Korea University

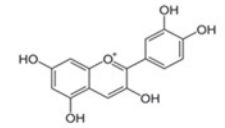
3rd International Conference and Exhibition on
Nutrition & Food Sciences
September 23-25, 2014 Valencia, Spain

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

Food Biomedical Science Lab.
Yaoyao Jia
Sep 23th, 2014

Background

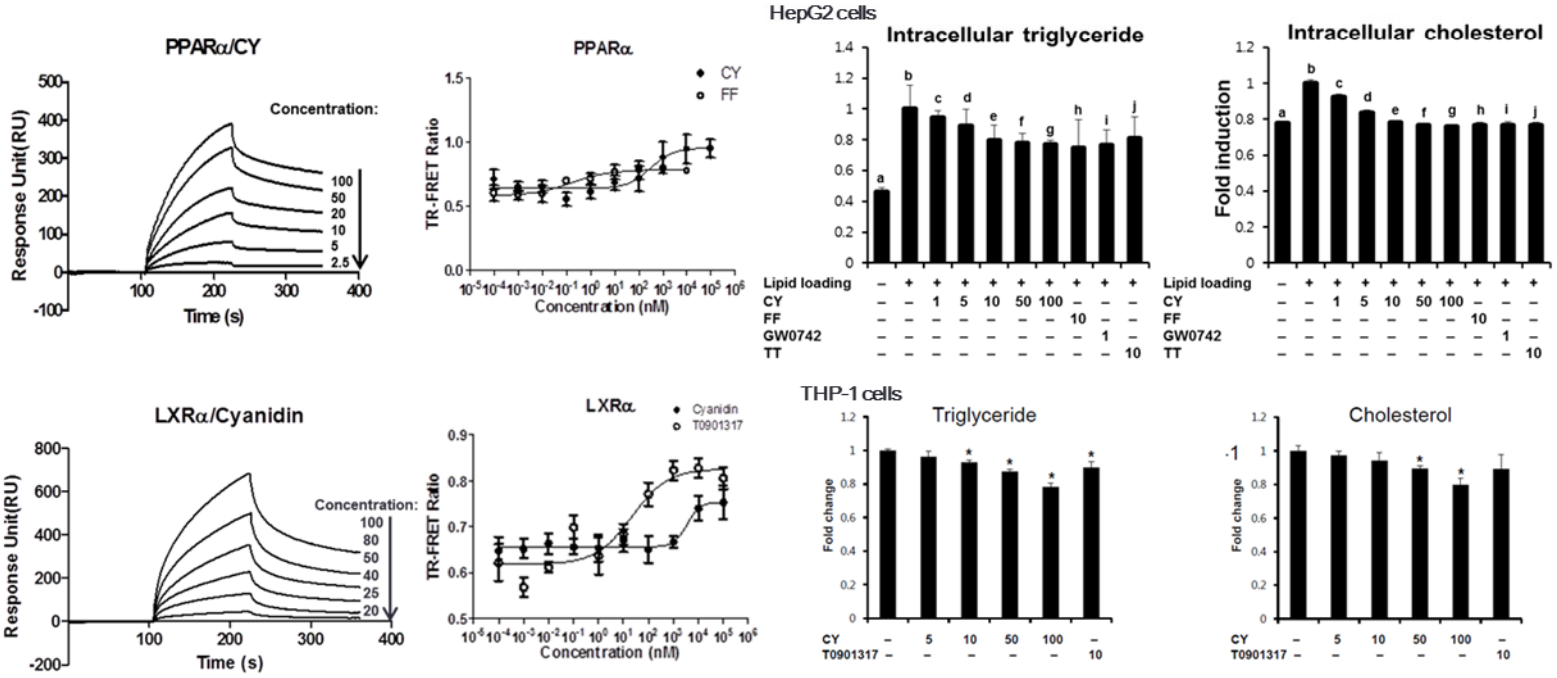
Previous study about cyanidin (CY)



Molecular mass: 287.24g/mol

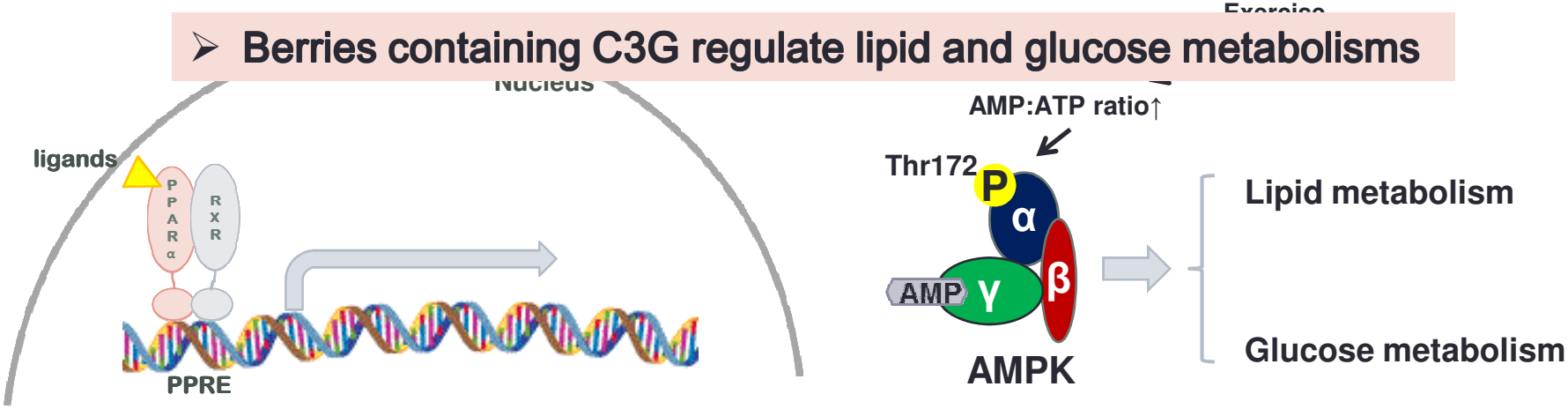
K_D values and EC_{50} values of cyanidin (CY)

	C3G	
	PPARα	LXRα
SPR	3.08 μM	2.2 μM
TR-FRET	3.03 μM	3.5 μM



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

HepG2 cells

Lipid-loading for 24 h → Treatment with C3G for 24 h

- Lipid contents
- Fatty acit oxidation/synthesis
- Autophagy analysis
- Gluconeogenesis

