Egg protein bioactive compounds: looking for a wellness gastronomy

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“Foods or dietary compounds that may provide a health benefit beyond the traditional nutrients”
Introduction: Functional Foods

- Omega-3
- Prebiotics
- Fiber
- Phytochemicals
- Vitamins
- Probiotics
- Proteins and peptides

Cardiovascular

Digestive

Endocrine

Immune system

Nervous system
Introduction: bioactive peptides

“specific amino acid sequences released by parent protein after in vivo or in vitro hydrolisis, which may act as regulatory compounds with physiological activities”

- Antioxidant
- Antihypertensive
- Hypcholesterolemic
- Opioid
- Antimicrobial
- Immunomodulator
- Mineral binding
- Antiproliferative
Introduction: Egg protein derived peptides

Nutritive value

- High protein content
- Vitamins A, B, D, E and K
- Rich in minerals
- Lecithin

<table>
<thead>
<tr>
<th>Activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACEI</td>
<td>Miguel et al., 2004; Miguel et al., 2005; Miguel et al., 2007; Yu et al., 2011; Ahmad et al., 2012</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>Jung et al., 2001; Dávalos et al., 2004</td>
</tr>
<tr>
<td>Vasodilator</td>
<td>Fujita et al., 1995; Scruggs et al., 2004; Miguel et al., 2005; Miguel et al., 2006; Miguel et al., 2007; García-Redondo et al., 2010</td>
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<td>Oxidative stress</td>
<td>Manso et al., 2008; Huang et al., 2010</td>
</tr>
<tr>
<td>Antidiabetic</td>
<td>Yu et al., 2012; Wang et al., 2012</td>
</tr>
</tbody>
</table>

ACEI: Angiotensin converting enzyme inhibitory activity
The oral administration of egg white hydrolysates, containing bioactive peptides, would allow the control of some pathologies.
Hypertension

2013-

In 2030 ≈ 23 mill. of people will die by cardiovascular diseases (CVD)

Hypertension is one of the main risk factor for CVD development

Healthy diet

Exercise

Drugs

Egg derived peptides may represent a novel strategy for the prevention and/or treatment of hypertension
Functional foods with antihypertensive activity

Angiotensin-I
DRVYIHPFHL

Angiotensin-II
DRVYIHPF

Potent vasopressor

Bradykinin

ACE

ACE inhibitors

Bradykinin inactivation

Bradykinin hypotensive activity

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Origin</th>
<th>ACE-inhibition (IC&lt;sub&gt;50&lt;/sub&gt;µM)</th>
<th>References</th>
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<tbody>
<tr>
<td>IPP</td>
<td>Milk</td>
<td>5</td>
<td>Nakamura et al., 1995</td>
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<td>VPP</td>
<td>Milk</td>
<td>9</td>
<td>Nakamura et al., 1995</td>
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<td>LW</td>
<td>Egg</td>
<td>6.8</td>
<td>Fujita et al., 2000</td>
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<td>LKPNM</td>
<td>Bonito</td>
<td>2.4</td>
<td>Fujita y Yoshikawa, 1999</td>
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<td>IKW</td>
<td>Chicken</td>
<td>0.21</td>
<td>Fujita y Yoshikawa, 1999</td>
</tr>
<tr>
<td>LRP</td>
<td>α-zein</td>
<td>0.27</td>
<td>Miyoshi et al., 1991</td>
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</tbody>
</table>
To obtain egg-derived peptides with ACEI and/or antioxidant activity which could be used in the prevention and/or control of hypertension.
Egg white hydrolysates with ACEI and/or antioxidant activity

UF membrane 3000 Da cut-off

IC₅₀ = 55.3 μg/ml
Retentate

IC₅₀ = 298 μg/ml
Egg-white with pepsin (3h)

IC₅₀ = 34.5 μg/ml
Permeate

HPLC-MS/MS
Peptide identification with ACEI activity

Increased ORAC value

HEW = 381 μmol Trolox eq/g of protein
Permeate = 2780 μmol Trolox eq/g of protein

HEW ad peptides caused a delay in lag time of LDL oxidation
Antihypertensive activity in animal models

**Experimental model**

- SHR
- Wistar-Kyoto

**Tail cuff method**

**Short-term**

**Intragastric administration**

- Water (negative control)
- Captopril (positive control)
- Hydrolysate or peptides

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>24</th>
</tr>
</thead>
</table>

Measurements after administration

Start measurement
Antihypertensive activity in animal models: **short-term**

Miguel et al. Br J Nutr. 94:731-7, 2005
Antihypertensive activity in animal models: long-term

