan SLC (Shizuoka, Japan). They were fed rodent chow (solid type of certified diet MF, Oriental Yeast, Tokyo, Japan), kept under a 12 h light-dark cycle at 23–25 °C and 40–60% relative humidity. For 1 week of acclimatization, the rats had free access to food and water and were housed three rats per cage. The rats were divided into five groups: vehicle (n = 3), amino acid 1 h (n = 3), amino acid 2 h (n = 3), YLDNY 1 h (n = 3), and YLDNY 2 h (n = 3). Before the administration of the samples, the rats were fasted overnight for 18 h.

The rats were administered distilled water, amino acid mixture, or YLDNY solution at 4 mL/kg body weight. One and two hours after administration, the rats were euthanized by withdrawing blood from the portal and abdominal vein using heparinized syringes under isoflurane-induced anesthesia. Collected blood was centrifuged at 800 × *g* for 10 min at 4 °C to obtain plasma. The plasma was mixed with 3 volumes of ethanol and centrifuged at 12,000 × *g* at 4 °C for 10 min. The supernatant was collected as serum samples and stored at -20 °C. Liver was collected and perfused with PBS through the intrahepatic vascular system to remove blood and stored at -20 °C for further experiments. The stomach was collected, and the stomach luminal contents were immediately flushed out with 10 mL of PBS and stored at -20 °C. The remaining stomach tissue was stored at -20 °C. Subsequently, the small intestine was collected from the anterior to the pyloric sphincter and the anterior to the cecum and cut into three portions in the ratio 3:4:3. Luminal contents in the anterior parts of the small intestine were immediately flushed with 3 mL of PBS stored at -20 °C. Effluents from the stomach and the anterior parts of the small intestine were mixed with 3 volumes of ethanol and centrifuged at 12,000 × *g* at 4 °C for 10 min to obtain the supernatants used for the following experiments as samples for luminal contents of the stomach and the anterior parts of the small intestine.

Water-soluble/insoluble metabolites in the organs were extracted using the modified Bligh and Dyer method (Naz et al., 2014; Gómez et al., 2020). Aliquots of liver tissue (100 mg) from the left lateral lobe of liver were homogenized with 100 µL of PBS using a Biomasher II (Nippi, Tokyo, Japan). The homogenate was mixed with 200 µL of chloroform and 600 µL of ethanol to form monophase and then centrifuged at 12,000 × *g* at 4 °C for 10 min. The supernatant was collected and used as the liver tissue extracts for subsequent experiments. The stomach tissue extracts were prepared as described above.

2.5. Determination of YLDNY and fragment peptides

Aliquots of synthetic peptide standard solutions (10 µL), *in vitro* protease digests of YLDNY (20 µL), and animal samples, including the stomach luminal contents (10 µL), the anterior parts of the small intestine luminal contents (10 µL), liver tissue extracts (10 µL), stomach tissue extracts (100 µL), and the serum samples (10 µL), were dried under vacuum. Residue was dissolved in 80 µL of 50 mM sodium borate buffer (pH 8.8), added with 20 µL of 0.3% AccQ acetonitrile solution, and incubated at 55 °C for 15 min to achieve the derivatization with AccQ for the amino group of the peptides. The AccQ reactants were clarified using centrifugation at 10,000 × *g* for 10 min at 5 °C or a Cosmonice filter (4 mm I.D., 0.45 µm, Nacalai Tesque). In some cases, the AccQ reactants were first mixed with the same volume of 5 mM sodium phosphate buffer containing 10% acetonitrile before clarification. Aliquots (5 or 10 µL) were injected to an LC-MS/MS equipped with an Inertsil ODS-3 column (2.1 mm I.D. × 250 mm, GL science, Tokyo, Japan). Binary linear gradient elution was performed using 0.1% formic acid (solvent A) and 0.1% formic acid in 80% acetonitrile (solvent B) at a flow rate of 0.2 mL/min. The gradient program and column temperatures are described in the Figure legends. Peptides were analyzed by precursor ion scanning, targeting the b1 ion from the AccQ moiety (mass-to-charge ratio *m/z* 171) or multi-reaction monitoring (MRM) in the positive mode (Miyauchi et al., 2022; Sri Wijanarti et al., 2024). The MRM conditions were optimized by LabSolutions LCMS Ver. 5.5 (Shimadzu, Kyoto, Japan) using synthetic peptides derivatized with AccQ.

According to a previous study (Nagao et al., 2024), amino group-blocked peptides fraction was prepared from the anterior parts of the small intestine luminal contents by solid phase extraction using a strong cation exchanger (AG50W-×8, hydrogen form, 100−200 mesh, Bio-Rad Laboratories, Hercules, CA, USA). The resin was prewashed with distilled water and 50% ethanol then packed into a centrifugal filter unit (5.0 µm pore size, Ultrafree-MC, Merck, Darmstadt, Germany). Two hundred microliters of 50% ethanol were added to the resin and eluted by centrifugation at 815 × *g* (five times). An aliquot (400 µL) of the anterior parts of the small intestine luminal contents sample was loaded onto the resin and stood for 60 min then eluted by centrifugation at 815 × *g*. The eluent was collected and referred to as the AG50 nonabsorbed fraction. The eluent (400 µL) was dried under vacuum. The residue was dissolved with distilled water (100 µL) and then clarified using centrifugation at 10,000 × *g* for 10

min at 5 °C. The supernatant (10 µL) was injected directly into an LC-MS system equipped with an Inertsil ODS-3 column. The column was maintained at 40 °C. Binary linear gradient was performed using 0.1% formic acid (solvent A) and 0.1% formic acid in 80% acetonitrile (solvent B) at a flow rate of 0.2 mL/min: 0−30 min, 0−30% B; 30−40 min, 30−100% B; 40−50 min, 100% B; 50−50.01 min, 100−0% B; 50.01−60 min, 0% B. Amino group-blocked peptides in the AG50 nonabsorbed fraction were assessed in the positive and negative modes by monitoring the total ions in the scan ranges of *m/z* 100–200, 200–250, 250–300, 300–400, 400–500, and 500–1,000.

2.6. Determination of uric acid and its precursors

Hypoxanthine, xanthine, and uric acid were dissolved in distilled water by sonication to obtain 1 mg/10 mL and used as stock solutions. Three stock solutions (100 µL) were mixed and 700 µL of distilled water was added just before LC-MS/MS analysis. Aliquots (20 µL) from liver tissue extracts were dried under vacuum. The pellet was reconstituted in 200 µL of distilled water and then vortexed for 30 min at room temperature. The sample solutions were then clarified using the Cosmonice filter. Aliquots (10 µL) of the standard and sample solutions were injected to LC-MS/MS equipped with the Inertsil ODS-3 column. A binary linear gradient was performed using 0.1% formic acid (solvent A) and 0.1% formic acid in 100% acetonitrile (solvent B), at a flow rate of 0.2 mL/min. The column was maintained at 40 °C. The gradient program was as follows: 0−5 min, 0−5% B; 5−10 min, 5%−15% B; 10−15 min, 15−30% B; 15−20 min, 30−50% B; 20−25 min, 50−80% B; 25−30 min, 80−100% B; 30−40 min, 100% B; 40−40.01 min, 100−0% B; 40.01−50 min, 0% B. Uric acid and its precursors were determined by MRM mode in negative mode as reported by previous studies (Svistounov et al., 2022). The MRM conditions were optimized by LabSolutions LCMS Ver. 5.5 using standards respectively.

