Possible new mechanisms for the benefit of bitter gourd to metabolic health

Ching-jang Huang  
Department of Biochemical Science and Technology  
National Taiwan University  
Taipei, Taiwan
Metabolic Syndrome (Met S)

Clustering of several cardiovascular disease risk factors in an individual.

Met S: 2X risk mortality from CVD, 3X risk heart attack/Stroke, 5X risk Type 2 DM
Central Obesity

Diet  Activity  Genetics  Environment

Bisphenol A

Inflammatory Cytokines

Adiponectin

Hypertension  Atherosclerosis

Diabetes  Insulin Resistance  Hyperlipidemia

Metabolic Syndrome
Obesity = excessive fat storage in adipose tissue
Long-term **positive** energy balance

- Energy Balance =
  \[ \text{Energy Intake} - \text{Energy Expenditure} \]
- Positive (\(>0\)): Energy Intake > Energy Expenditure
  - ↑ Fat storage in adipose, Gain weight
- Balance (=0): Energy Intake = Energy Expenditure
- Negative (\(<0\)): Energy Intake < Energy Expenditure
  - ↓ Fat storage in adipose, Lose weight

To reduce obesity, one need to have long-term **negative** energy balance, ↓ Energy Intake, ↑ Energy expenditure, or both!
Increase Energy Expenditure

- Basal Metabolism: \(\downarrow\) w/ decreased energy intake. \(\uparrow\) w/ lean body mass.
- Physical Activity/Aerobic Exercise: best way to increase energy expenditure, might also increase lean tissue!
- Thermogenesis Through Brown adipose??
Brown Fat Thermogenesis

• Brown adipose tissue - high vascularity, abundant mitochondria

• Special mitochondria promote thermogenesis at the expense of ATP
  – Have H⁺ pores in inner membranes formed of uncoupling protein (UCP-1)

• Thermogenesis triggered by ingestion of food (overeating) or prolonged exposure to cold temperatures
Production of ATP in the Mitochondria Electron Transport Chain

Activated by:
- β-Adrenergic stimulation
- Cold stress
- Free fatty acids

Intermembrane space

NADH + H⁺ → NAD⁺
FADH₂ → FAD

½ O₂ + 2H⁺ → H₂O
ATP-Synthase

ADP + Pi → ATP

Inner Mitochondrial Membrane

F₀
F₁
Brown Adipose Tissue (BAT)

• Stimulated by sympathetic nerve neurotransmitters, Norepinephrine

• Uncoupling Protein (UCP-1) or Thermogenin: A protein on the inner membrane of mitochondria of BAT, cause leaking of the proton gradient, uncouple the energy produced and phosphorylation of ATP

• BAT in Adult Human body?
Significance of UCP-1 expressing (Brown-like) adipocytes in the white adipose tissues

**Nature Medicine** 19(10) : 1252, OCTOBER 2013

---

<table>
<thead>
<tr>
<th>Immunohistochemistry with anti-Ucp1</th>
<th>Location in humans</th>
<th>Location in mice</th>
<th>Developmental origin in mice</th>
<th>Enriched markers</th>
<th>Key transcription factors</th>
<th>Activators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown</td>
<td>Neck Interscapular (newborns) (Perirenal?)</td>
<td>Interscapular Cervical Axillary Perirenal (Endocardial?)</td>
<td>Myf5⁺ cells (dermomyotome)</td>
<td>Zic1 Lhx8 Eva1 Pdk4 Epst1 miR-206, miR-133b</td>
<td>C/ebpβ Prdm16 Pgc-1α Ppar-α Ebf2 TR</td>
<td>Cold Thiazolidinediones Natriuretic peptides Thyroid hormone Fgf21, Bmp7, Bmp8b Orexin</td>
</tr>
<tr>
<td>Beige</td>
<td>Supraclavicular (Paraspinal?)</td>
<td>Interspersed within WAT subcutaneous fat &gt; visceral fat</td>
<td>Myf5⁻ cells Pdgfr-α⁺ (perigonadal)</td>
<td>Cd137 Tbx1 Tmem26 Cited1 Shox2</td>
<td>C/ebpβ Prdm16 Pgc-1α (Ppar-α?)</td>
<td>Cold Thiazolidinediones Natriuretic peptides (Thyroid hormone?) Fgf21 Insin</td>
</tr>
</tbody>
</table>

