Role of Dietary Peptides in Human Health

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Peptides with health-beneficial biological activities

Peptides are ubiquitous bio-molecules in human body regulating biological functions

Insulin

- Helps in maintaining blood sugar level

Irisin

- Helps in browning of adipocytes, reduces lipid accumulation

Apelin

- Increases lipolysis, regulates blood pressure by influencing renin–angiotensin system (RAS)

- Synthesize inside the body
- Receptor activation, G-protein coupled receptor (GPCR)
Dietary peptides with health-beneficial biological activities

Food-derived dietary bioactive peptides (BAPs)

- **Inert** inside the native protein but exert biological activities once released from the parental protein

**Major Challenges with BAPs**
- Stability in the gastrointestinal tract
- Bitter taste

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**Peptidomic screening of bitter and nonbitter casein hydrolysate fractions for insulinogenic peptides**

Niamh M. Murray,* Dolores O’Riordan,* Jean-Christophe Jacquier,* Michael O’Sullivan,* Thérèse A. Holton,† Kieran Wynne,‡ Randall C. Robinson,§ Daniela Barile,‡ Seren D. Nielsen,§ and David C. Dallas§

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J. Dairy Sci. 101:2826–2837
https://doi.org/10.3168/jds.2017-13853
Dietary peptides - intestinal absorption

Proteins in the diet and in GI secretions are hydrolyzed initially by gastric pepsin and hydrochloric acid, then by pancreatic proteases and peptidases in the small bowel and cytoplasmic domain of the enterocyte.

Dietary proteins after ingestion breaks down to amino acids and transport into the circulation.

90% of the absorbed dietary proteins is represented in the circulation by amino acids and 10% as dipeptides and tripeptides.

Substantial quantity of undigested nitrogenous material (water insoluble undigested proteins and oligopeptides) is transferred from the small intestine to the large intestine.

Absorption of peptides is structure dependent.

After simulated gastrointestinal (GI) digestion, 65.1% of protein from egg white (EW) can transfer to the large intestine.

~7% of the digested EW proteins are free amino acids and ~70% are peptides (2-10 aa long)

Kiela et al., 2016, Best Practice & Research Clinical Gastroenterology
PMID: 27086882, PMID: 10820518, PMID: 21933113, PMID: 1103167

Other/ New Thoughts

Data from Food BIOPEP Lab

Val-Tyr Transport

MS detection | Apical | Basolateral
---|---|---
Val-Tyr | | |
Tyr | | |

PMID: 29756574, PMID: 28215168

Number of identifications

Peptide length

EW-GID

0 2 4 6 8 10 12 14 16 18 20 22 24
0
100
200
300
400
500
Dietary bioactive peptides - intestinal absorption

Transport of BAPs across the intestinal epithelium

BAPs cross the Blood-Brain Barrier

Tyr-Pro were transported across the BBB with $K_i$ value of $3.53 \pm 0.74 \mu L/g\cdot min$, and accumulated in the mouse brain parenchyma.

PMID: 33003506

PMID: 30962462
Dietary peptides with health-beneficial biological activities

**Dietary γ-glutamyl peptides**

- Occur naturally in foods – dry beans, cheeses, onion, soy sauce
- Major contributors of the **Kokumi sensation** (mouth fullness, continuity, and thickness) through their interaction with the **Calcium-Sensing Receptors (CASR)** in the tongue

  **Umami**-delicious taste/pleasant flavor taste;  
  **Kokumi**-rich taste, taste enhancer, longer lasting.
- Generally resistant to gastrointestinal digestion due to the γ-bond

**Iso-peptide bond**: Amide bonds between **sidechain amines or carbonyl carbons** on the side chain rather than α-amine or α-carbonyl carbon

Glutathione
Dietary γ-glutamyl peptides and interaction with taste cells

Kokumi Substances, Enhancers of Basic Tastes, Induce Responses in Calcium-Sensing Receptor Expressing Taste Cells

Yuta Takayama, Reiko Yasuda, Motonaka Kuroda, Yuzuru Eto
Published: April 12, 2012 • https://doi.org/10.1371/journal.pone.0034489

Confocal images showing colocalization of CaSR and the taste cell markers in taste cells from mouse circumvallate papillae.

CASR expression in PLCβ2 in type II taste cells (receptor cells) and of neural cell adhesion molecule (NCAM) in type III taste cells (presynaptic cells)

- γ-glutamyl peptides can **allosterically activates CaSR** after binding it to the Venus-Flytrap (VFT) Domain
- **CaSR omnipotently present** in various mammalian tissues
- CaSR activation by can γ-glutamyl peptides **reduce inflammation**

Intervention of Dietary Dipeptide Gamma-L-Glutamyl-l-Valine (γ-EV) Ameliorates Inflammatory Response in a Mouse Model of LPS-Induced Sepsis

Hua Zhang, Toshihiro Kodera, Yuzuru Eto, Yoshinori Mine

γ-Glutamyl valine supplementation-induced mitigation of gut inflammation in a porcine model of colitis

Ajinomoto Co., Ltd., 1-2 Saitozaki-cho, Kawasaki-shi, Kawasaki-ken, Kanagawa-ken, 210-8503, Japan
Chronic vascular inflammation is the major contributor of pathophysiological conditions such as atherosclerosis and the progression of cardiovascular diseases (CVDs).