2.7. Statistical analysis

Data were analyzed using GraphPad Prism version 10.5.0 (GraphPad Software, Boston, MA, USA). Student's *t*-test or Welch's *t*-test was used to determine the differences between two groups. In cases with more than two groups, one-way analysis of variance (ANOVA) with Tukey's post-hoc test was employed. A *p*-value less than 0.05 was considered statistically significant.

3. Results

3.1. In vitro digestion of YLDNY

The enzymatic susceptibility of YLDNY was assessed by *in vitro* digestion using endoproteinases and exoproteinases. Figure 1 shows the mass spectrometry chromatograms of AccQ derivatives of YLDNY pepsin digest (a), followed by pancreatin and leucine aminopeptidase digests (b). Almost the same amount of intact YLDNY remained after pepsin digestion (Figure 2), whereas fewer YLDNY fragment peptides and constituent amino acids were observed (Figures 1 and 2). In contrast, pancreatin and leucine aminopeptidase digestion completely degraded YLDNY into consistent amino acids, and smaller amounts of Leu-Asp (LD) and Asp-Asn (DN) (Figures 1 and 2). The presence of NH_4^+ in the digest originated from leucine aminopeptidase preparation, which contained ammonium sulfate.

3.2. In vivo digestion of YLDNY

We examined the metabolic fate of YLDNY in the luminal contents of the stomach and the anterior parts of the small intestine, portal blood, abdominal blood, and tissue extracts of the stomach and liver. In the stomach, YLDNY, rather than its peptide fragments, was the predominant form detected at 1 and 2 h after administration (Figure 3), indicating that YLDNY survived in the luminal contents of the stomach. YLDNY was not detected in stomach tissue extracts (Figure 4). Thus, YLDNY cannot be absorbed from the stomach although it was present in the luminal contents of the stomach. In contrast, in the luminal contents of the anterior parts of the small intestine, bloods, and the liver tissue extracts, YLDNY completely disappeared (Figures 5–7). These findings indicate that YLDNY was completely degraded by exopeptidases in the pancreatic juice and small intestinal mucosa. These *in vivo* results were consistent with the *in vitro* results. For peptide fragments derived from YLDNY, only Tyr-Leu-Asp-Asn (YLDN) was significantly increased in the luminal contents of the stomach 2 h after the administration of YLDNY compared to the administration of YLDNY-constituent amino acids (Figure 3). Furthermore, Asp-Asn-Tyr (DNY), Tyr-Leu-Asp (YLD), and Leu-Asp-Asn-Tyr (LDNY) and Tyr-Leu-Asp-Asn (YLDN) were detected in the luminal contents of the stomach and the luminal contents of the anterior parts of the small intestine only after YLDNY administration, respectively (Figures 3 and 5). While some other peptide fragments, especially dipeptides, were also detected in the luminal contents of the stomach and the anterior parts of the small intestine and tissue extracts of the liver and stomach, these peptides were also present in the control groups and did not significantly increase after YLDNY administration (Figures 3–6). Therefore, these peptide fragments could be generated by the degradation of endogenous proteins. In both portal and abdominal blood samples, YLDNY and all the peptide fragments were not detected (Figure 7).

Presence of YLDNY and its fragment peptides with D-Asp residue and *β*-Asp peptide bond was examined, as it has been reported that such modification occurs in Asp-containing peptides in fermented foods (Nagao et al., 2024). All Asp-containing peptides, YLDNY, and their fragments were synthesized. The MRM conditions for all isomers were determined. When the MS/MS chromatograms of all the isomers of the synthetic peptide were compared with those of the samples, no isomerized peptides were detected in any sample. Representative LC-MS/MS chromatograms of all isomers of YLDNY and Leu-Asp-Asn (LDN) in blood samples are shown in Figure 7. The AG50 nonabsorbed fraction of the luminal contents of the anterior parts of the small intestine was examined using LC-MS in both positive and negative modes. After the administration of YLDNY, no significant increase in the area of any peaks was observed compared to the vehicle and amino acid controls (data not shown). Therefore, it can be concluded that the amino-terminal blocked peptide derived from YLDNY was not produced in significant amounts during the digestive and absorptive processes in rats.

3.3. Effect of YLDNY administration on liver uric acid precursors

To evaluate the effect of YLDNY on xanthine oxidase activity, the concentrations of uric acid and its precursors, hypoxanthine

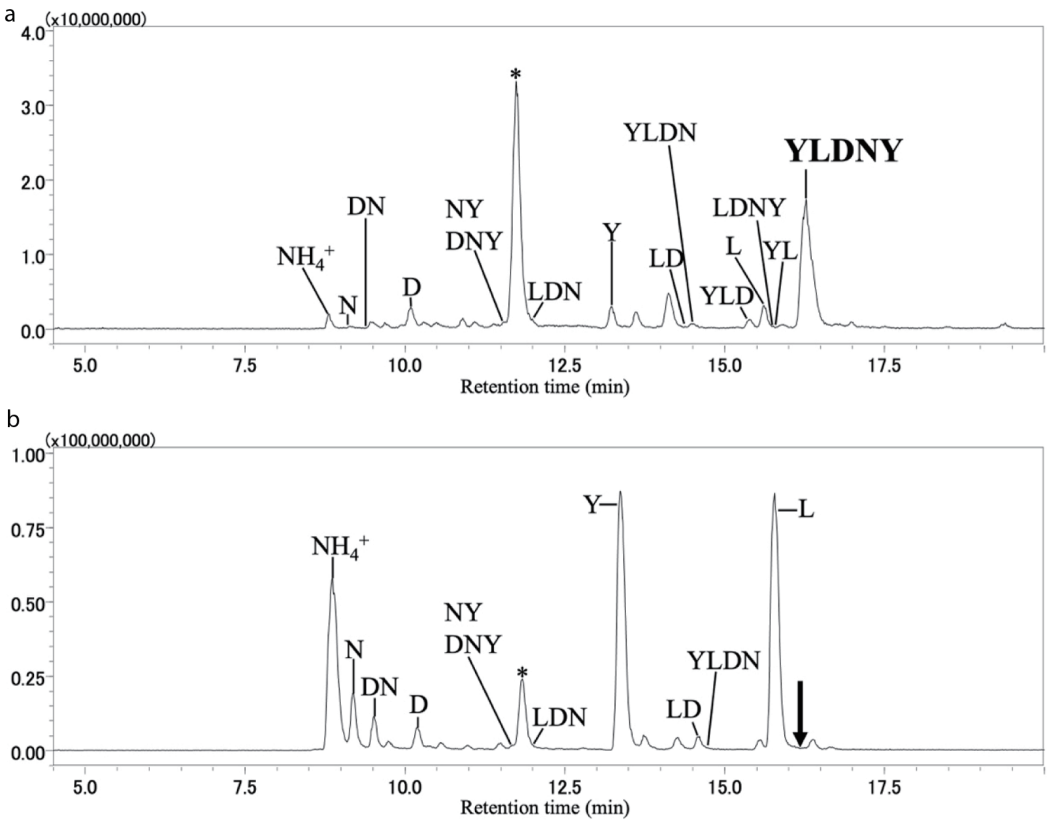

Figure 1. Mass spectrometry chromatograms of AccQ derivatives of pepsin (a) and pancreatin-leucine aminopeptidase (b) digests of YLDNY. The AccQ derivatives were analyzed using LC-MS/MS in the precursor ion scan mode (positive) targeting the b1 ion (*m/z* 171.1) generated from the AccQ moiety in the scanning range of *m/z* 100–1,000. The gradient program was as follows: 0–20 min, 0–50% B; 20–30 min, 50%–100% B; 30–35 min, 100% B; 35–35.1 min, 100–0% B; 35.1–45 min, 0% B. The column was maintained at 40 °C. Constituting amino acids and peptides were identified by molecular weight based on the *m/z* of the precursor ions and the retention time and labeled with one letter abbreviation of amino acids. The peaks marked with asterisks represent reagent peaks. Peaks without markers could not be assigned to any of the YLDNY fragments. Bold black arrow indicates the retention time of YLDNY (panel b).