Beige Adipocyte, Brite (Brown in White) Adipocyte, iBAT (inducible Brown Adipose)

Existence of Brown Fat in Adult Human which is negatively correlated with adiposity!
Brown and beige fat: development, function and therapeutic potential
Matthew Harms & Patrick Seale

Adipose tissue, best known for its role in fat storage, can also suppress weight gain and metabolic disease through the action of specialized, heat-producing adipocytes. Brown adipocytes are located in dedicated depots and express constitutively high levels of thermogenic genes, whereas inducible ‘brown-like’ adipocytes, also known as beige cells, develop in white fat in response to various activators. The activities of brown and beige fat cells reduce metabolic disease, including obesity, in mice and correlate with leanness in humans. Many genes and pathways that regulate brown and beige adipocyte biology have now been identified, providing a variety of promising therapeutic targets for metabolic disease.
<table>
<thead>
<tr>
<th>Agent/signal</th>
<th>BAT activation</th>
<th>WAT browning</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-adrenergic agonists</td>
<td>+</td>
<td>+</td>
<td>[14–18,138]</td>
</tr>
<tr>
<td>Leptin</td>
<td>+</td>
<td>+</td>
<td>[123–127,130,131,139]</td>
</tr>
<tr>
<td>TLQP-21</td>
<td>+</td>
<td>++</td>
<td>[128]</td>
</tr>
<tr>
<td>Brain-derived neurotrophic factor</td>
<td>+</td>
<td>++</td>
<td>[129]</td>
</tr>
<tr>
<td>Prostaglandins</td>
<td>−</td>
<td>+</td>
<td>[140,141]</td>
</tr>
<tr>
<td>Cardiac natriuretic peptides</td>
<td>+</td>
<td>+</td>
<td>[143]</td>
</tr>
<tr>
<td>PPARγ ligands</td>
<td>+</td>
<td>+</td>
<td>[19,43,144–153]</td>
</tr>
<tr>
<td>PPARα ligands</td>
<td>+</td>
<td>+</td>
<td>[44,78,138,157]</td>
</tr>
<tr>
<td>Retinoids</td>
<td>+</td>
<td>+</td>
<td>[29,110,158,159,163]</td>
</tr>
<tr>
<td>Thyroid hormones</td>
<td>+</td>
<td>+</td>
<td>[78,165]</td>
</tr>
<tr>
<td>AMPK activators</td>
<td>−</td>
<td>?*</td>
<td>[168–170]</td>
</tr>
<tr>
<td>Irisin</td>
<td>−</td>
<td>+</td>
<td>[171]</td>
</tr>
<tr>
<td>Fibroblast growth factor 21</td>
<td>+</td>
<td>++</td>
<td>[177]</td>
</tr>
<tr>
<td>Bone morphogenetic protein 7</td>
<td>+</td>
<td>?**</td>
<td>[178,179]</td>
</tr>
</tbody>
</table>
Previous studies: Bitter gourd (*Momordica charantia*, BG)

- EAE extracts of BG Powder contain PPAR $\alpha$/ PPAR $\gamma$ agonists, 10 active compounds have been isolated. (Chuang et al., 2006)

- In high fat-fed rodents, BG juice up-regulated UCP-1 mRNA in BAT (brown adipose tissue), and PGC-1$\alpha$ in BAT and skeletal muscle (Chan et al., 2005)

- BG seed oil up-regulated PGC-1$\alpha$ and UCP-1 in WAT (white adipose tissue) (Chen et al., 2012)
Peroxisome proliferator-activated receptor γ coactivator-1 (PGC-1α)

- PPAR-γ co-activator-1 α
- Critical transcriptional regulator of oxidative metabolism and adaptive thermogenesis.
- Stimulates mitochondrial biogenesis.
- Anti-obese & anti-diabetic effects.

