Plant bioactives for the regulation of metabolism and energy expenditure.

Karsten Kristiansen
Professor of Molecular Biology
Department of Biology
University of Copenhagen
and
BGI-Shenzhen
The global obesity map

Eventually, the problem will solve itself

http://www.neatorama.com/2007/05/15/the-world-fatness-chart/
### Medical Complications of Obesity

- **Pulmonary disease**: abnormal function, obstructive sleep apnea, hypoventilation syndrome
- **Nonalcoholic fatty liver disease**: steatosis, steatohepatitis, cirrhosis
- **Gall bladder disease**
- **Gynecologic abnormalities**: abnormal menses, infertility, polycystic ovarian syndrome
- **Osteoarthritis**
- **Skin**
- **Gout**
- **Idiopathic intracranial hypertension**
- **Stroke**
- **Cataracts**
- **Coronary heart disease**
- **Diabetes**
- **Dyslipidemia**
- **Hypertension**
- **Severe pancreatitis**
- **Cancer**: breast, uterus, cervix, colon, esophagus, pancreas, kidney, prostate
- **Phlebitis**: venous stasis

### The problems of modern Pharma

**Very inefficient R&D paradigm**
- 250 man-years/compound that enters clinic
- 9 in 10 clinical entrants then fail
- No real improvements, despite massive investment

**Graph**

- Y-axis: NCEs
- X-axis: Spend ($bn in USA)
- Legend: New Chemical Entities Approved, R&D Spend
Nature has a strong track record in drugs

61% of the 877 new drugs launched in last 20 years originate from nature

- Nature provides different structures than organic chemists
  - Rigid structures with complex motifs, useful structural blueprints
  - Often outside "rules" of structure guided drug design

Type 2 diabetes

- Type 2 Diabetes (T2D) = "Inability to effectively use the insulin that the body produces"

More than 180 mill people suffer from T2D today! This number is estimated by WHO to be more than doubled by 2030!

Less than 50 % of the people who have T2D have actually been diagnosed

Initial compensatory increased insulin secretion to maintain euglycaemia

Inability to produce sufficient insulin (β-cell failure) => hyperglycaemia

Non-insulin-dependent diabetes (overt type 2 diabetes)
Prevalence of T2D

The PPARs – Peroxisome proliferator-activated receptors
Regulators of lipid and glucose metabolism

*Adapted from www.who.int

Rosen and Spiegelman, 2001
Insulin sensitivity and energy expenditure

- Two targets:
  - PPARγ agonists: improve insulin sensitivity and lower blood glucose
  - PPARδ agonists:
    - Increase fatty acid β-oxidation and enhances energy expenditure in muscle and fat
    - Depletes fat stores
    - Improve HDL/LDL ratio in primates
  - But: Concern that PPARδ agonists may promote cellular proliferation and cause cancers

The glitazones as insulin sensitizers and antidiabetic drugs

- Bind PPARγ with very high affinity
- Enhance insulin sensitivity and lower blood glucose
  - but
- Promotes adipogenesis, i.e. patients gain weight
- Heart enlargement and liver failure have been observed
- Enhance fluid retention - edema

*Still a serious need for novel activators of PPARγ for the treatment of type 2 diabetes*
Desirable PPAR profiles

- Insulin sensitizers with better safety profiles

- Partial PPAR\(\gamma\) or dual partial PPAR\(\gamma\)/PPAR\(\delta\) agonists
  - High selectivity
  - Full insulin sensitizing effect
  - No or attenuated adipogenic activity
  - No or reduced fluid retention
  - No heart enlargement
  - No liver toxicity

- Bioactive molecules in plants are interesting and promising candidates

Why partial PPAR\(\gamma\) agonists and what is a partial PPAR\(\gamma\) agonist

Available evidence indicate that partial PPAR\(\gamma\) agonists cause less:
- fat deposition
- Plasma volume expansion
- Myalgia
- Hepatotoxicity

Natural products are promising candidates as partial PPAR\(\gamma\) agonists and modulators of other nuclear receptors
Plants as anti-diabetics

- Plants have been used in the traditional treatment of diabetes for centuries
- More than 1200 different species have been used or tested for this purpose

French lilac (*Galega officinalis*)  
Stinging nettle (*Urtica dioica*)  
Sage (*Salvia officinalis*)