and xanthine, in the liver tissue extracts were measured. As shown in Figure 8, the uric acid levels were considerably lower than the precursor concentrations in all samples. No significant differences were observed in hypoxanthine and xanthine concentrations among the vehicle, amino acid mixture, and YLDNY groups 1 h after administration. However, 2 h after the administration of YLDNY, xanthine concentrations were significantly higher than those after the administration of the amino acid mixture. Hypoxanthine exhibited a similar trend, although the difference was not statistically significant.

4. Discussion

Oligopeptides from dipeptides to pentapeptides exhibit notable resistance to endopeptidases, such as pepsin and trypsin, but are susceptible to exopeptidases, such as leucine aminopeptidase and carboxypeptidase A (Ejima et al., 2018; Chen et al., 2019), which is consistent with the present data. Pepsin preferentially cleaves peptide bonds between hydrophobic amino acid residues, such as Tyr-Leu (YL) found in YLDNY; however, it has been reported that its efficiency in cleaving such peptide bonds near both termini is limited (Fruton 2002; Ahn et al., 2013). YLDNY has a cleavage site only at the amino-terminus, such as Tyr-Leu (YL). Thus, the majority of YLDNY remain intact after pepsin digestion. Other endoproteinases such as trypsin and chymotrypsin have also been reported to be unable to cleave the preferred peptide bond near the terminus (Fruton 2002). This explains why oligopeptides shorter than pentapeptides with certain cleavage sites are able to resist endoproteinase digestion. In contrast to its resistance to pepsin, YLDNY is completely hydrolyzed by exopeptidases in pancreatin and leucine aminopeptidase. The present *in vitro* digestion results are consistent with intact YLDNY remaining in the stomach, but completely degraded in the anterior parts of the small intestine, as pancreatic juice and the small intestinal mucosa have strong exopeptidase activity (Hooton et al., 2015; Sri Wijanarti et al., 2024).

Certain short-chain peptides, such as Pro-Gly and Pro-Hyp, are generated by the gastrointestinal digestion of large peptides in foods (Iwai et al., 2005; Shigemura et al., 2012; Sri Wijanarti et al. 2024). Therefore, we examined the generation of peptide fragments by YLDNY during digestion. Some YLDNY fragment tetrapeptide and tripeptides were detected in luminal contents of the stomach and anterior parts of the small intestine only after the administration of YLDNY. However, these YLDNY fragment peptides were not detected in both portal and abdominal bloods and the liver tissue extracts. Some dipeptides potentially derived from YLDNY, such as Asn-Tyr (NY), Tyr-Leu (YL), and Leu-Asp (LD), were observed in the luminal contents and tissue extracts. However, YLDNY administration did not increase the levels of these di-

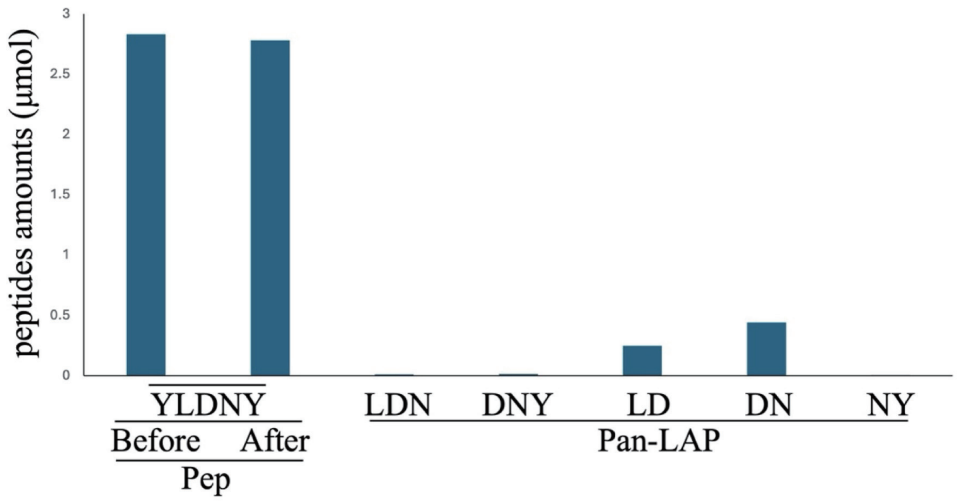

Figure 2. Amounts of peptides in pepsin digest (Pep) and pancreatin-leucine aminopeptidase (Pan-LAP) digests of YLDNY. The amount of YLDNY before and after pepsin digestion (Pep) was determined by LC-MS/MS in the precursor ion scan mode under the same conditions as shown in Figure 1. All values are converted to the amount would be contained in the initial solution volume. All YLDNY-derived peptides in pancreatin-leucine aminopeptidase digestion (Pan-LAP) were determined by LC-MS/MS in the MRM mode (positive). The gradient program was as follows: 0–15 min, 0–30% B; 15–20 min, 30%–50% B; 20–25 min, 50%–100% B; 25–30 min, 100% B; 30–30.1 min, 100–0% B; 30.1–40 min, 0% B. The column was maintained at 40 °C.

peptides. In contrast, the administration of a free amino acid mixture (Tyr (Y), Leu (L), Asn (N), and Asp (D)) significantly increased Tyr-Leu (YL) in the liver tissue extracts compared to that in the vehicle control. Kusubata et al. (2015) demonstrated that stable isotopes of Pro could be incorporated into Pro-Hyp within 2 h of oral ingestion of the Pro. Thus, the dipeptides found in the luminal contents and tissue extracts may be the degradation products of endogenous proteins rather than fragments of YLDNY. These facts indicate that YLDNY or its peptide fragments do not transfer significantly from portal blood to the liver after YLDNY administration.

Some modified peptides, such as isomerized and racemized aspartyl peptides (Ejima et al., 2019; Nagao et al., 2024; Rahmadian et al., 2025), pyroglutamyl peptides (Ejima et al., 2018; Miyauchi et al., 2022), and cyclic dipeptides (Shigemura et al., 2018), resist exopeptidase digestion and are present in the luminal contents of the small intestine and may enter the bloodstream. Thus, we first examined the generation of isomerized and racemized aspartyl peptides because YLDNY contains an Asp residue. However, neither YLDNY nor its fragment peptides containing aspartyl residues in D-stereoisomers and β-peptide bonds forms were observed in all blood, lumen, and tissue samples. Next, we examined amino-terminal-blocked peptide compounds in the AG50 nonabsorbed fraction, as it has been reported that amino group-blocking modifications, such as formylation, methylation, acetylation, and cyclization, can occur during food processing (Marino et al., 2015; Nagao et al., 2024). However, YLDNY administration did not result in the formation of amino-terminally blocked peptides in the rat body. Taken together, orally administered YLDNY was degraded into amino acids and was not converted to modified peptides in the small intestine, whereas intact and some fragment tetra- and tripeptides are present in the stomach and may be transiently present in the upper part of the small intestine.