(Scarpulla, 2011)
Wild bitter gourd increased metabolic rate and up-regulated genes related to mitochondria biogenesis and UCP-1 in mice

Kan-Ni Lu, Chin Hsu, Mei-Ling Chang, Ching-jang Huang
C57BL/6J male mice

BW 26.3 g, plasma glucose 202.5 mg/dL

Basal (n=6)  5% BGP (n=6)

Oxygen Consumption, CO2 production measurement

OGTT, 2 g glu./kg BW

ITT, 0.75 I.U/kg BW

Sacrifice at 22 week, Plasma Biochemistry, Tissue mRNA Expression
**O₂ Consumption (Week 5~8)**

- **A.** Graph showing O₂ consumption over time with Basal and BGP conditions.
- **B.** Area Under Curve (AUC) comparison between Basal and BGP during light and dark phases.

**CO₂ Production (Week 5~8)**

- **A.** Graph showing CO₂ production over time with Basal and BGP conditions.
- **B.** AUC comparison between Basal and BGP during light and dark phases.
BGP顯著增加黑暗期時間點呼吸商數及曲線下面積

RQ: VCO₂/VO₂
RQ趨近於1 表示增加葡萄糖氧化
RQ 趨近於0.7 表示增加脂肪的氧化

BGP的呼吸商接近0.9，Basal則接近0.8→顯示出BGP增加葡萄糖利用。

綜合上述結果指出, BGP組:
(1)降低攝食利用率 (2)增加葡萄糖氧化

⇒↓體重、體脂並有較佳之血糖調控現象。

山苦瓜各種萃取物改善血糖調節之活性比較
**Table 1** The energy expenditure of mice fed BGP or basal diets for 22 wk.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Basal</th>
<th>BGP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy expenditure (TEE, kcal/day)</td>
<td>6.77±0.10</td>
<td>6.87±0.05</td>
</tr>
<tr>
<td>Energy expenditure at dark phase (kcal/12 hr)</td>
<td>3.53±0.03</td>
<td>3.62±0.01*</td>
</tr>
<tr>
<td>Energy expenditure at light phase (kcal/12 hr)</td>
<td>3.24±0.11</td>
<td>3.25±0.05</td>
</tr>
</tbody>
</table>
OGTT at week 9

**Glucose**

- Basal: 29.7 ± 10.4
- BGP: 8.34 ± 2.63

**Insulin**

HOMA-IR index

- Basal: 29.7 ± 10.4
- BGP: 8.34 ± 2.63

AUC
Insulin Tolerance Test at week 16
**Table 1** The food intake, final body weight, body weight gain, relative adipose tissue weight, fasting blood glucose and lipids, % HbA1C of mice fed test diets for 22 wks.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Basal</th>
<th>BGP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food intake (g/day) and body weight (g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food intake</td>
<td>3.20±0.18&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3.03±0.13</td>
</tr>
<tr>
<td>Final body weight</td>
<td>36.19±7.38</td>
<td>29.11±4.69&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body weight gain</td>
<td>9.91±5.64</td>
<td>3.35±3.05&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Feed efficiency&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2.08±1.15</td>
<td>0.89±0.69&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Relative adipose tissue weight&lt;sup&gt;3&lt;/sup&gt; (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RWAT&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2.05±0.66</td>
<td>1.13±0.51&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>EWAT&lt;sup&gt;4&lt;/sup&gt;</td>
<td>4.69±0.95</td>
<td>3.62±1.69</td>
</tr>
<tr>
<td>BAT&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.78±0.12</td>
<td>0.48±0.09&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Fasting Blood chemistry (mg/dL)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>glucose</td>
<td>183.90±65.40</td>
<td>83.09±30.62&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>80.77±15.15</td>
<td>73.71±14.93</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>141.60±43.59</td>
<td>119.62±63.61</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>9.51±0.66</td>
<td>8.36±1.02&lt;sup&gt;★&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
mRNA expression in epididymal white adipose tissue

Mitochondria Biogenesis
brown adipose
mRNA expression gastrocnemius muscle

Mitochondria Biogenesis
mRNA expression in liver

![Graph showing mRNA expression in liver for different genes, including PPARα, PPARδ, PGC1α, GK, CPT1α, ACADL1, ACSL3, SREBP1, and NrF1, compared to basal and BGP conditions.](image)
Better glucose homeostasis control and reduced adiposity in mice fed BGP are associated with higher metabolic rate and up-regulated mRNA of genes related to mitochondria biogenesis and UCP-1 in WAT. We speculate that BGP might increase mitochondria biogenesis and induce “browning” of WAT, which then increase energy expenditure and decrease fat accumulation.
Role of GLP-1 in the Hypoglycemic Effects of Wild Bitter Gourd