C57BL/6J

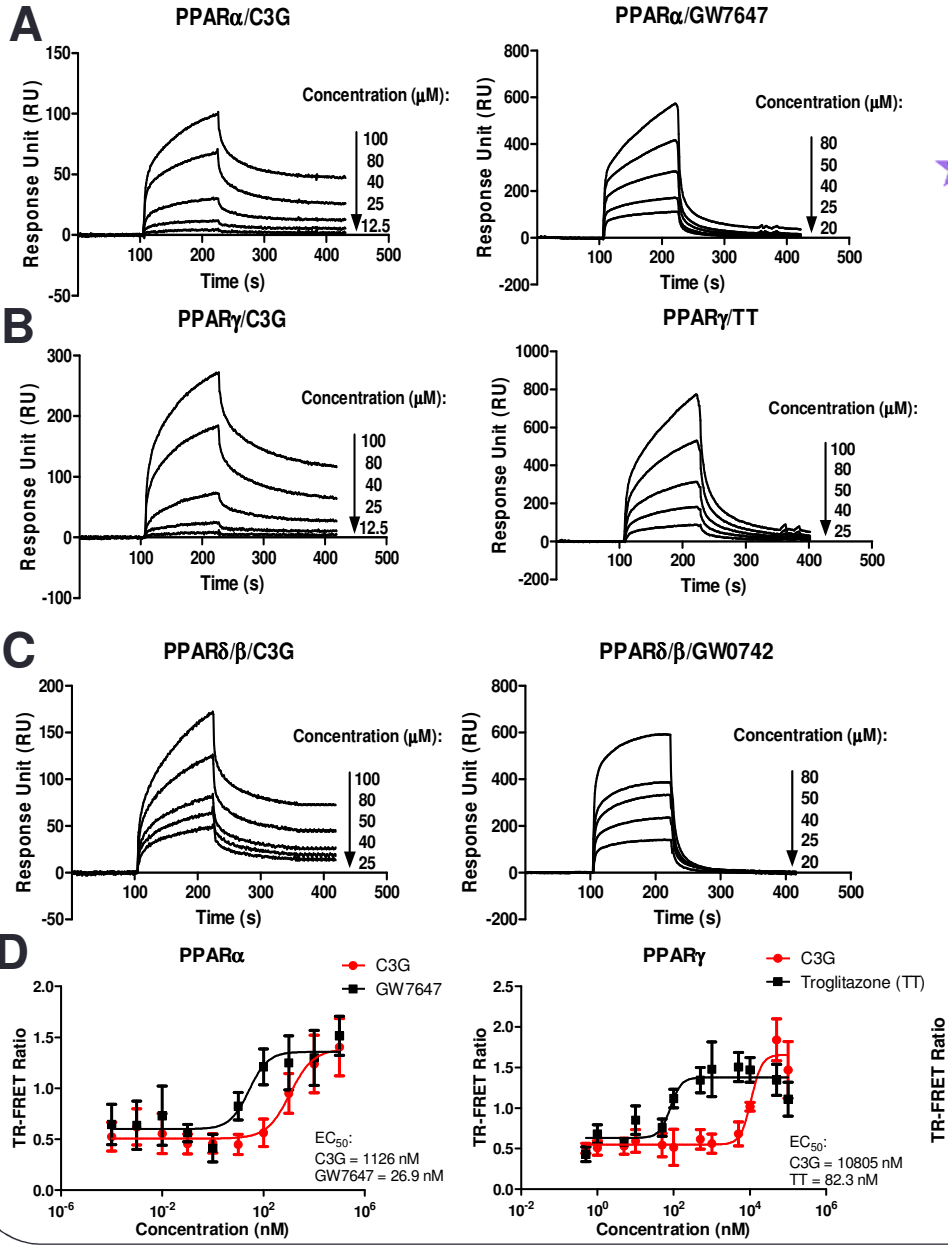
4 weeks HFD →
- 8 weeks HFD
- 8 weeks HFD+FF
- 8 weeks HFD+C3G

- Sacrifice
- Sample (plasma & organs) collection

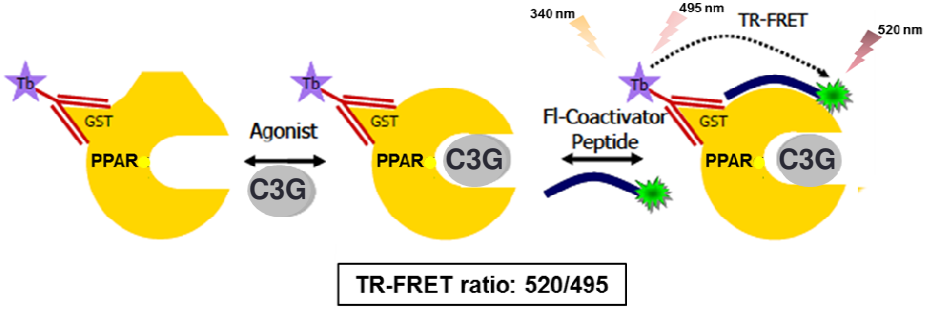
✓ HFD: High fat diet (45%)
✓ FF: 100 mg/kg body weight of fenofibrate (FF)
✓ C3G: 100 mg/kg body weight of cyanidin-3-O-glucoside (C3G)

❑ 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



TR-FRET ratio: 520/495

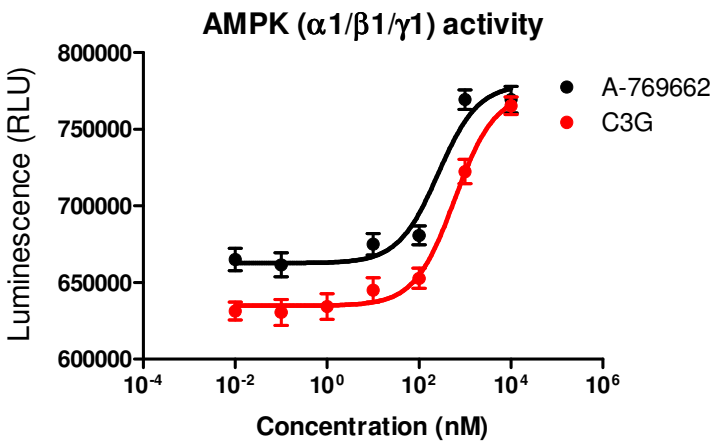
K_D values and EC_{50} values of C3G and positive controls

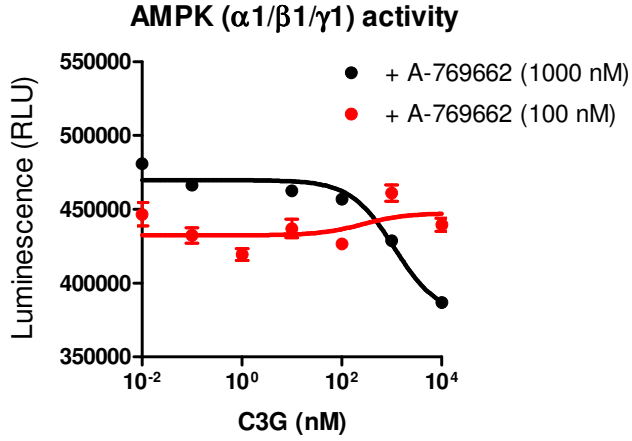
	C3G			Positive controls		
	PPARα	PPARγ	PPARδ	PPARα GW7647	PPARγ TT	PPARδ GW0742
SPR	456 nM	1.36 μM	4.96 μM	13.2 nM	377 nM	102 nM
TR-FRET	1126 nM	10.8 μM	31.05 μM	26.9 nM	82.3 nM	10.25 nM

K_D, the equilibrium dissociation constant ('binding constant');
EC_{50}, Half maximal effective concentration

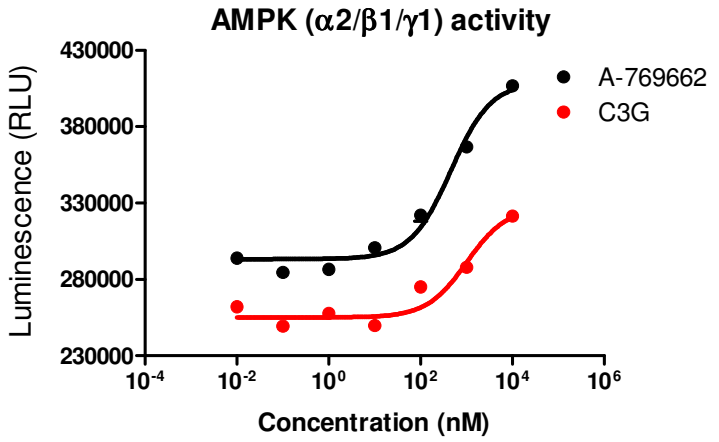
C3G induces AMPKα1 activity via direct interaction with AMPKα1