The liver plays a crucial role in purine metabolism, where purine bases are successively metabolized to hypoxanthine and xanthine and further metabolized to uric acid by xanthine oxidase (Chandel 2021). Normal rats did not show hyperuricemia (Figure 8) because uric acid is rapidly metabolized to allantoin by uricase in rats, which is consistent with the results of a previous study (Kim et al., 2012). Murota et al. (2014) suggested that oral administration of YLDNY reduces blood uric acid levels, possibly via the modulation of xanthine oxidase activity in potassium oxonate-induced hyperuricemic rats. Potassium oxonate is an uricase inhibitor. In the present study, we used normal rats to evaluate the digestion and absorption of YLDNY under normal conditions. Despite no increase in YLDNY and its fragment peptides in the bloods and liver, hepatic xanthine levels significantly increased 2 h after the administration of YLDNY compared to the administration of the amino acid mixture. Hepatic hypoxanthine levels showed a similar trend without significant changes because hypoxanthine can undergo additional pathway to generate inosine monophosphate in the purine salvage pathway in mammals (Chandel 2021). These facts indicate that xanthine oxidase activity or its protein level were decreased by YLDNY administration, which may be associated with the antihyperuricemia activity of YLDNY reported in a previous study (Murota et al., 2014). To confirm it, it is necessary to measure xanthine oxidase activity and protein amount in the liver.

Several studies have shown that food-derived peptides, including Val-Tyr and Ile-Pro-Pro, exert significant biological activities, such as antihypertensive effects, by oral ingestion, despite their blood concentrations being far below the effective levels determined by *in vitro* assays based on their proposed mechanism of inhibition against angiotensin-converting enzymes (Sato 2022). However, this discrepancy remains unresolved. Recently, it has been reported that oral ingestion of relatively small doses of long peptides, such as Leu-Ser-Ser-Thr-Gln-Ala-Gln-Gln-Ser-Tyr, exerts antidepressant-like activity via gut–brain communication, possibly through the vagus nerve system. However, the presence of these peptides in the luminal contents of the small intestine has not been demonstrated (Mori et al., 2018). The present study showed that orally administered YLDNY affected hepatic xanthine levels, despite no increase in YLDNY and its peptide fragments in the bloods and liver. No modified peptides potentially derived from YLDNY were detected. In contrast, YLDNY and some peptide fragments were present in the stomach, even 2 h after administration. Based on these data, we hypothesized that

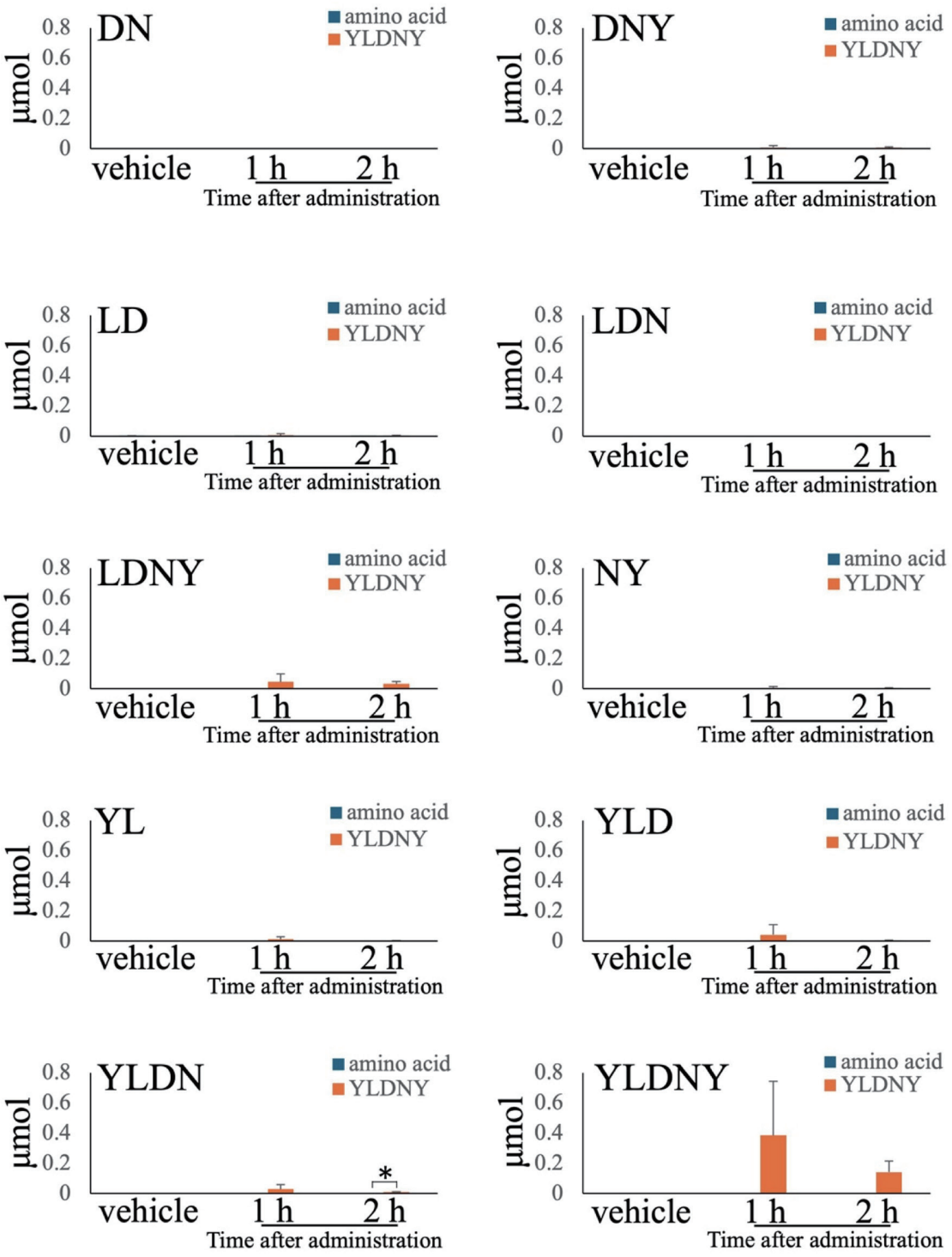

Figure 3. Amounts of YLDNY and its fragment peptides in the luminal contents of stomach 1 and 2 h after administration of YLDNY or the amino acid mixture. All peptides were determined using LC-MS/MS in the MRM mode (positive). The gradient program was as follows: 0–35 min, 0–35% B; 35–45 min, 35%–100% B; 45–55 min, 100% B; 55–55.01 min, 100–0% B; 55.01–65 min, 0% B. The column was maintained at 40 °C. The results were presented as the mean ± standard deviation. Asterisks (*) represent a significant difference, $p < 0.05$, between the YLDNY group and amino acid mixture group, according to Welch's *t*-test.