Ting-ni Huang, Kan-Ni Lu, Yi-Ping Pai, Chin Hsu, and Ching-jang Huang
Medications Available to Treat T2DM

AMPK

GLP-1

BG

PPARγ

Dopamine agonists

DPP-4 inhibitors

Amylin

GLP-1 receptor agonists

Meglitinides

TZDs

AGIs

Biguanides

SUs

Insulin

Year available for clinical use

Medication classes, n
Incretin effect

- In response to food ingestion, enteroendocrine cells in the intestinal mucosa release hormones that can stimulate insulin secretion from endocrine pancreas and thereby reduce blood glucose.

Bioactive GLP-1(7-36)amide and GIP (1-42) are released from the small intestine after meal ingestion and enhance glucose-stimulated insulin secretion (incretin action). DPP-4 rapidly converts GLP-1 and GIP to their inactive metabolites GLP-1 (9-36) and GIP (3-42) in vivo. Inhibition of DPP-4 activity prevents GLP-1 and GIP degradation, thereby enhancing incretin action.
GLP-1

- produced and secreted by the enteroendocrine L cells
- stimulate glucose-dependent insulin secretion from pancreatic β-cells.
- Stimulate insulin biosynthesis and insulin sensitivity
- Enhance pancreatic β-cell proliferation and protection against apoptosis
- Inhibit glucagon secretion and gastric emptying
- Inhibit food intake.

GLP-1 actions in peripheral tissues. The majority of the effects of GLP-1 are mediated by direct interaction with GLP-1Rs on specific tissues. However, the actions of GLP-1 in liver, fat, and muscle most likely occur through indirect mechanisms.

Drucker, 2007
Does BG exert an Incretin Effect?

Does BG evoke higher insulin secretion?
Oral glucose tolerance and insulinogenic index of mice fed the BGP diet for 5 weeks

**Insulinogenic index**

\[
\text{Insulinogenic index} = \Delta \text{serum insulin (15 min–0 min)} / \Delta \text{serum glucose (15 min–0 min)}
\]

(value of the basal group was taken as 1)
Does BG exert an Incretin Effect?

Yes, mice fed a BG diet secret more insulin when challenged by a oral glucose dose!

Does BG stimulate GLP-1 secretion?
STC-1 cell

• A murine enteroendocrine cell line
• Can secret GLP-1 in response to various stimuli.
• Has been used to study the effects of nutrients and secretagogues on the GLP-1 secretion and the mechanism underlying
• Expresses T2R family members of Bitter Taste Receptors: R4 (mT2R7), rT2R6 (mT2R30), rT2R8 (mT2R2), rT2R9 (mT2R5), and rT2R12 (mT2R26).

Wu et al., 200
Preparation of various BG extracts

- Bitter gourd powder (BGP)
  - Bitter compounds rich fraction of BGP (BGP-bi (0.7%))
  - Water extract of BGP (WE (27%))
    - P fraction (Pf (4.7%))
    - Large molecule fraction of WE (>3 kD) (WEL (14%))
  - Ethanol extract of BGP (EE (16.4%))
    - Small molecule fraction of WE (<3 kD) (WES (86%))
  - Ethyl acetate extract of BGP (EAE (4.8%))
BG extracts stimulate GLP-1 secretion in STC-1

**BGP-bi: bitter compounds rich fraction**

**EC$_{50}$**

- Control
- 20, 100, 500, 1000 µg/mL
- 100, 250, 400 µg/mL
- 20, 100 µg/mL

**GLP-1 secretion (cell/fold)**

- EC$_{50} = 133$ µg/mL
- EC$_{50} = 17$ µg/mL
- EC$_{50} = 271$ µg/mL
- EC$_{50} = 77$ µg/mL

**BGP-bi (µg/mL)**

- Control
- 20, 50, 100 µg/mL

- (µg/mL)

- WEL
- WES
- Pf
Does BG stimulate GLP-1 secretion?