Intervention of γ-glutamyl peptides will reduce vascular inflammation and development of atherosclerosis via allosteric activation of CaSR.

γ-glutamyl valine (γ-EV) used as the model γ-glutamyl peptide.
Presence of CaSR in vascular endothelium

Can γ-EV exhibit anti-inflammatory effect via activation of vascular endothelial CaSR?

Confirmed the presence of CaSR in Human aortic endothelial cells (HAoECs) and its localization in the cell

Guha et al, JAFC, 2020, 68 (34), 9139-9149
Evaluating the biological activity of γ-EV on HAoECs

**Cell type:** HAoECs (Human aortic endothelial cells)

**Negative Control**

**Positive Control**

0.01 mM 0.1 mM 1 mM

γ-EV pre-treatment (2 h)

TNF-α treatment (5 ng/mL, 6 h)
Anti-inflammatory activity of γ-EV on HAOECs

Cell adhesion molecules

VCAM-1

E-Selectin

What is the role of vascular endothelial CaSR?

Chemokine

MCP-1

Guha et al, JAFC, 2020, 68 (34), 9139-9149
What is the transport efficiency and mechanism of γ-EV across the intestinal cells?
Transport efficiency of γ-EV across intestinal Caco-2 cells

1 mM γ-EV

Caco-2 cells grown for 21 days until differentiation with the TEER values being checked regularly until it was over 500 Ωcm²

Transepithelial electrical resistance (TEER) measures the integrity of tight junction dynamics

Transport efficiency of γ-EV was evaluated and the apparent permeability ($P_{app}$) of the peptide was found to be $1.56 \times 10^{-6}$ cm/sec.

Guha et al, Nutrients, 2021, 13 (5), 1448
Transport mechanism of γ-EV across intestinal Caco-2 cells

3 ways peptide transport
- Trans-cellular
- Para-cellular
- Transporter dependent

Route of transport of γ-EV is through PepT1 and para-cellular pathway
Major findings - on γ-EV in-vitro studies

• γ-EV (1mM) pretreatment significantly reduced the TNF-α induced upregulation of inflammatory adhesion molecules, VCAM-1 and E-selectin, by 44% and 57%, respectively in HAoECs

• Significantly reduced the production of TNF-α induced cytokines IL-8 and IL-6 by 40 and 51%, respectively, and chemokine MCP-1 by 68%

• The anti-inflammatory effect of γ-EV was attenuated by the treatment of the CaSR-specific inhibitor, NPS-2143, suggesting the involvement of CaSR-mediated pathways

• γ-EV was able to transport across intestinal Caco-2 cells with a Papp of 1.56 x 10⁻⁶ cm/sec via PepT-1 and paracellular pathway
γ-EV in-vivo studies - Bioavailability

γ-EV Mixed Water
100 mg per kg body weight (Daily)

γ-EV Gavage
100 mg per kg body weight

Blood Draw: Jugular vein

5.26 µM

γ-EV is bio-available in rodent model

Unpublished Data: FoodBioPepLAB UNL
γ-EV in-vivo studies – vascular inflammation - Atherosclerosis

- **Group 1 (PC)** – Positive control on Low-Fat diet (n=9) (20 kcal% protein, 70 kcal% carbohydrates, and 10 kcal% fat)
- **Group 2 (PCF)** – Positive control on High-Fat diet (n=9)
- **Group 3 (LD)** – Low-Dose γ-EV treatment (50 mg/Kg BW) on High-Fat diet (n=9)
- **Group 4 (HD)** – High-Dose γ-EV treatment (150 mg/Kg BW) on High-Fat diet (n=9)

(20 kcal% protein, 40 kcal% carbohydrate, and 40 kcal% fat, 1.25% cholesterol)

Apolipoprotein E knockout (ApoE\(^{-/-}\)) mice

γ-EV in drinking water (50 mg/Kg BW – G3 and 160 mg/Kg BW – G4)

- Aortic root and arch
- Thoracic aorta
- Abdominal aorta

Lipid accumulation through Oil Red O staining
Expression of inflammatory biomarkers via Immunohistochemistry of frozen tissue sections
Expression of inflammatory biomarkers via immunoblotting

Plasma
Spleen
Cultured splenocytes
γ-EV in-vivo studies – vascular inflammation- Atherosclerosis

1. Lipid accumulation in ApoE-/ mice aortas

γ-EV intervention reduces lipid accumulation and plaque formation in aorta

2. Effect of macrophage infiltration in ApoE-/ mice aortas

Unpublished Data: FoodBioPepLAB UNL
4. Anti-inflammatory effect of γ-EV in ApoE-/- mice thoracic aortas