YLDNY may act on xanthine oxidase in the liver via the stomach-liver axis. However, YLDNY and its peptide fragments were not directly absorbed from the stomach (Figure 4). In contrast, the stomach epithelium and enteroendocrine cells expressed cell surface receptors, such as taste receptor type 2 (Tas2R) (Sternini and Rozengurt, 2025) and other related receptors, such as the

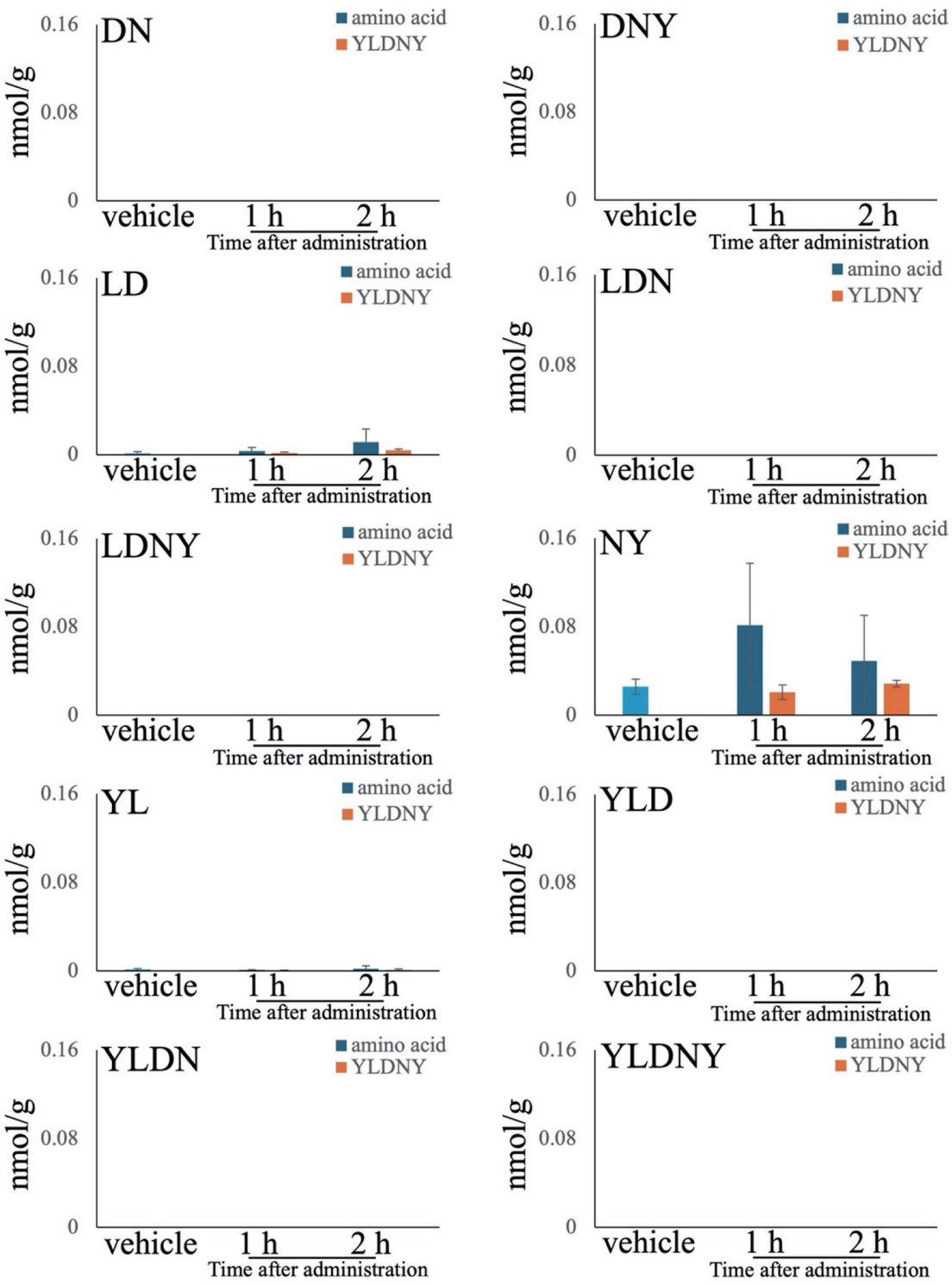

Figure 4. YLDNY and its derived peptides in the stomach tissue. All peptides were determined using the method described in Figure 3. The results are presented as the mean ± standard deviation.

farnesoid X receptor (FXR), calcium-sensing receptor (CaSR), and lysophosphatidic acid receptor 5 (LPA5R) (Fiorucci et al., 2011; Steensels and Depoortere, 2018). We assumed that YLDNY, a hydrophobic peptide, may activate some surface receptors, on gastric cells and/or the upper part of the small intestine, which triggers hormonal secretion or neural signal transduction, which can alter liver homeostasis (Maehashi and Huang, 2009; Fiorucci et al., 2011; Calvo and Egan, 2015; Kok et al., 2018;