Yes, it does in the cell culture.

How about \textit{in vivo}?
Acute effects of WES on plasma glucose, insulin, and GLP-1 in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Vehicle</th>
<th>WES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>395.7 ± 24.1</td>
<td>377.6 ± 40.9</td>
</tr>
<tr>
<td>30 min</td>
<td>411.5 ± 22.4</td>
<td>282.6 ± 24.2 ***</td>
</tr>
<tr>
<td>30 min–0 min</td>
<td>015.8 ± 24.1</td>
<td>−95.0 ± 39.3 ***,##</td>
</tr>
<tr>
<td>Plasma insulin (ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>0.78 ± 0.29</td>
<td>0.66 ± 0.34</td>
</tr>
<tr>
<td>30 min</td>
<td>0.46 ± 0.09</td>
<td>0.80 ± 0.35 **</td>
</tr>
<tr>
<td>30 min–0 min</td>
<td>−0.32 ± 0.32 #</td>
<td>0.15 ± 0.26 *</td>
</tr>
<tr>
<td>Plasma GLP-1 (pM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>4.69 ± 1.96</td>
<td>4.60 ± 1.46</td>
</tr>
<tr>
<td>30 min</td>
<td>4.71 ± 1.86</td>
<td>8.18 ± 2.50 ***</td>
</tr>
<tr>
<td>30 min–0 min</td>
<td>0.02 ± 0.88</td>
<td>3.58 ± 1.38 ***,###</td>
</tr>
</tbody>
</table>
How about *in vivo*?

BG WES evoked increases in Insulin and GLP-1 after 30 min!

Is the hypoglycemic effects of the BG WES mediated by the GLP-1 after 30 min?
Effects of exendin-9 (GLP-1 receptor antagonist) on the acute hypoglycemic effect of WES.
exendin-9 abolished the hypoglycemic effect of BG WES

Is the hypoglycemic effects of the BG WES mediated by the GLP-1 after 30 min? Yes!
Effects of probenecid or U73122 on the GLP-1 secretion stimulated by WE or BGP-Pi in STC-1

- Probenecid, an inhibitor of human TAS2R16. Probenecid could inhibit human TAS2R16, TAS2R38 and TAS2R43, but not TAS2R31 and other non-TAS2R GPCR
- U73122, PLCβ2 inhibitor.
- Bitter taste receptors signal transducers: G protein α-gustducin, phospholipase Cbeta 2 (PLCβ2), inositol 1,4,5-trisphosphate receptor type, 3 (IP3R3), and transient receptor potential (TRP) channels → depolarize the cell through elevation of intracellular Ca2+ concentration
BG compounds stimulate GLP-1 secretion in STC-1
Summary

• This study provides evidences that BGP can exert an **incretin effect** which might contribute, at least in part, to a better glucose homeostasis control. To our knowledge, this is the first study demonstrating an incretin effect of BG.
Large X: T2DM pathologies directly addressed by GLP-1 receptor agonists.
Small x: Anecdotal evidence suggests that weight loss induced by GLP-1 receptor agonists may correspond with positive changes in food and exercise choices.

Abbreviations: GLP-1, glucagon-like peptide-1; T2DM, type 2 diabetes mellitus.
long lasting endogeneous GLP-1 agonist
Transactivation assay of PPARα and PPARγ by six cucurbitane-type triterpenoids (compounds 1 – 6) (A–F), loliolide(G) and CLN(H)
Phytol

PPARα

(A)

EC₅₀ = 214 μM

(B)

EC₅₀ = 294 μM

Lutein

PPARα

(A-1)

EC₅₀ = 1.2 μM

(B-1)

EC₅₀ = 6.5 μM

PPARγ
WES is mainly constituted by polar molecules, such as sugars, amino acids, small peptides, water soluble alkaloids and plant secondary metabolites.

WEL might contain proteins and water soluble dietary fibers. Sugars, amino acids, protein and soluble/fermentable fibers are known to stimulate GLP-1 secretion [11].