<table>
<thead>
<tr>
<th>WEEKS OF LIFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
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</tbody>
</table>

**TREATMENT PERIOD**

- GROUP 1: 0.5 g/kg/day OF EGG WHITE PROTEIN WITH PEPSIN
- GROUP 2: 1 g/kg/day OF EGG WHITE PROTEIN WITH PEPSIN
- GROUP 3: 1 g/kg/day OF EGG WHITE
- GROUP 4: 100 mg/kg/day OF CAPTOPRIL
- GROUP 5: WATER

**FOLLOW-UP PERIOD** (ALL GROUPS TAKE WATER)

**WEEKLY CONTROL OF BODY WEIGHT, DRY FOOD AND LIQUID CONSUMPTION**

**SBP AND DBP WERE MEASURED WEEKLY BY THE TAIL CUFF METHOD**
Antihypertensive activity in animal models: long-term

Miguel et al. Life Sci. 78:2960-6, 2006
Antihypertensive mechanisms: in vivo ACEI activity

ACEI activity  
Miguel et al. Mol Nutr Food Res. 51:555-63, 2007

Antioxidant activity  

Antioxidant capacity

Lipid peroxidation

Vasodilator activity  

Anti-inflammatory activity  
Garcés-Rimón et al., in preparation
Publications and International Patents


Short-term effect of egg white hydrolysates on the vascular function in rat aorta. The sequence NV, RADHP, and VAERVP (0.1 mM) induced vasodilatation in intact aortic rings, with the maximum percentage of dilation corresponding to RADHP (40.5±7.0%). Two of the end products of the gastrointestiinal digestion, RADHP and YPL also showed vasodilator activity with degrees of dilation higher than 20%. However, all these
Egg derived peptides, selected by their *in vitro* biological multi-activity, could treat and/or prevent simultaneously some cardiometabolic complications associated to metabolic syndrome, such as obesity, dyslipidemia, diabetes and hypertension.
Egg peptides with multifunctional activity: selection

EGG WHITE

HYDROLYSIS

Different food grade enzymes

HYDROLYSATES

0, 2, 4, 8, 12, 24, 36 y 48 h

HYDROLYSATE SELECTION USING IN VITRO STUDIES

ACE inhibitory activity
Quirós et al., Peptides 30: 1848-53, 2009

Antioxidant activity
Dávalos et al. J Food Prot 67: 1939-44, 2004

Hypocholesterolemic Activity
Yoshie-Stark y Wasche, Food Chem 88: 179-84, 2004

DPP IV Inhibitory activity
DPPIV Inhibitor Screening Assay Kit, Cayman Chemical

Most activities simultaneously
The hydrolysate of egg white with pepsin for 8 hours presented a high ACE inhibitory activity, as well as important peroxyl radical scavenging capacity and bile acid-binding activity, and a moderate DDP IV inhibitory activity.

<table>
<thead>
<tr>
<th>Incubation period (h)</th>
<th>IACE (IC$_{50}$ µg/ml)</th>
<th>Antioxidant activity (µmol Trolox/ g protein)</th>
<th>Hypcholesterolemic activity (mmol bounded/ mg protein)</th>
<th>DPP IV Inhibition (IC$_{50}$ mg/ml)</th>
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<tbody>
<tr>
<td>0</td>
<td>&gt;1000</td>
<td>75.7</td>
<td>0.028</td>
<td>&gt;1000</td>
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<td>2</td>
<td>81.9</td>
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<td>4</td>
<td>50.5</td>
<td>624.0</td>
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<td><strong>8</strong></td>
<td><strong>46.7</strong></td>
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<td>5.55</td>
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<td>48</td>
<td>47.2</td>
<td>457.0</td>
<td>0.029</td>
<td>4.82</td>
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</table>
Egg peptides with multifunctional activity: selection

The hydrolysate with aminopeptidase for 24 hours stood out for its peroxyl radical scavenging capacity, and its hypocholesterolemic activity. This hydrolysates also exhibited a moderate DDP IV inhibitory activity.