Natural products as modulators of nuclear receptors

- **PPAR modulators (FAs and FA-like compounds)**
  - 9Z,11E,13E conj. linolenic acid: PPARβ agonist from bitter melon
  - Ajulemic acid: Selective PPARγ agonist related to cannabinoids

- **PPAR modulators (Terpenoids)**
  - 20(S)-protopanaxatriol: PPARγ activator from ginseng
  - Carnosic acid (1) and 12-O-methyl carnosic acid (2): PPARγ activators from sage
  - 6-shogaol: PPARγ agonist from ginger
Natural products as modulators of nuclear receptors

- **PPAR modulators (Flavonoid derivatives)**

  - **Kaempferol**
  - **Genistein**
  - **Auraptene**

- **PPAR modulators (Alkaloids)**

  - **Harmine**

Harmine is one of the most well-studied PPARγ modulators. It is not a true agonist but has \textit{in vivo} effects comparable to those of true agonists although it does not affect body weight and hepatic lipid accumulation.

---

Health promoting effects of bioactive compounds in plants

**Partners**
- University of Copenhagen
- Aarhus University
- University of Southern Denmark
- Visiopharm A/S
- Developmental Center Aarslev

**Supported by the Strategic Research Council**
<table>
<thead>
<tr>
<th>Plants</th>
<th>Indications from literature and own preliminary results, prevention/treatment</th>
<th>Classes of compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Food plants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cabbage, many kinds (<em>Brassica oleracea</em> ssp.)</td>
<td>Cancer</td>
<td>Glucosinolates (e.g., glucotropaeolin, glucobrassicin) and degradation products (e.g., indole-3-carbinol, benzyl isothiocyanate)</td>
</tr>
<tr>
<td>Carrot (<em>Daucus carota</em>)</td>
<td>Cancer, cardiovascular disorders, inflammation</td>
<td>Carotenes, polyacetylenes (e.g., falcarinol, falcarindiol)</td>
</tr>
<tr>
<td>Buckwheat (<em>Fagopyrum esculentum/tartaricum</em>)</td>
<td>Cardiovascular disorders, obesity, insulin resistance</td>
<td>Flavonoids (e.g., rutin, quercetin)</td>
</tr>
<tr>
<td>2. Herbs and spices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Savory (<em>Satureja hortensis/montana</em>)</td>
<td>Diabetes, inflammation</td>
<td>Essential oils/ flavonoids, phenolic acids, polyacetylenes</td>
</tr>
<tr>
<td>Sage (<em>Salvia officinalis</em>)</td>
<td>Insulin resistance, Inflammation, Alzheimer</td>
<td>Essential oils/ flavonoids, phenolic acids</td>
</tr>
<tr>
<td>Oregano (<em>Origanum vulgare</em>)</td>
<td>Inflammation</td>
<td>Essential oils/ flavonoids, phenolic acids</td>
</tr>
<tr>
<td>Rosemary (<em>Rosmarinus officinalis</em>)</td>
<td>Inflammation, Alzheimer</td>
<td>Essential oils/ flavonoids, phenolic acids</td>
</tr>
<tr>
<td>3. Medicinal plants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purple coneflower (<em>Echinacea</em> ssp.)</td>
<td>Immunostimulatory, neuroprotective, obesity, insulin resistance</td>
<td>Alkamides, polyacetylenes, phenolic acids (e.g., chicoric acid)</td>
</tr>
<tr>
<td>Elderflower, Elderberry (<em>Sambucus nigra</em>)</td>
<td>Insulin resistance, obesity</td>
<td>Flavonoids (e.g., rutin, quercetin), phenolic acids (e.g., chlorogenic acid)</td>
</tr>
<tr>
<td>Ginseng (<em>Panax ginseng/quinquefolium</em>)</td>
<td>Insulin resistance, immunomodulatory, cancer, inflammation, CNS modulatory</td>
<td>Ginsenosides, (e.g., Rb1, Rg1, Re), polyacetylenes (e.g., falcarinol, panaxydol)</td>
</tr>
<tr>
<td>Thyme (<em>Thymus vulgaris</em>)</td>
<td>Inflammation</td>
<td>Essential oils, flavonoids</td>
</tr>
<tr>
<td>Rosenrod (<em>Rhodiola rosea</em>)</td>
<td>Obesity, insulin resistance, cancer</td>
<td>Phenylpropanoid glycosides (e.g., salidroside, rosavin) flavonoids, phenolic acids</td>
</tr>
</tbody>
</table>
Plants of current particular interest

- Purple coneflower
- Elder flower
- Vinter savory

From plants to bioactive compounds

1. Selection of plants
2. Extraction of bioactive compounds
3. Bioassays
4. Structure analysis
5. Pure compound
6. Animal testing
7. Purification
High-throughput analysis of cellular growth

Effects of compounds on cellular growth:
Promoting or inhibiting cellular growth

High-throughput fluorometric determination of DNA accumulation.
Microtiterplate format.