Pf fraction also stimulated GLP-1 secretion but is less potent than the WEL. Intact proteins such as casein, codfish, egg, and wheat, but not soybean, ovomucoid and some peas have been shown to have pronounced effects on GLP-1 release in the STC-1 cells [50].
• The bitter taste receptor TAS2R16 mediates bitter taste in response to $\beta$-glucopyranosides.  
  Bufel et al., 2002
• The bitter principles in BG fruits, momordicosides K, L. are $\beta$-glucopyranosides of cucurbitane triterpenoids.
• Our BGP-Bi fraction presumably contained quite a few compounds of this type. As expected, this fraction exhibited highest folds of maximal stimulation (Figure 2(c)) among the BG extracts/fractions tested in this study.
• Moreover, the stimulation was inhibited by probenecid, an inhibitor of human TAS2R16 (Figure 3(b)). Probenecid could inhibit human TAS2R16, TAS2R38 and TAS2R43, but not TAS2R31 and other non-TAS2R GPCR [45].  
  Greene et al., 2011
• Our speculation that bitter taste receptors might be involved in the stimulating GLP-1 secretion need further investigations, since the orthologues of these human bitter taste receptors in mice and STC-1 cells and their specific agonists/antagonists remain unknown.
Preparation
Acute effects of WE, WEL, and WES on serum glucose concentrations in an intraperitoneal glucose tolerance test (ipGTT).

Hi-fat diet fed Mice were fasted for 6 hr before being oral gavaged with Vehicle, WE, WES, or WEL. 30 min later, Ip GTT (intra-peritoneally injected a glucose solution, 1 g/kg body weight) was performed. The changes of blood glucose concentrations were measured over time.

Water Extract of BG

Graphs show the changes in blood glucose levels over time for each condition: Vehicle, WE, WES, and WEL. The area under the curve (AUC) for each condition is also presented.
a) Cells in somite (Myf5⁺) → Commitment → Brown preadipocyte → Prdm16 → Differentiation → Brown adipocyte → Activation → Pgc-1α → Activated brown adipocyte.

b) Inguinal WAT (subcutaneous) → Precursor → White adipocyte → Prdm16 → PPAR-γ → Sirt1 → TZD → Cold or β-agonist → Beige adipocyte.

Epididymal WAT (intra-abdominal) → Pdgfr-α⁺, bipotent preadipocyte → High-fat diet → Pgc-1α → Beige adipocyte → Cold or β-agonist → Pdgfr-α⁺, bipotent preadipocyte → White adipocyte.
Figure 3 Catecholamine and natriuretic induction of thermogenesis.
Figure 4 Secreted factors that recruit brown adipocytes, beige adipocytes or both.
Mitochondrial biogenesis
WAT & BAT

Sympathetic nerve → Noradrenaline → βAR

White → Decreased body fat

Beige → Increased energy expenditure

Brown → Uncoupling of oxidative phosphorylation

TRP (V1, M8, A1)

Cold

Food ingredients

(Masayuki Saito, 2013)
PPARs
(Peroxisome-proliferator activation receptors)

• Fatty acid sensor and controlling metabolism.
• 3 isoforms:
  1) PPAR α: expressed in liver, heart, BAT.
  2) PPAR β/δ: ubiquitously expressed.
  3) PPAR γ: highly expressed in WAT & BAT.
  4) Thiazolidinediones (TZDs): PPAR γ agonist.
    insulin sensitizer → antidiabetic & adipogenic effects
Uncoupling protein (UCP)

3 isoforms:
• UCP-1
  Thermogenin: non-shivering thermogenesis
  Control body weight and temper oxidative damage.
• UCP-2, UCP-3
  Protect against oxidative damage

Dulloo et al., 1999
Bitter gourd (Momordica charantia, BG)

- Momordica charantia L., also known as bitter gourd, bitter melon.
- Cucurbitaceae family, Momordica genus
- Abundant in South Africa, India, Southeast Asia, China and Taiwan and other sub-tropical regions
- Common tropical vegetable that has also been used to manage diabetes in oriental traditional medicine.
The antidiabetic effects and mechanism of *Momordica charantia*
O₂ consumption and CO₂ production measured at week 5
Oral glucose tolerance test (OGTT) performed at week 9 and insulin tolerance test (ITT) performed at week 16
A working model for nutrient-stimulated GLP-1 release Upper panel: enteroendocrine cells in the intestinal epithelium (grey) detect nutrients passing in the lumen, but also respond to paracrine, neural and hormonal stimuli.