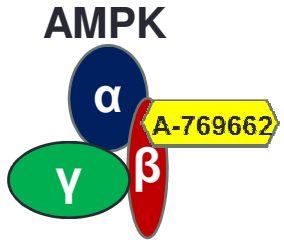
A

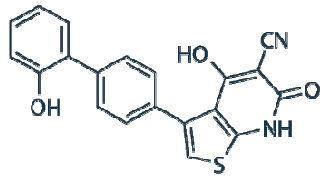




B





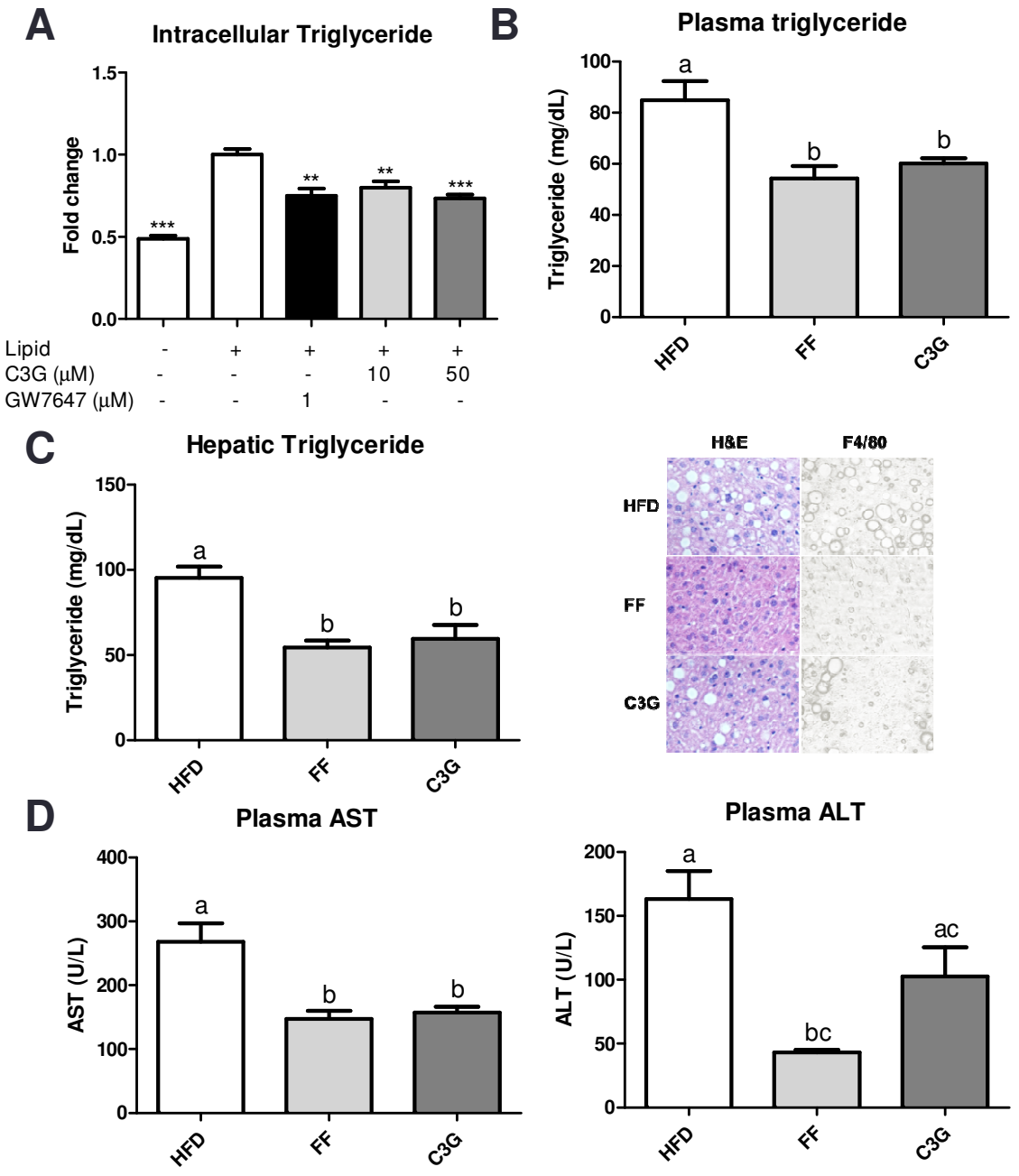


A-769662

EC_{50} (nM)	A1/B1/G1	A2/B1/G1
A-769662	262	457
C3G	599	1010

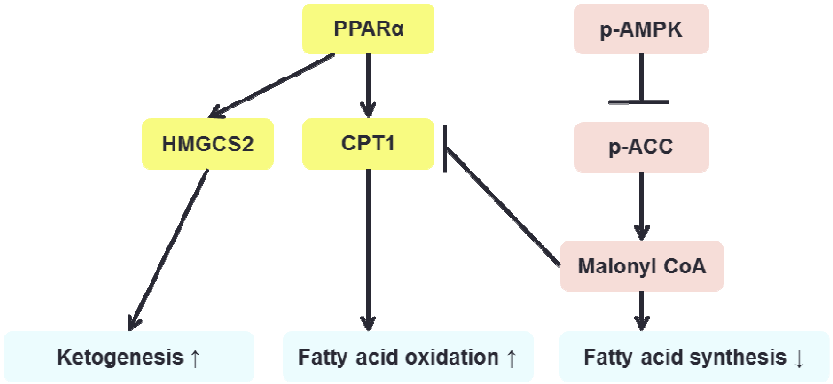
➢ **C3G directly activates PPARα and AMPK**

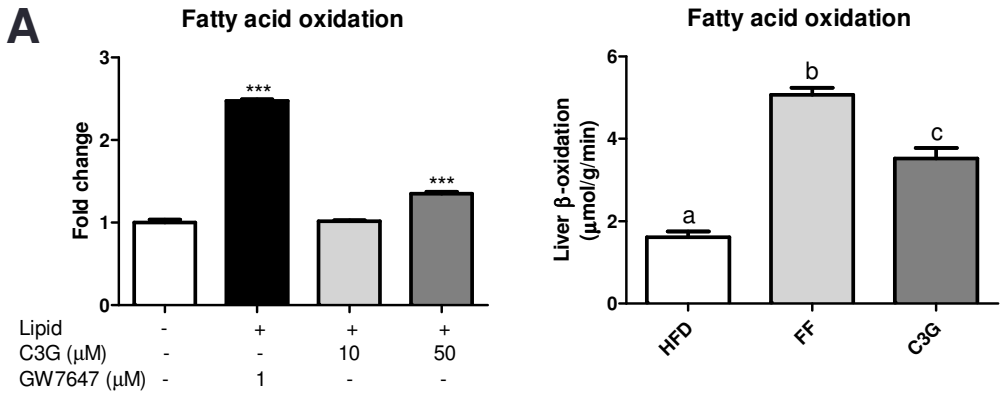
C3G reduces lipid accumulation in mouse livers & hepatocytes