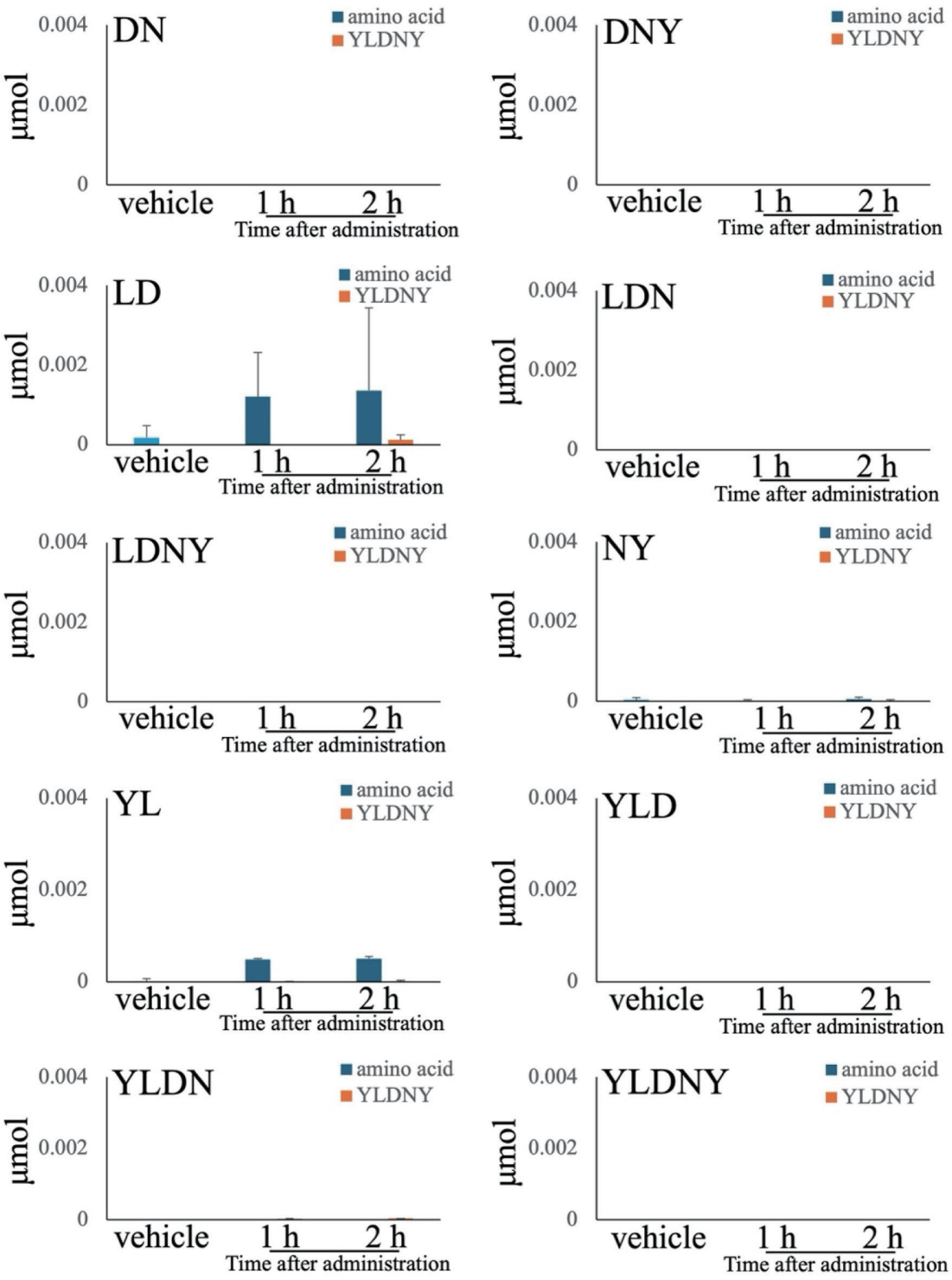

Figure 5. Amounts for YLDNY and its derived peptides in the anterior parts of the small intestine luminal contents. All peptides were determined using the method described in Figure 3. The results were presented as the mean ± standard deviation.

Engevik et al., 2020; Anderson and Gayer, 2021; Sternini and Rozengurt, 2025). This is a new concept for understanding the underlying mechanism of orally administered peptide activity but requires further confirmation. In our preliminary experiment, we observed that conjugated bile acid levels in the liver were altered by YLDNY administration, which can affect xanthine oxidase expression (Kanemitsu et al., 2017). Further studies on the activation of Tas2R by YLDNY and its subsequent events are

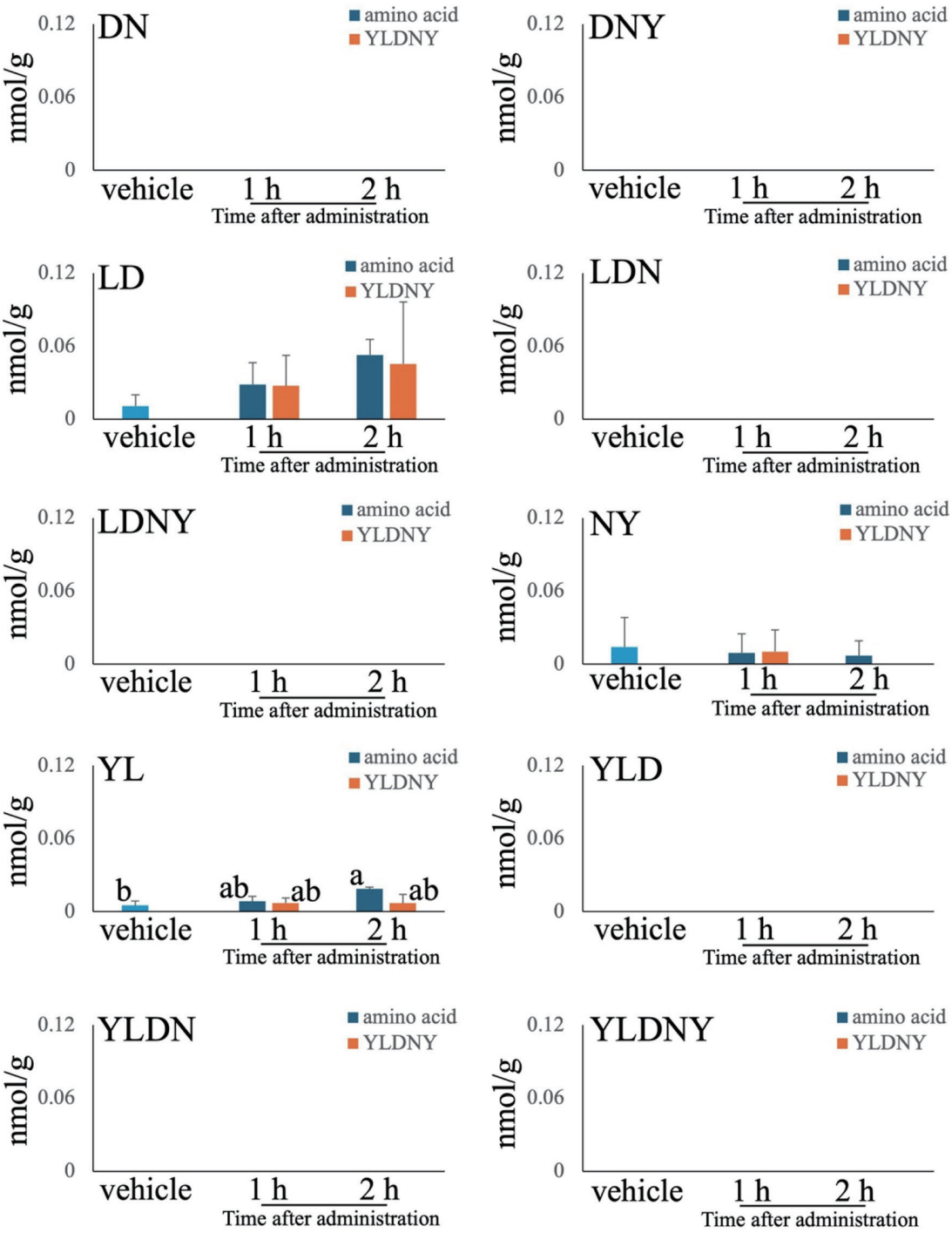

Figure 6. YLDNY and its derived peptides in the liver. All peptides were determined using the method described in Figure 3. The results were presented as the mean ± standard deviation. Different letters indicate a significant difference ($p < 0.05$) using ANOVA with Tukey's post-hoc test among all five groups.

currently in progress.

5. Conclusion

After the oral administration of YLDNY, intact YLDNY remained after gastric pepsin digestion and was completely degraded by the exopeptidases present in the small intestine. YLDNY, its fragment peptides, and their modified peptides were not detected in bloods or liver. However, hepatic xanthine oxidase activity was suppressed, as evidenced by significantly increased hepatic xanthine levels. These findings suggest that YLDNY exerts its antihyperuricemic effects

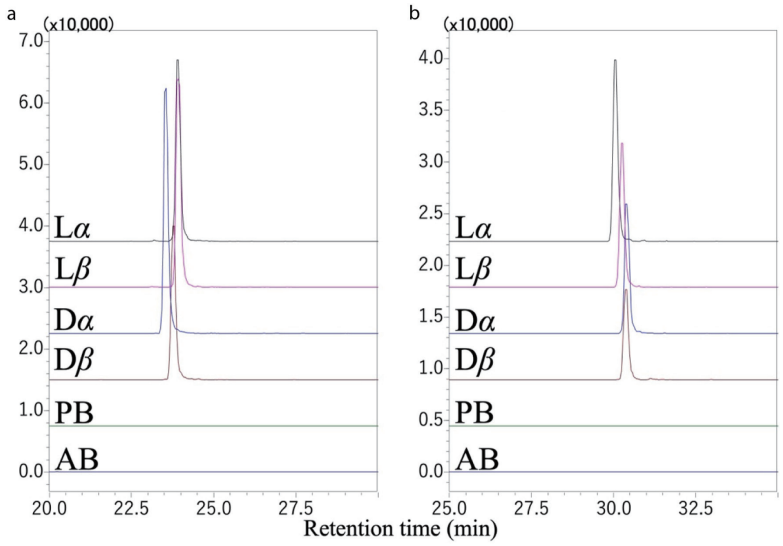

Figure 7. Representative MS chromatograms of the AccQ derivatives of four isomers of Leu-Asp-Asn (LDN) (a) and YLDNY (b) standards and AccQ derivatives of portal and abdominal blood samples. Samples of portal blood (PB), abdominal blood (AB), and synthetic standard peptides were detected by LC-MS/MS in the positive mode under MRM. The gradient program is the same as that shown in Figure 3. The column was maintained at 45 °C. L*α*, L*β*, D*α*, and D*β* represent peptides containing L-*α*-aspartyl residue, L-*β*-aspartyl residue, D-*α*-aspartyl residue, and D-*β*-aspartyl residue, respectively.