<table>
<thead>
<tr>
<th>Nutrient Sensing</th>
<th>L Cell Receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>TAS1R, $K_{ATP}$ channel, SGLT1, GLUT2</td>
</tr>
<tr>
<td>Fatty acid/ bile acid</td>
<td>GPR40, GPR120, GPR119, TGR5</td>
</tr>
<tr>
<td>Amino acid</td>
<td>SLC38A2</td>
</tr>
</tbody>
</table>
Bitter gourd (*Momordica charantia*, BG)

- *Momordica charantia* L., also known as bitter gourd, bitter melon.
- *Cucurbitaceae* family, *Momordica* genus
- Abundant in South Africa, India, Southeast Asia, China and Taiwan and other sub-tropical regions
- Common tropical vegetable that has also been used to manage diabetes in oriental traditional medicine.
The antidiabetic effects and mechanism of *Momordica charantia*

Fang et al., 2011
DPP-4 inhibitors can replace GLP-1 receptor agonists in injection-averse patients. Long-acting SUs can replace long-acting insulin in injection-averse patients. The borders of each step are imprecise, depending on comorbidities; the pathophysiologic defects (see Figure 1) addressed at each stage are indicated. The extent of “regression” along the continuum will also vary from patient to patient.

Abbreviations: DPP-4, dipeptidyl peptidase-4; GLP-1 RA, glucagon-like peptide-1 receptor agonist; HbA₁c, glycated hemoglobin; MGI, metformin, glucagon-like peptide-1 receptor agonist, and insulin; SU, sulfonylurea
Momordica charantia active components

- Glycosides (momordin and charantin),
- Alkaloids (momordicin)
- Polypeptide-P
- Oils from the seeds (linoleic, stearic and oleic acids)
- Glycoproteins (alpha-momorcharin, beta-momorcharin and lecitins)
- Protein MAP30 and vicine (pyrimidine nuclease)
- Of these constituents, charantin, insulin-like peptides and alkaloids possess hypoglycemic properties. They are more effective when they are combined and they produce effects almost similar to the crude water soluble extract.
Momordica charantia active components

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Model</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charantin (a saponin analog)</td>
<td>Rat ($^{22}$)</td>
<td>Postprandial hypoglycemic effect</td>
</tr>
<tr>
<td>Vicine (an alkaloid glycoside) (2,6-diaminopyrimidinol-5-β-D-glucopyranoside)</td>
<td>Male Wistar rat ($^{29}$)</td>
<td>Blood glucose and serum neutral fat-lowering effects (inhibits disaccharidase and pancreatic lipase activities)</td>
</tr>
<tr>
<td>Polypeptide-P</td>
<td>Albino rat ($^{11}$)</td>
<td>Hypoglycemic effect, favism</td>
</tr>
<tr>
<td>D-(+)-Trehalose</td>
<td>Gerbils, langurs and humans ($^{12}$)</td>
<td>Insulin-like effect</td>
</tr>
<tr>
<td>9c,11t,13t-Conjugated linolenic acid</td>
<td>Enzyme (from rat intestinal acetone powder) ($^{13}$)</td>
<td>α-Glucosidase inhibitor</td>
</tr>
<tr>
<td>Momordin</td>
<td>Cell-based transactivation assay ($^{4}$)</td>
<td>PPAR-α and -γ agonist</td>
</tr>
<tr>
<td>Cucurbitane triterpenoid glycosides (momordicosides Q, R, S, and T), karaviloside XI, and their aglycones</td>
<td>HepG2 cells ($^{6}$)</td>
<td>Increases PPAR-β/δ expression</td>
</tr>
<tr>
<td>Cucurbitane-type triterpene glycosides</td>
<td>L6 myotubes and 3T3-L1 adipocytes ($^{14}$)</td>
<td>Translocates GLUT4 to cell membranes</td>
</tr>
<tr>
<td>Momordicoside U (cucurbitane-type triterpenoid glycoside)</td>
<td>Rat intestinal α-glucosidase ($^{17}$)</td>
<td>α-Glucosidase inhibitor</td>
</tr>
<tr>
<td></td>
<td>Insulin secretion assay ($^{18}$)</td>
<td>Moderates insulin secretion activity</td>
</tr>
</tbody>
</table>

PPAR, peroxisome proliferator-activated receptor; GLUT4, glucose transporter 4; PGE$_3$, prostaglandin E$_3$.  