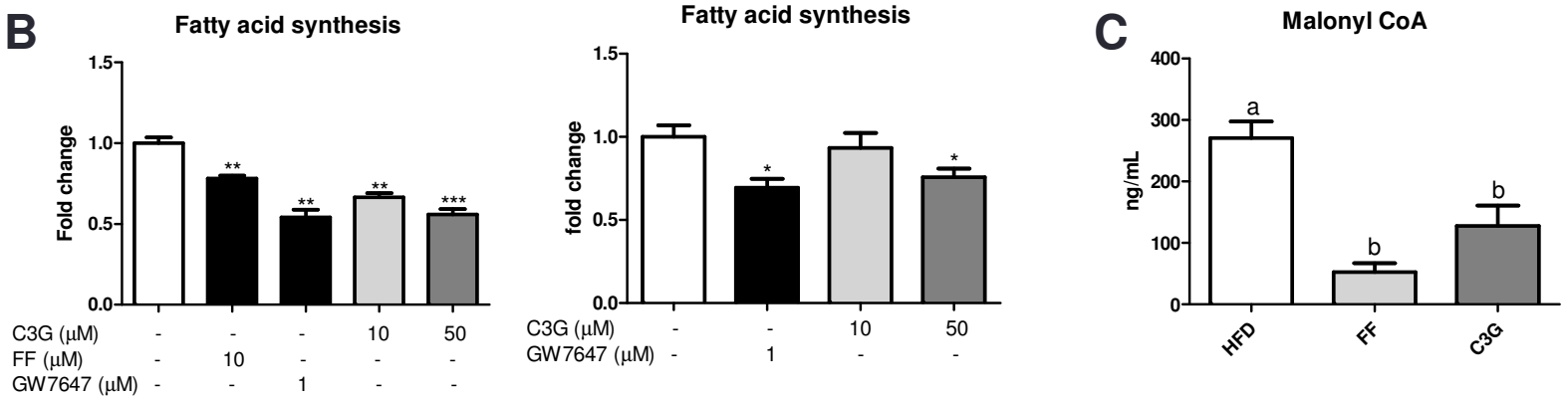


AST, Aspartate Aminotransferase;
ALT, Alanine Aminotransferase

C3G induces hepatic fatty acid oxidation and ketogenesis while decreases fatty acid synthesis via regulation of PPARα & AMPKα1

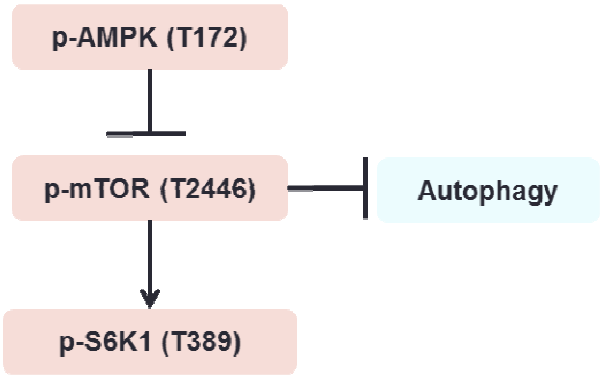


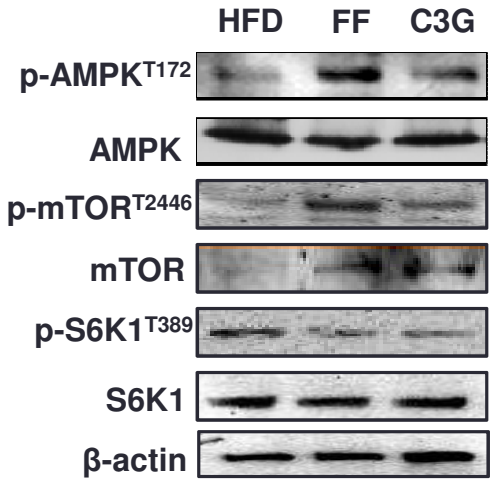


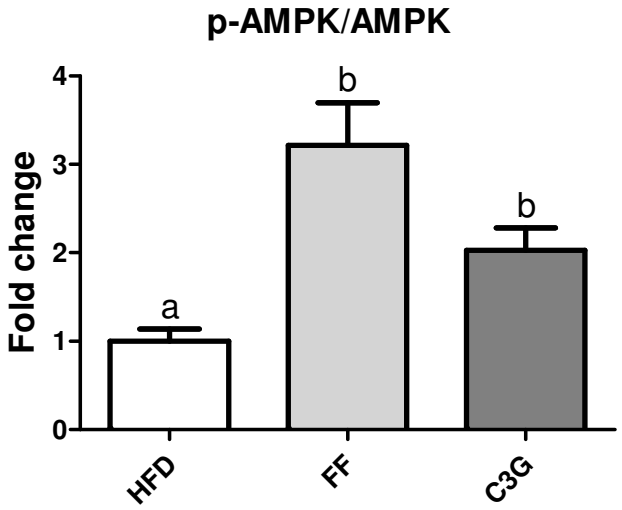


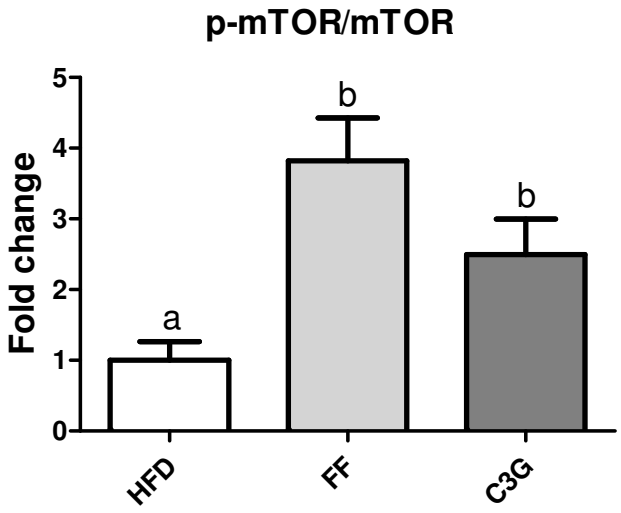
➢ C3G reduces lipid accumulation via increases fatty acid oxidation, ketogenesis, whereas inhibits fatty acid synthesis

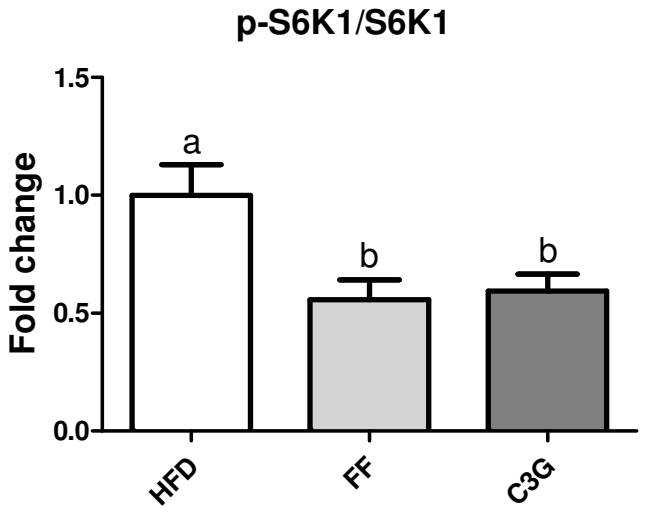
C3G induces phosphorylation of AMPK thus blocks the mTOR-S6K1 axis