through indirect mechanisms. We propose that YLDNY activates cell surface receptors, such as bitter taste receptors expressed in the stomach or upper small intestine, resulting in hormone release and neural signaling, which may attenuate xanthine oxidase activity by regulating liver homeostasis. This study suggests a new target for peptides that affect hepatic purine metabolism.

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Supplementary material

Figure S1. Chromatograms and mass spectra of the second purification of YLDNY. The left panel shows the purification of YLDNY by small column (4.6 mm in diameter) for LC–MS/MS standard. The right panel shows purification of YLDNY by large column (10 mm in diameter) for animal experiment and *in vitro* digestion. Each peak was collected and analyzed by mass spec-

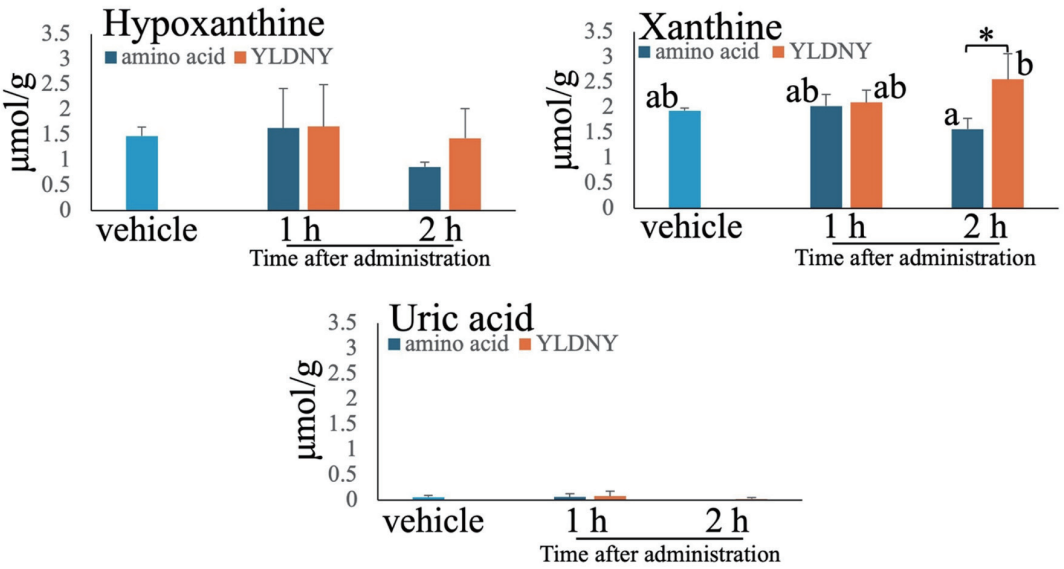

Figure 8. Uric acid and its precursors levels in the liver 1 and 2 h after the administration of YLDNY and amino acid mixture. The results are presented as the mean ± standard deviation. Different letters indicate a significant difference ($p < 0.05$) using ANOVA with Tukey's post-hoc test among all five groups. Asterisks (*) represent a significant difference ($p < 0.05$) between the YLDNY group and the amino acid mixture group as determined using Student's *t*-test.

trometer. No other peptides or amino acids were detected. Only few reagents peak was observed in the preparation for LC-MS/MS standard. Thus, its concentration was determined by amino acid analysis. Preparation for animal experiment showed only ions corresponding to YLDNY monovalent and divalent ions. Except for LDN and DN, other synthetic peptides were not contaminated with other amino acids or peptides. LDN and DN were contaminated with traces of L, LD and N, respectively. However, these peptides were not detected in animals after administration of YLDNY.

References

Ahn, J., Cao, M.J., Yu, Y.Q., and Engen, J.R. (2013). Accessing the reproducibility and specificity of pepsin and other aspartic proteases. Biochim. Biophys. Acta Proteins Proteomics 1834(6): 1222–1229.

Anderson, K.M., and Gayer, C.P. (2021). The pathophysiology of farnesoid X receptor (FXR) in the GI tract: inflammation, barrier function and innate immunity. Cells 10(11): 3206.

Calvo, S.S.C., and Egan, J.M. (2015). The endocrinology of taste receptors. Nat. Rev. Endocrinol. 11(4): 213–227.

Chandel, N.S. (2021). Nucleotide metabolism. Cold Spring Harb. Perspect. Biol. 13(7): a040592.

Chen, L., Ejima, A., Gu, R., Lu, J., Cai, M., and Sato, K. (2019). Presence of exopeptidase-resistant and susceptible peptides in a bacterial protease digest of corn gluten. J. Agric. Food Chem. 67(43): 11948–11954.

Dalbeth, N., Choi, H.K., Joosten, L.A., Khanna, P.P., Matsuo, H., Perez-Ruiz, F., and Stamp, L.K. (2019). Gout (primer). Nat. Rev. Dis. Primers 5(1): 69.

Ejima, A., Nakamura, M., Suzuki, Y.A., and Sato, K. (2018). Identification of food-derived peptides in human blood after ingestion of corn and wheat gluten hydrolysates. J. Food Bioact. 2: 104–111.

Ejima, A., Yamada, K., and Sato, K. (2019). Presence of isomerized aspartic dipeptides in a porcine liver protein hydrolysate and their bioavailability upon ingestion. Bioact. Compd. Health Dis. 2(7): 155–169.

Engevik, A.C., Kaji, I., and Goldenring, J.R. (2020). The physiology of the gastric parietal cell. Physiol. Rev. 100(2): 573–602.

Fiorucci, S., Mencarelli, A., Cipriani, S., Renga, B., Palladino, G., Santucci, L., and Distrutti, E. (2011). Activation of the farnesoid-X receptor protects against gastrointestinal injury caused by non-steroidal anti-inflammatory drugs in mice. Br. J. Pharmacol. 164(8): 1929–1938.

Fruton, J.S. (2002). A history of pepsin and related enzymes. Q. Rev. Biol. 77(2): 127–147.

Gómez, C., Stücheli, S., Kratschmar, D.V., Bouitbir, J., and Odermatt, A. (2020). Development and validation of a highly sensitive LC-MS/MS method for the analysis of bile acids in serum, plasma, and liver tissue samples. Metabolites 10(7): 282.

Hooton, D., Lentle, R., Monro, J., Wickham, M., and Simpson, R. (2015). The secretion and action of brush border enzymes in the mammalian small intestine. Rev. Physiol. Biochem. Pharmacol. 168: 59–118.

Iwai, K., Hasegawa, T., Taguchi, Y., Morimatsu, F., Sato, K., Nakamura, Y., Higashi, A., Kido, Y., Nakabo, Y., and Ohtsuki, K. (2005). Identification of food-derived collagen peptides in human blood after oral ingestion of gelatin hydrolysates. J. Agric. Food Chem. 53(16): 6531–6536.