γ-EV intervention reduces the expression of pro-inflammatory biomarkers in thoracic aorta

Unpublished Data: FoodBioPepLAB UNL
Major findings - γ-EV *in-vivo* studies - Atherosclerosis

- γ-EV exhibits **anti-inflammatory effect** in high fat-fed ApoE\(^{-/-}\) mice aorta

- γ-EV intervention reduces **lipid accumulation** in high fat-fed ApoE\(^{-/-}\) mice aortas

- The **anti-inflammatory effects** can reduce the atherosclerotic **plaque** development in high fat-fed ApoE\(^{-/-}\) mice

- Like *in-vitro* study, possible role of **CaSR in modulating** the observed biological activity
γ-EV in-vivo studies – Type-2-diabetes (T2D)

Activation of CaSR and potential role in T2D modulation

**db/db Mice**

- db/db mice are used to model phase 1 to 3 of diabetes type II and obesity
- Spontaneous mutation (Lepr$^{db}$) demonstrate morbid obesity, chronic hyperglycemia, pancreatic beta cell atrophy and come to be hypoinsulinemic

Intervention of γ-glutamyl peptides will improve type-2 diabetes conditions via allosteric activation of Enteroendocrine CaSR
γ-EV *in-vivo* studies – Type-2-diabetes (T2D)

Male, *db/db* mice

- *n* = 8/group
- H$_2$O Chow diet
- 5 weeks
- AIN-93G diet
- 2 weeks
- 3 weeks
- Body composition
- Metabolic cage

Blood and tissue collection

Dosage: 500mg/kg BW
γ-EV *in-vivo* studies – Type-2-diabetes (T2D)

** γ-EV intervention normalized water intake 

![Graph showing water intake over time](image)

** Acclimation** | **Intervention**
---|---
| Ctrl | γ-EV |

Water Intake (mL) Per Gram Biomass

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<th>Time (days)</th>
<th>0</th>
<th>4</th>
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<td>Ctrl</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
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</tr>
<tr>
<td>γ-EV</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
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</tbody>
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CS7BL/6J ~1.5 mL/day (per gram biomass) (Tordoff, 2007)

Unpublished Data: FoodBioPepLAB UNL
γ-EV in-vivo studies – Type-2-diabetes (T2D)

γ-EV intervention lower blood glucose, lower glycogen content in liver and Skeletal muscle
γ-EV *in-vivo* studies – Type-2-diabetes (T2D)

γ-EV intervention in renal function to circadian rhythm

Unpublished Data: FoodBioPepLAB UNL

Metabolic cage study

γ-EV intervention modulating the respiratory exchange ratio as well as energy expenditure in the early half of the light phase—potentially affecting the Circadian rhythm?
Major findings- γ-EV in-vivo studies – Type-2-diabetes (T2D)

- In *db/db* mice, γ-EV intervention improved high blood glucose and diuresis, suggesting an anti-diabetic effect

- γ-EV intervention stimulated hepatic glucose catabolism (lower glycogen, higher AMPK activity) vs untreated mice.

- γ-EV intervention is potentially modulating circadian rhythm
γ-EV in foods - Great Northern Beans

- 11 γ-glutamyl peptides identified in the Nebraskan Great-Northern Beans (GNBs), these peptides were resistant to GI digestion and naturally present in the beans.

\[ \text{γ-Glu-Val} \, \text{[52.64ng/mg of bean protein]} \]

Challenges and Solutions

- γ-EV or γ-glutamyl peptides present in a very low amount in edible beans, thus eating beans and observing these bioactivities are not realistic.

- Food processing techniques can be used to enhance the abundance of γ-glutamyl peptides that can be used as flavor enhancer with additional health beneficial properties.
Increasing the abundance of γ-glutamyl peptides in Nebraskan Great Northern Beans—Fermentation

Glutamyl cysteine ligases (GCL) from lactobacilli

*L. reuteri* LTH5448 express GCL, which utilizes L-glutamine, L-glutamic acid, and other acceptor amino acids from GN beans to synthesize γ-glutamyl peptides

*Limosilactobacillus reuteri* (formerly *Lactobacillus reuteri*)

L-glutamic acid and L-glutamine (collectively abbreviated as Glx) ranges in most beans between 1.2-1.8 g/100g;

Fermentation with GNB paste

LTH5448 WT

Unpublished Data: FoodBioPepLAB UNL
Biological activity of the fermented beans

Cell type: HUVECs (Human umbilical vein endothelial cells)

Glutathione (GSH) as a treatment control

VCAM-1 Expression

MCP-1 Expression

GNB fermented with *L. reuteri* LTH5448 increases the abundance of γ-glutamyl peptides and fermented bean paste intervention can reduce vascular inflammation.

Unpublished Data: FoodBioPepLAB UNL
**γ-glutamyl peptides (GPs)/ fermented beans – What’s next – flavor development**

- Increases **kokumi active peptides**
- Isolated **peptide fractions can be used as natural flavor ingredients**
Acknowledgements

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