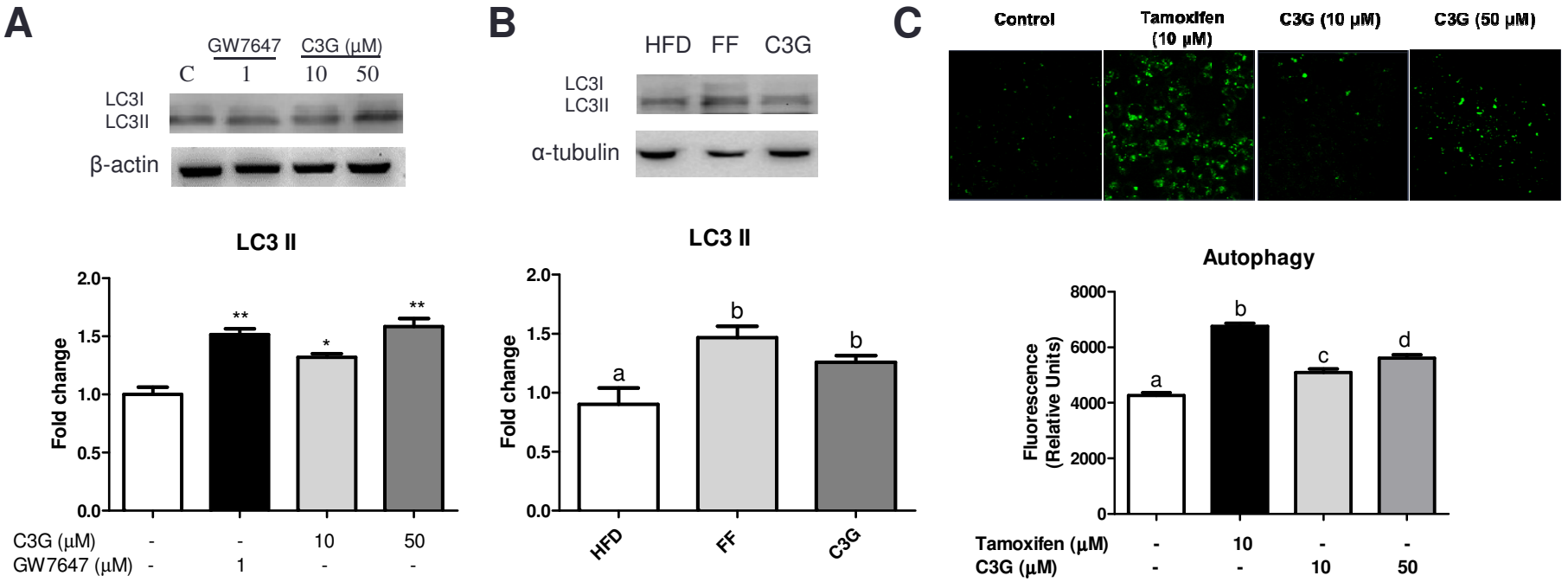




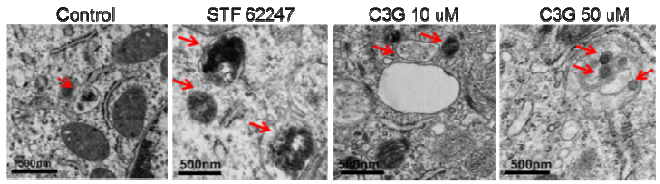


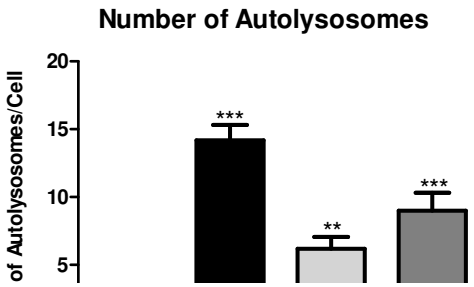
mTOR, mammalian target of rapamycin; S6K1, P70-S6 Kinase 1

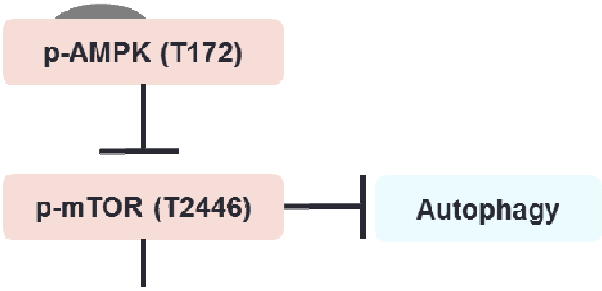
C3G induces hepatic autophagy pathway



D

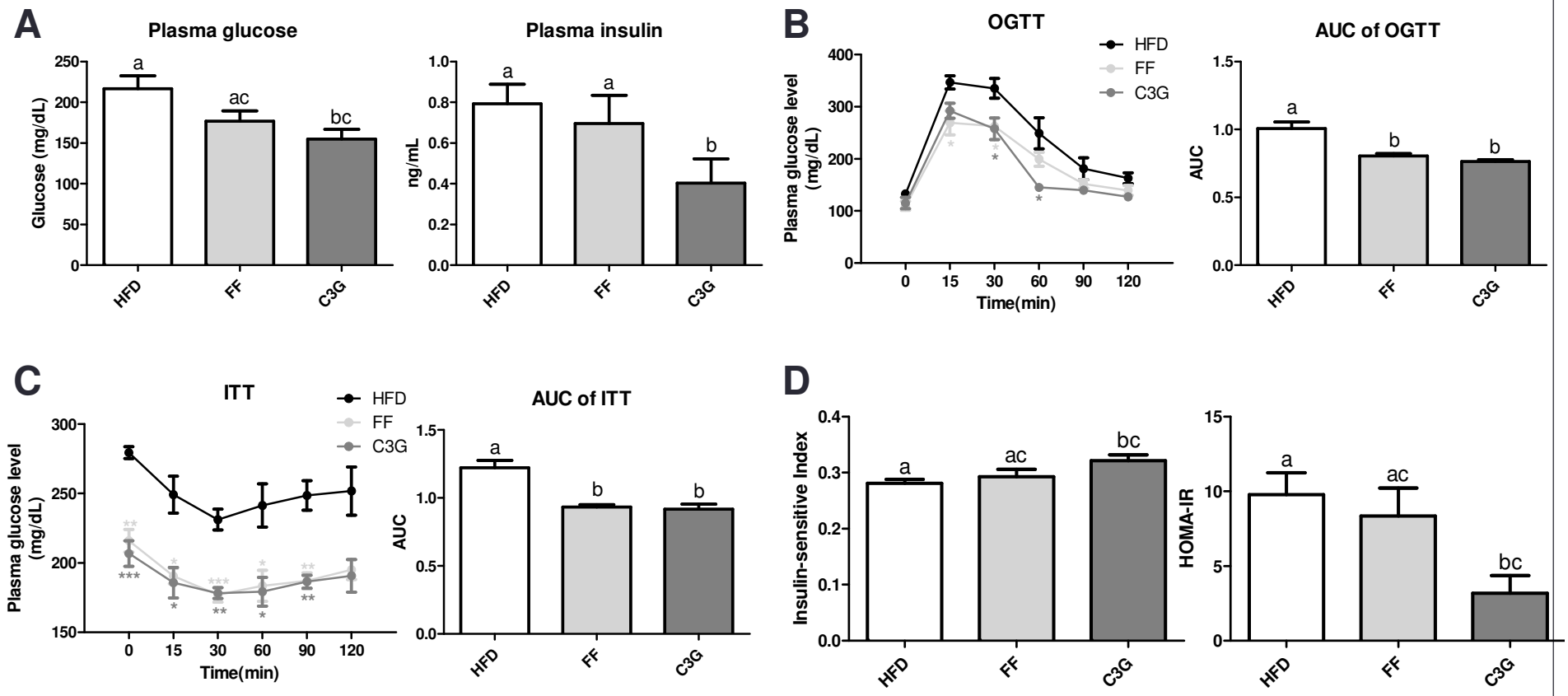






STF, ST ➢ **C3G reduces lipid accumulation via activates hepatic autophagy pathway**