Tsai et al., 2010
Previously reported

Bitter Gourd (Momordica charantia) Extract Activates Peroxisome Proliferator-Activated Receptors and Upregulates the Expression of the Acyl CoA Oxidase Gene in H4IIEC3 Hepatoma Cells

Che-Yi Chao, Ching-jang Huang

Laboratory of Nutritional Biochemistry, Department of Biochemical Science and Technology and Institute of Microbiology and Biochemistry, National Taiwan University, Taipei, Taiwan, ROC

Fractionation and identification of 9c, 11t, 13t-conjugated linolenic acid as an activator of PPARα in bitter gourd (Momordica charantia L.)

Chia-Ying Chuang¹, Chin Hsu¹, Che-Yi Chao¹,², Yung-Shung Wein³, Yueh-Hsiong Kuo¹,²,⁵ & Ching-jang Huang¹,²,⁵*

¹Nutritional Biochemistry Laboratory, Institute of Microbiology and Biochemistry, National Taiwan University, I, Sec. 4, Roosevelt Rd., Taipei, 106, Taiwan; ²Department of Applied Life Science, Asia University, 500 Lieufeng Road, Wufong Township, Taichung County, 413, Taiwan; ³Department of Chemistry, National Taiwan University, I, Sec. 4, Roosevelt Rd., Taipei, 106, Taiwan; ⁴Department of Biochemical Science and Technology, National Taiwan University, I, Sec. 4, Roosevelt Rd., Taipei, 106, Taiwan; ⁵Institute of Bio-Agricultural Sciences, Academia Sinica, Taipei, 115, Taiwan

Received 12 April 2006; accepted 25 July 2006
© 2006 National Science Council, Taipei

Key words: PPARα, 9cis, 11trans, 13trans-conjugated linolenic acid, wild bitter gourd, transactivation assay, Acyl CoA Oxidase

Wild bitter gourd improves metabolic syndrome: A preliminary dietary supplementation trial

Chung-Huang Tsai¹,², Emily Chin-Fun Chen², Hsin-Sheng Tsay³* and Ching-jang Huang³,⁴*
Effects of various BG compounds on GLP-1 secretion in STC-1

• The aglycone form of momordicoside L has been reported to have hypoglycemic activity
  Cheng et al., 2008, Harinantenaina et al., 2006

• In addition, aglycone form of cucurbitane triterpenoid, such as Cucurbitacin B and E, also elicit bitter response through TAS2R10 and TAS2R14.
  Meyerhof et al., 2009

• Compound 5 showed especially high efficacy (Figure 4(e)). This compound is characterized by an aromatic B ring. Compound 6 is characterized by an epoxy linkage between C19 and C5 and a hydroxyl group at side chain C23.
• A large number of lipophilic plant secondary metabolites existed as glycoside form (saponin in a broad sense) and the solubility in water depends on the chain length and number of sugar moiety.
  Hostettmann et al., 1995

• Numerous phytosteroidal glycosides and triterpenoid glycosides have been isolated and identified from BG. The aglycone part might be C27 phytosteroid, oleanolic acid type or cucurbitane type C30 triterpenoids.

• The cucurbitane type triterpenoid glycosidies have been named goyaglycosideds, momordicicosides, karavilosides, Kuguagly-cosides, and so forth.

• Indeed, many aglycone triterpenoid compounds isolated from BG were obtained by hydrolyzing the sugar moiety.
  Harinantenaina et al., 2006, Tan et al., 2008
Chronic exposure to cold

Long-term overeating (diet-induced thermogenesis)

Sympathetic nervous system

Uncoupling protein (thermogenin)

Retinoic acid

Thermogenesis

Mitochondrion

Intermembrane space

F$_{0}$F$_{1}$ ATP-synthase

NADH + H$^+$

Electron transport

$\frac{1}{2}$ O$_2$

NAD$^+$

H$^+$

H$_2$O