Cyanidin-3-O-glucoside ameliorates lipid and glucose accumulation in C57BL/6J mice via activation of PPAR-α and AMPK

Food Biomedical Science Lab.
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Sep 23th, 2014
Background

Previous study about cyanidin (CY)

<table>
<thead>
<tr>
<th>Kd values and EC50 values of cyanidin (CY) C3G</th>
<th>PPARα</th>
<th>LXRα</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPR</td>
<td>3.08 μM</td>
<td>2.2 μM</td>
</tr>
<tr>
<td>TR-FRET</td>
<td>3.03 μM</td>
<td>3.5 μM</td>
</tr>
</tbody>
</table>
Background

Berries containing C3G regulate lipid and glucose metabolisms

Peroxisome proliferator-activated receptors (PPARs):
- Nuclear receptors
- Containing 3 isoforms: PPARα, PPARγ, PPARδ/β

PPARα:
- A major regulator of lipid metabolism in the liver
  - Fatty acid uptake (fatty acid transport)
  - Fatty acid utilization (fatty binding and activation)
  - Fatty acid catabolism (peroxisomal and mitochondrial fatty acid β-oxidation)
  - Ketogenesis
  - Triglyceride turnover
- Ligands:
  - Synthetic ligands include the fibrate drugs (hyperlipidemia)
  - Endogenous ligands include fatty acids and various fatty acid-derived compounds

AMP-activated protein kinase (AMPK):
- An enzyme plays a role in cellular energy homeostasis
- Consists of three proteins (subunits): α, β, and γ
- Three subunits together make a functional enzyme

AMPK:
- Stimulate:
  - Fatty acid oxidation
  - Ketogenesis
- Inhibit:
  - Lipogenesis
  - Triglyceride synthesis
  - Gluconeogenesis

* RXR: retinoid X receptor
PPRE: peroxisome proliferator hormone response elements
### Experimental design

**Molecular targets of C3G**
- BIACore Surface plasmon resonance (SPR)
- Time resolution-fluorescence resonance energy transfer (TR-FRET) coactivator assay
- AMPK activity assay

**Animal/cell experimental design**

<table>
<thead>
<tr>
<th>Time Points</th>
<th>Assays/Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid-loading for 24 h</td>
<td>• Lipid contents</td>
</tr>
<tr>
<td>Treatment with C3G for 24 h</td>
<td>• Fatty acit oxidation/synthesis</td>
</tr>
<tr>
<td>8 weeks HFD</td>
<td>• Autophagy analysis</td>
</tr>
<tr>
<td>8 weeks HFD+FF</td>
<td>• Gluconeogenesis</td>
</tr>
<tr>
<td>8 weeks HFD+C3G</td>
<td><strong>HFD:</strong> High fat diet (45%)</td>
</tr>
<tr>
<td>• Sacrifice</td>
<td><strong>FF:</strong> 100 mg/kg body weight of fenofibrate (FF)</td>
</tr>
<tr>
<td>• Sample (plasma &amp; organs) collection</td>
<td><strong>C3G:</strong> 100 mg/kg body weight of cyanidin-3-O-glucoside (C3G)</td>
</tr>
</tbody>
</table>

**Physiological relevance & molecular mechanisms of C3G**
- Body & organ weight measurement
- Plasma lipid, glucose, insulin & hormone measurement
- Liver & adipose tissue histology & analysis
- Liver lipid concentration measurement
- Oral glucose tolerance test (OGTT)
- Insulin tolerance test (ITT)
- Autophagy pathway analysis
- qPCR & immunobloting
C3G induces PPARα coactivator activity via direct binding to PPARα

Time resolution-fluorescence resonance energy transfer (TR-FRET) coactivator assay

K_D values and EC_{50} values of C3G and positive controls

<table>
<thead>
<tr>
<th>C3G</th>
<th>PPARα</th>
<th>PPARγ</th>
<th>PPARδ</th>
<th>PPARα</th>
<th>PPARγ</th>
<th>PPARδ</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPR</td>
<td>456 nM</td>
<td>1.36μM</td>
<td>4.96μM</td>
<td>13.2 nM</td>
<td>377 nM</td>
<td>102 nM</td>
</tr>
<tr>
<td>TR-FRET</td>
<td>1126 nM</td>
<td>10.8μM</td>
<td>31.05μM</td>
<td>26.9 nM</td>
<td>82.3 nM</td>
<td>10.25 nM</td>
</tr>
</tbody>
</table>

K_D, the equilibrium dissociation constant ('binding constant'); EC_{50}, Half maximal effective concentration
C3G induces AMPKα1 activity via direct interaction with AMPKα1

A

AMPK (α1/β1/γ1) activity

B

AMPK (α2/β1/γ1) activity

C3G directly activates PPARα and AMPK
C3G reduces lipid accumulation in mouse livers & hepatocytes

A) Intracellular Triglyceride

B) Plasma triglyceride

C) Hepatic Triglyceride

D) Plasma AST

D) Plasma ALT

AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase.
C3G induces hepatic fatty acid oxidation and ketogenesis while decreases fatty acid synthesis via regulation of PPARα & AMPKα1