C3G reduces plasma glucose & insulin concentrations and improves insulin sensitivity



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

C3G reduces gluconeogenesis

A

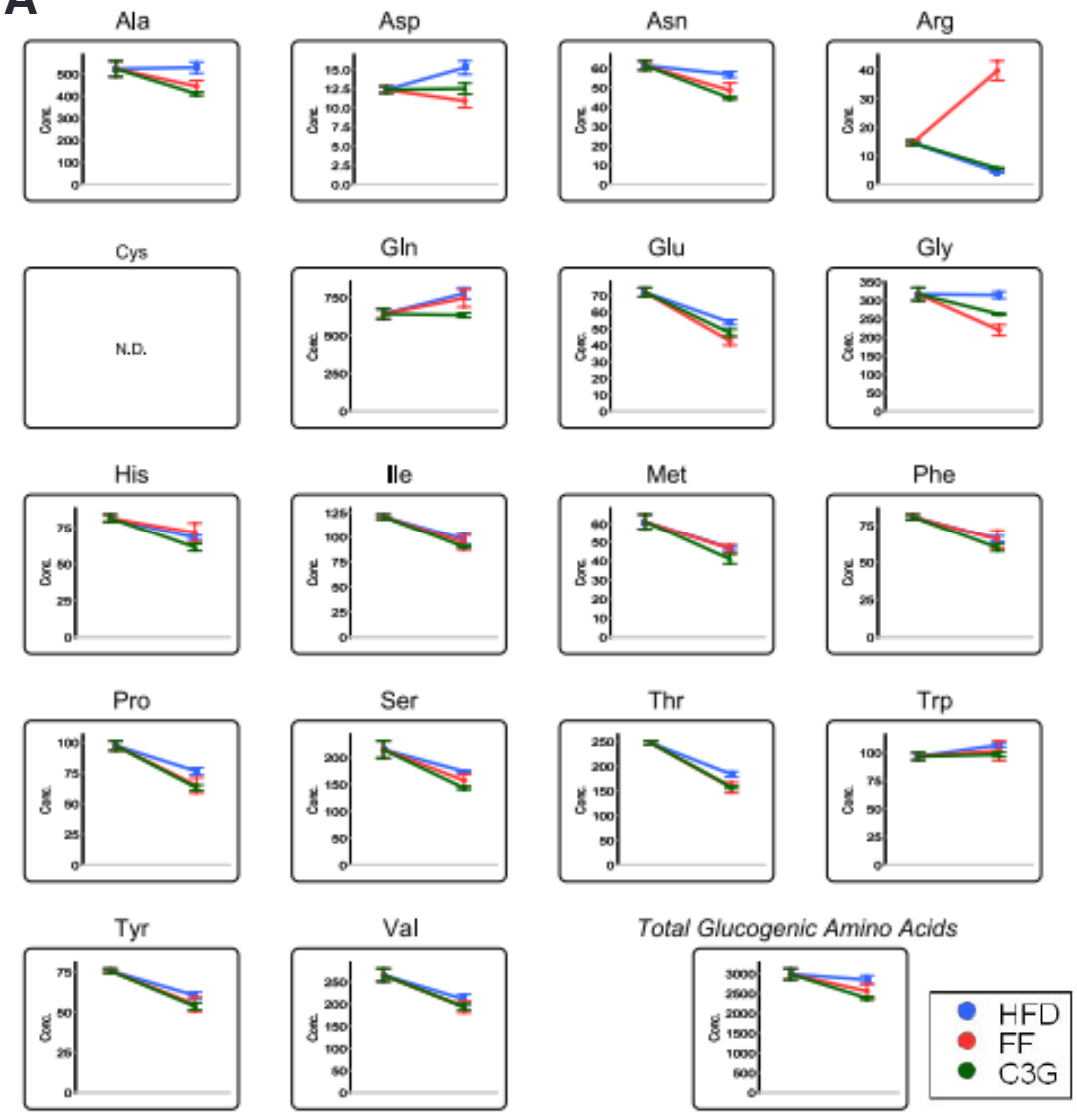
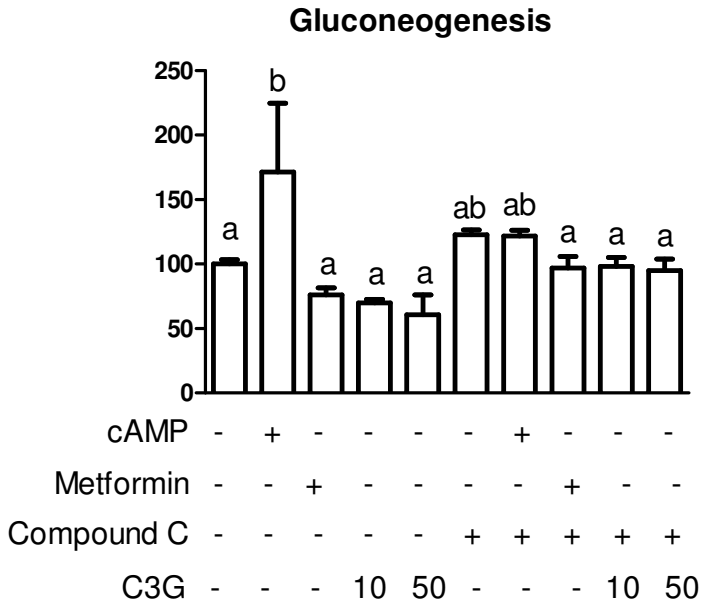
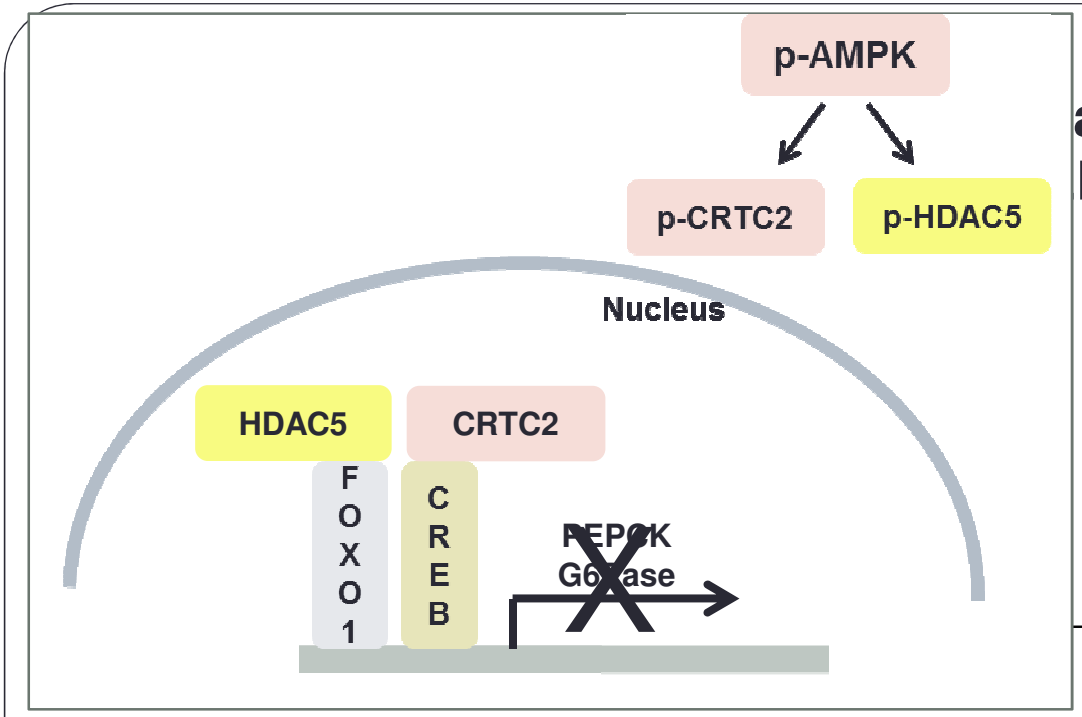