Iwasaki, Y., Sato, M., Katakura, Y., Sugawara, Y., and Shigemura, Y. (2024). Increase in blood-transferable linear and cyclic dipeptides in human plasma following ingestion of elastin hydrolysate. Food Hydrocoll. Health 6: 100188.

Kanemitsu, T., Tsurudome, Y., Kusunose, N., Oda, M., Matsunaga, N., Koyanagi, S., and Ohdo, S. (2017). Periodic variation in bile acids controls circadian changes in uric acid via regulation of xanthine oxidase by the orphan nuclear receptor PPARα. J. Biol. Chem. 292(52): 21397–21406.

Kim, K.H., Iguchi, E., Funaba, M., and Matsui, T. (2012). Effects of magnesium deficiency on purine-base metabolism in rats. Trace Nutr. Res. 29: 47–50.

Kok, B.P., Galmozzi, A., Littlejohn, N.K., Albert, V., Godio, C., Kim, W., Kim, S.M., Bland, J.S., Grayson, N., Fang, M., Meyerhof, W., Siuzdak, G., Srinivasan, S., Behrens, M., and Saez, E. (2018). Intestinal bitter taste receptor activation alters hormone secretion and imparts metabolic benefits. Mol. Metab. 16: 76–87.

Kusubata, M., Koyama, Y.I., Tometsuka, C., Shigemura, Y., and Sato, K. (2015). Detection of endogenous and food-derived collagen dipeptide prolylhydroxyproline (Pro-Hyp) in allergic contact dermatitis-affected mouse ear. Biosci. Biotechnol. Biochem. 79(8): 1356–1361.

Li, Q., Kang, X., Shi, C., Li, Y., Majumder, K., Ning, Z., and Ren, J. (2018). Moderation of hyperuricemia in rats via consuming walnut protein hydrolysate diet and identification of new antihyperuricemic peptides. Food Funct. 9(1): 107–116.

Liu, N., Wang, Y., Zeng, L., Yin, S., Hu, Y., Li, S., Fu, Y., Zhang, X., Xie, C., Shu, L., Li, Y., Sun, H., Yang, M., Sun, J., and Yang, X. (2020). RDP3, a novel antigout peptide derived from water extract of rice. J. Agric. Food Chem. 68(27): 7143–7151.

Liu, X., Zhang, L., Lai, B., Li, J., Zang, J., and Ma, L. (2024). Harnessing protein hydrolysates and peptides for hyperuricemia management: Insights into sources, mechanisms, techniques, and future directions. J. Agric. Food Chem. 72(34): 18758–18773.

Maehashi, K., and Huang, L. (2009). Bitter peptides and bitter taste receptors. Cell. Mol. Life Sci. 66(10): 1661–1671.

Marino, G., Eckhard, U., and Overall, C.M. (2015). Protein termini and their modifications revealed by positional proteomics. ACS Chem. Biol. 10(8): 1754–1764.

Mehmood, A., Iftikhar, A., and Chen, X. (2024). Food-derived bioactive peptides with anti-hyperuricemic activity: A comprehensive review. Food Chem. 451: 139444.

Miyauchi, S., Kajiwara, S., and Sato, K. (2022). Metabolic fate of peptides in a rice protein hydrolysate in rat intestine and blood after oral administration. J. Food Bioact. 20: 40–55.

Mori, Y., Asakura, S., Yamamoto, A., Odagiri, S., Yamada, D., Sekiguchi, M., Wada, K., Sato, M., Kurabayashi, A., Suzuki, H., Kanamoto, R., and Ohinata, K. (2018). Characterization of soy-deprestatin, a novel orally active decapeptide that exerts antidepressant-like effects via gut–brain communication. FASEB J. 32(2): 568–575.

Murota, I., Taguchi, S., Sato, N., Park, E.Y., Nakamura, Y., and Sato, K. (2014). Identification of antihyperuricemic peptides in the proteolytic digest of shark cartilage water extract using in vivo activity-guided fractionation. J. Agric. Food Chem. 62(11): 2392–2397.

Nagao, A., Nakamoto, Y., Miyauchi, S., and Sato, K. (2024). Presence of modified peptides with high bioavailability and angiotensin-converting enzyme inhibitory activity in Japanese fermented soybean paste (miso). J. Agric. Food Chem. 72(34): 18942–18956.

Naz, S., Moreira dos Santos, D.C., Garcia, A., and Barbas, C. (2014). Analytical protocols based on LC–MS, GC–MS and CE–MS for nontargeted metabolomics of biological tissues. Bioanalysis 6(12): 1657–1677.

Rahmadian, Y., Miyauchi, S., Wijanarti, S., Asai, T., and Sato, K. (2025). Presence of γ-glutamyl and β-aspartyl isopeptides, diketopiperazines, pyroglutamyl peptides, in addition to normal peptides in fish and soy sauces: Structures, contents and their bioavailability. J. Food Bioact. 29: 60–76.

Sato, K. (2022). Metabolic fate and bioavailability of food-derived peptides: Are normal peptides passed through the intestinal layer to exert biological effects via proposed mechanisms? J. Agric. Food Chem. 70(5): 1461–1466.

Shigemura, Y., Nakaba, M., Shiratsuchi, E., Suyama, M., Yamada, M., Kiyono, T., Fukamizu, K., Park, E.Y., Nakamura, Y., and Sato, K. (2012). Identification of food-derived elastin peptide, prolyl-glycine (Pro-Gly), in human blood after ingestion of elastin hydrolysate. J. Agric. Food Chem. 60(20): 5128–5133.

Shigemura, Y., Iwasaki, Y., Tateno, M., Suzuki, A., Kurokawa, M., Sato, Y., and Sato, K. (2018). A pilot study for the detection of cyclic prolylhydroxyproline (Pro-Hyp) in human blood after ingestion of collagen hydrolysate. Nutrients 10(10): 1356.

Sri Wijanarti, S., Gu, R., Chen, L., Liu, W., Cai, M., Suzuki, R., and Sato, K. (2024). Substrate specificity of exopeptidases in small intestinal mucosa determines the structure of food-derived collagen peptides in rat lumen and blood. J. Food Bioact. 26: 29–41.

Steensels, S., and Depoortere, I. (2018). Chemoreceptors in the gut. Annu. Rev. Physiol. 80(1): 117–141.

Sternini, C., and Rozengurt, E. (2025). Bitter taste receptors as sensors of

gut luminal contents. Nat. Rev. Gastroenterol. Hepatol. 22(1): 39–53.
Svistounov, D., Solbu, M.D., Jenssen, T.G., Mathisen, U.D., Hansen, T., Elgstøen, K.B.P., and Zykova, S.N. (2022). Development of quantitative assay for simultaneous measurement of purine metabolites and creatinine in biobanked urine by liquid chromatography-tandem mass spectrometry. Scand. J. Clin. Lab. Invest. 82(1): 37–49.
Wang, Q., Stagos, D., and Liu, M. (2025). Antihyperuricemia bioactive substances derived from marine organisms. J. Agric. Food Chem. 73(23): 14192–14211.
Zhang, X., Cui, J., Hou, J., and Wang, W. (2022). Research progress of natural active substances with uric-acid-reducing activity. J. Agric. Food Chem. 70(50): 15647–15664.