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<tr>
<th>Incubation period (h)</th>
<th>IACE (IC$_{50}$ µg/ml)</th>
<th>Antioxidant activity (µmol Trolox/ g protein)</th>
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<th>DPP IV Inhibition (IC$_{50}$ mg/ml)</th>
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<tr>
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<td>79.4</td>
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<td>12</td>
<td>506.6</td>
<td>491.7</td>
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<tr>
<td>24</td>
<td>151.4</td>
<td>1099.9</td>
<td>0.259</td>
<td>8.07</td>
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<tr>
<td>36</td>
<td>229.8</td>
<td>610.1</td>
<td>0.138</td>
<td>10.31</td>
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<tr>
<td>48</td>
<td>121.5</td>
<td>904.8</td>
<td>0.103</td>
<td>6.67</td>
</tr>
</tbody>
</table>
Effect of the administration of egg hydrolysates on an experimental model of metabolic syndrome

- Obesity
- Hyperphagia
- Dyslipidemia (↑Tg, Cho and FFA)
- Insulin resistance
- Normal postprandial glycemia
- Proinflammatory state (↑TNF-α)
- Oxidative stress (↑superoxide anion)
- Renal damage and pre-hypertension
Egg peptides with multifunctional activity: in vivo study

### TREATMENT

- 10 Rats. Water
- 10 Rats. 750 mg/kg/day pepsin-egg white hydrolysate
- 10 Zucker Lean rats

### PERIPHERAL NEUROPATHY

- Feces and urine collection

### FOLLOW UP: WEIGHT, FOOD AND LIQUID INTAKE PER WEEK

### DIRECT BLOOD PRESSURE MEASUREMENTS

### SACRIFICE

### HISTOLOGICAL STUDIES

- Liver (Reduced Glutathione)
- Epididymal adipose tissue
- Brown adipose tissue
- Pancreas
- Kidneys

### PLASMA SAMPLES

- Total Cholesterol
- Triglycerides
- Free fatty acids
- Lipase Activity
- Adiponectin
- TNF-α
- Malondialdehyde
- Antioxidant Capacity
- Glucose
- Insulin
- ACE activity

### HEART ISOLATION AND PERFUSION

- Coronary perfusion pressure
- Left ventricular pressure
- Final Diastolic pressure
- Heart rate
- Cardiac mass Index
- Left Ventricular mass index

### VASCULAR REACTIVITY

- Aorta
- Mesenteric arteries

### TISSUE SAMPLES

- Liver (Reduced Glutathione)
- Epididymal adipose tissue
- Brown adipose tissue
- Pancreas
- Kidneys

Statistical analysis.
* Lean vs obese water
# Obese water vs obese treated
Compared to the lean control group, obese Zucker rats showed mechanical allodynia. The consumption of pepsin egg hydrolysate significantly increased the threshold for mechanical sensitivity.
The intake of pepsin hydrolysate lowered the plasma levels of insulin, causing a clear improvement in the quantitative indices of insulin resistance, insulin sensitivity and pancreatic β cells functionality.
The consumption of egg hydrolysate significantly reduced the epididymal adipose tissue, improved the hepatic steatosis, and lowered the plasmatic concentration of free fatty acids.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Lean</th>
<th>Obese</th>
<th>Obese-pepsin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight (g)</td>
<td>1.45±0.06</td>
<td>1.43±0.06</td>
<td>1.50±0.09</td>
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<tr>
<td>Liver weight (g)</td>
<td>14.87±0.73</td>
<td>28.64±0.95*</td>
<td>26.06±1.02</td>
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<tr>
<td>Relative liver weight</td>
<td>3.70±0.13</td>
<td>5.40±0.19*</td>
<td>4.90±0.19</td>
</tr>
<tr>
<td>Kidneys weight (g)</td>
<td>2.89±0.18</td>
<td>2.84±0.21</td>
<td>2.59±0.05</td>
</tr>
<tr>
<td>Relative Kidneys weight</td>
<td>0.72±0.04</td>
<td>0.53±0.03*</td>
<td>0.48±0.01</td>
</tr>
<tr>
<td>Epididymal adipose tissue (g)</td>
<td>6.37±0.31</td>
<td>14.80±0.24*</td>
<td>13.41±0.2#</td>
</tr>
<tr>
<td>Relative epididymal adipose tissue weight</td>
<td>1.60±0.006</td>
<td>2.72±0.07*</td>
<td>2.47±0.02#</td>
</tr>
</tbody>
</table>

Liver histology

- A: Lean
- B: Obese
- C: Obese-pepsin

Graph showing the effect on free fatty acids (µM):
- *: Significant difference
- #: Marked difference
The administration of the pepsin hydrolysate also decreased the plasma levels of tumor necrosis factor alpha and reduced the oxidative stress.
The administration of the pepsin hydrolysate improved the vascular function in mesenteric resistance arteries through an increase in the levels and/or the bioavailability of nitric oxide.
Conclusion

Hydrolysates and peptides from egg proteins could be used as health enhancing ingredients in functional foods, nutraceuticals and pharmaceutical preparations to reduce the risk of hypertension and other complications associated to metabolic syndrome.
Bioactive components in eggs

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Support and collaborators

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