Fig. 22: Total Glucogenic Amino Acids

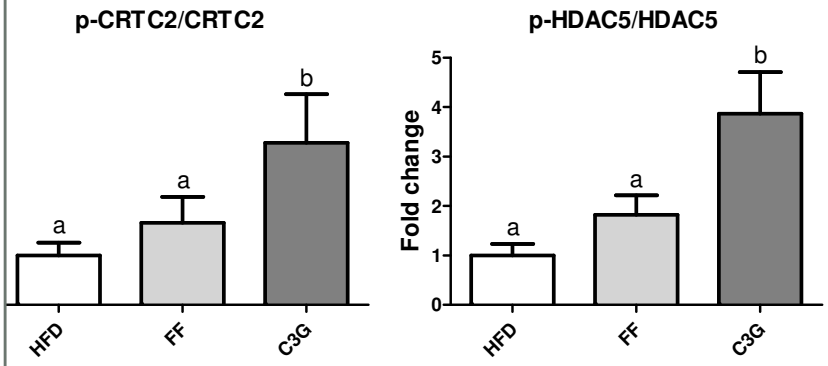
B

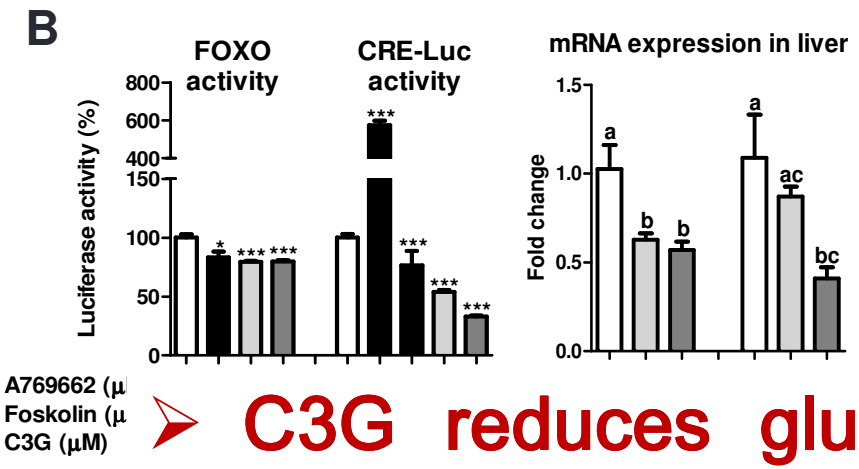


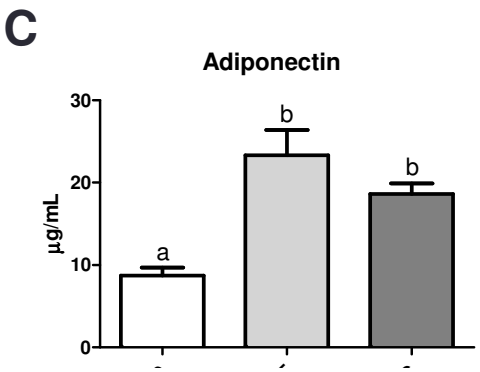
CE-TOF & QqQMS：Selected component analysis



ases plasma adiponectin
B activity



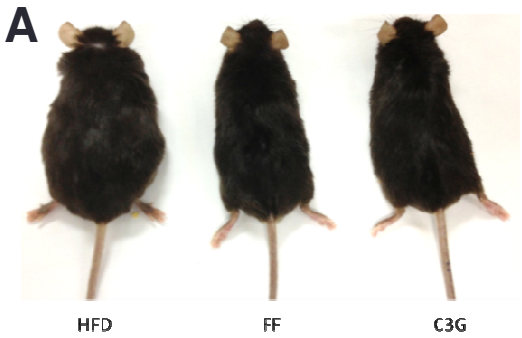


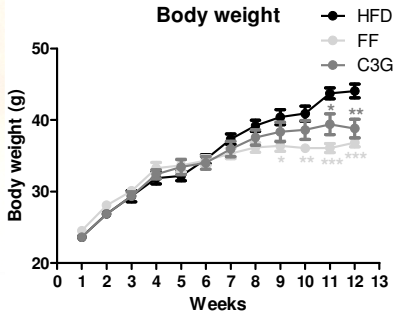


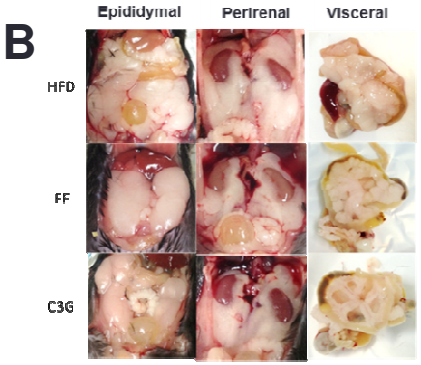
➢ **C3G reduces glucose accumulation via inhibits hepatic gluconeogenesis**

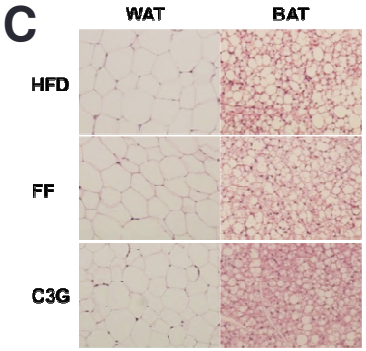
FOXO1, Forkhead box protein O1; CREB, cAMP response element-binding protein; HDAC5, Histone deacetylase 5; CRTC2, CREB regulated transcription coactivator 2; PEPCK, Phosphoenolpyruvate carboxykinas; G6Pase, Glucose 6-phosphatase

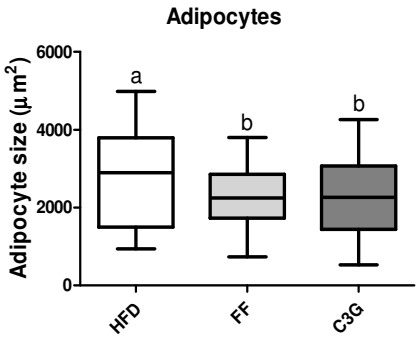
C3G reduces body weight, visceral fat weight & adipocyte size