- C3G reduces lipid accumulation via increases fatty acid oxidation, ketogenesis, whereas inhibits fatty acid synthesis
C3G induces phosphorylation of AMPK thus blocks the mTOR-S6K1 axis

mTOR, mammalian target of rapamycin; S6K1, P70-S6 Kinase 1
C3G induces hepatic autophagy pathway

C3G reduces lipid accumulation via activates hepatic autophagy pathway
C3G reduces plasma glucose & insulin concentrations and improves insulin sensitivity

AUC of OGTT

HFD
FF
C3G

AUC of ITT

HFD
FF
C3G

AUC

ITT

HFD
FF
C3G

AUC of ITT

Insulin-sensitive Index

HOMA-IR

HOMA-IR, Homeostatic Model Assessment - Insulin Resistance;
AUC, Area under the curve
C3G reduces gluconeogenesis

**A**

![Graphs of various amino acids showing effects on gluconeogenesis](image)

**B**

Bar graph showing gluconeogenesis levels with different compounds:
- **cAMP**: - + - - - + - - -
- **Metformin**: - + - - - + - - -
- **Compound C**: - - - - + + + + +
- **C3G**: - - 10 50 - - 10 50

Fig. 22: Total Glucogenic Amino Acids

CE-TOF & QqQMS: Selected component analysis
C3G reduces glucose accumulation via inhibits hepatic gluconeogenesis

FOXO1, Forkhead box protein O1; CREB, cAMP response element-binding protein; HDAC5, Histone deacetylase 5; CRTC2, CREB regulated transcription coactivator 2; PEPCK, Phosphoenolpyruvate carboxykinas; G6Pase, Glucose 6-phosphatase
C3G reduces body weight, visceral fat weight & adipocyte size

**A**

**B**

**C**

<table>
<thead>
<tr>
<th>Organ weight of mice</th>
<th>HFD</th>
<th>FF</th>
<th>C3G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epididymal Fat (g)</td>
<td>2.45 ± 0.16a</td>
<td>2.43 ± 0.26a</td>
<td>2.41 ± 0.19a</td>
</tr>
<tr>
<td>Visceral Fat (g)</td>
<td>1.67 ± 0.11a</td>
<td>0.71 ± 0.09bc</td>
<td>0.98 ± 0.19c</td>
</tr>
<tr>
<td>Perirenal Fat (g)</td>
<td>1.52 ± 0.10a</td>
<td>1.02 ± 0.09bc</td>
<td>1.19 ± 0.15ac</td>
</tr>
<tr>
<td>Total White Adipose Tissue (WAT, g)</td>
<td>5.63 ± 0.20a</td>
<td>4.16 ± 0.42bc</td>
<td>4.58 ± 0.52ac</td>
</tr>
<tr>
<td>Brown Adipose Tissue (BAT, g)</td>
<td>0.29 ± 0.03ab</td>
<td>0.22 ± 0.03a</td>
<td>0.36 ± 0.04b</td>
</tr>
<tr>
<td>WAT/BAT</td>
<td>20.81 ± 2.07a</td>
<td>19.90 ± 1.52a</td>
<td>12.90 ± 0.87b</td>
</tr>
<tr>
<td>Skeletal Muscle (g)</td>
<td>0.68 ± 0.04a</td>
<td>0.55 ± 0.08a</td>
<td>0.76 ± 0.06a</td>
</tr>
<tr>
<td>WAT/Skeletal Muscle</td>
<td>8.43 ± 0.49a</td>
<td>7.98 ± 0.58ab</td>
<td>5.85 ± 0.80b</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>1.59 ± 0.13a</td>
<td>1.46 ± 0.05a</td>
<td>1.37 ± 0.17a</td>
</tr>
<tr>
<td>Liver/Body weight</td>
<td>0.036 ± 0.002a</td>
<td>0.040 ± 0.001a</td>
<td>0.034 ± 0.003a</td>
</tr>
</tbody>
</table>

Note: Values are means ± standard error. Different letters indicate significant differences (a, b, c) among groups.
C3G increases energy expenditure via induces thermogenesis gene expressions in brown adipose tissue (BAT)

A

![Graphs showing VO2, RQ, and Energy Expenditure for CON and C3G groups in Total, Light, and Dark conditions.]

B

mRNA expression in BAT

- C3G reduces body weight via increases energy expenditure and thermogenesis in brown adipose tissue
Conclusion

- **Fatty acid oxidation**
- **Energy expenditure**
- **Autophagy**
- **Gluconeogenesis**

- **Fatty acid synthesis**
- **Insulin sensitivity**

- **Body weight, visceral fat weight & adipocyte size**
- **Lipid accumulation in liver**
- **Glucose & insulin concentrations in plasma**
- **Improves insulin sensitivity**

- ↓ Atherosclerosis
Acknowledgement

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✓ Minyoung So

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Thank you for your attention!