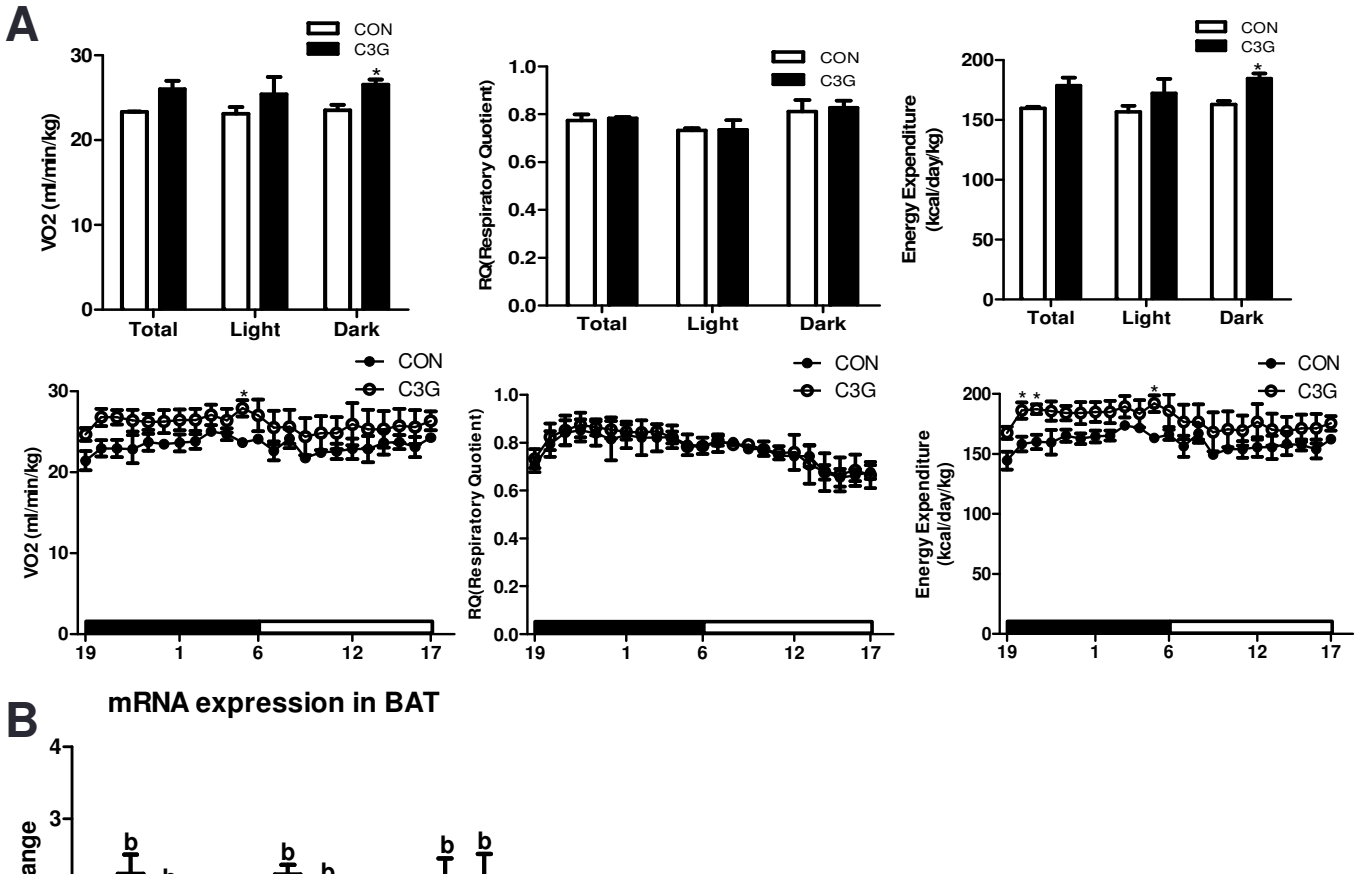




Organ weight of mice

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

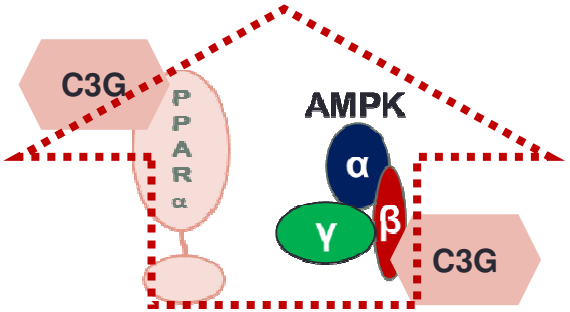
C3G increases energy expenditure via induces thermogenesis gene expressions in brown adipose tissue (BAT)

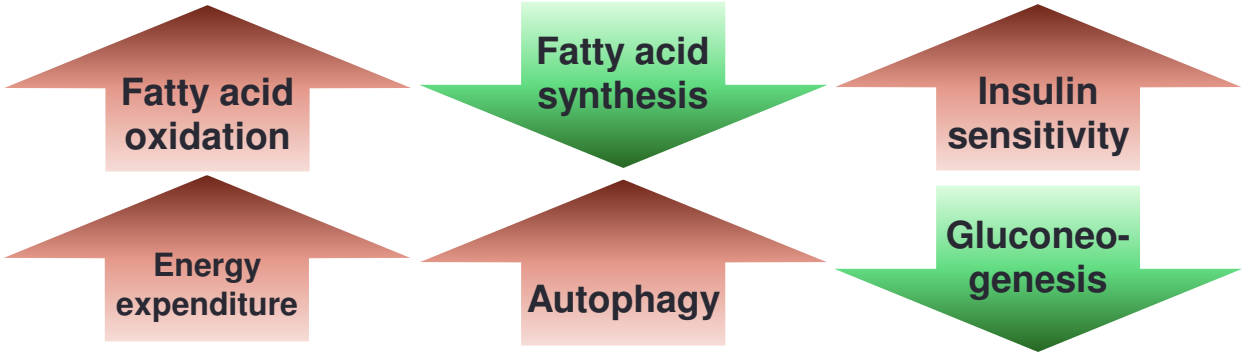


- C3G reduces body weight via increases energy expenditure and thermogenesis in brown adipose tissue

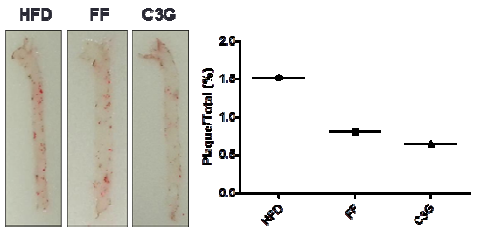
alpha;

Conclusion





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



↓ Atherosclerosis

Acknowledgement

Food Biomedical Science Lab.

- Supervisor

  Prof. Sung-Joon Lee

- FBS lab. Members
  - ✓ Ji Hae Lee
  - ✓ Chunyan Wu
  - ✓ Bobae kim
  - ✓ Ji Ah Kim
  - ✓ Soyoung Kim
  - ✓ Boram Mok

Ewha Women's University

- Prof. Young-Suk Kim
  - ✓ Minyoung So

- RDA 농촌진흥청 Rural Development Administration of Korea

Thank you for your attention!