Activation of nuclear receptors

High-throughput microtiterplate-based assay.
Determines the ability of extracts/components to Activate different nuclear receptors

Examples:
• Peroxisome proliferator-activated receptor
• Retinoic acid receptors
• Estrogen receptors
Recruitment of specific co-activators to nuclear receptors

Medium-throughput microtiterplate-based assays. Determines the ability of compounds to recruit subsets of co-activators to nuclear receptors.

Examples:
- Recruitment of TIF-2/GRIP-1 to PPARγ/RXRα
- Recruitment of SRC-1 to PPARγ/RXRα
- Recruitment of PGC-1α to PPARγ/RXRα
- Recruitment of TRAP220 to PPARγ/RXRα
- Recruitment of RIP140 to PPARγ/RXRα

Desired partial PPARγ agonist profile

- Partial PPARγ agonist
- Should recruit
  - SRC-1
    - Recruitment improves insulin stimulated glucose uptake
  - PGC-1α
    - Key regulator of glucose metabolism and energy expenditure
- Should not recruit
  - TIF2
    - Allows PPARγ induced expression of genes associated with lipid storage & weight gain
  - RIP140
    - Represses genes involved in energy expenditure

Example of desired ligand
Differentiation profiling

Determining the effect on cellular differentiation and function.

Available cell systems:

• Mouse and human fat cells
• Mouse and human bone cells
• Mouse muscle cells
• Mouse and human skin cells

Determination of insulin-dependent glucose uptake in adipocytes and muscle

• Medium-throughput microtiterplate-based assays.
• Determines the ability of compounds to stimulate glucose uptake in adipocytes or pig muscle cells
Mouse and rat models of obesity

Alternative test systems for evaluation of bioactivity

• C. elegans model system of obesity
  • The entire genome of this nematode is known
  • As fast and easy to perform as a microorganism-based system while offering features of higher organisms e.g. intestine and muscles
  • Optical transparency with easy visualization of lipid accumulation

• Zebrafish models
  • Sequencing of the genome has been completed
  • "Fertile" animal model with genetic and physiological similarities to mammals
  • Models for e.g. glucose metabolism, atherosclerosis, and certain forms of cancer

Identification of bioactive compounds from purple coneflower

- Preparations of purple coneflower (*Echinacea purpurea*) are some of the most used herbal medicinal products
- Preparations of *Echinacea* are primarily used for the treatment of upper respiratory tract infections due to their immunomodulatory activities
- Two other species are used for medicinal purposes: *E. pallida* and *E. angustifolia*
- Bioactive compounds are:
  - Polysaccharides
  - Caffeic acid derivatives
  - Alkamides

Results from general screening

KB Christensen et al. (2009) *J. Nat. Prod.* 72,
Bioassay-guided set-up for identification

Cultivation and harvest of plant material → Extraction → Screening → Fractionation and isolation → Identification → Pure bioactive metabolites

Isolation of bioactive metabolites

KB Christensen et al. (2009) J. Nat. Prod. 72, 933-37
Activation of PPARγ by alkamides

KB Christensen et al. (2009) J. Nat. Prod. 72, 933-37

Effect on adipocyte differentiation and glucose uptake

KB Christensen et al. (2009) J. Nat. Prod. 72, 933-37
Identification of bioactive compounds from elderflowers

- Elderflowers (*Sambucus nigra*) is used in traditional medicine as a diuretic and to treat colds, influenza, and inflammation
- The leaves of elder have been used traditionally to treat diabetes
- Elderflowers is a rich source of bioactive metabolites e.g.
  - Triterpenoids
  - Flavonoid derivatives
  - Phenolic acids

A study on aq. extracts of elderflowers showed that they exhibit insulin-like and insulin-releasing actions *in vitro*. However, the bioactive metabolites were not identified.

Activation of PPARs

![Activation of PPARs by extracts of elderflowers (*Sambucus nigra*)](image)

KB Christensen et al. (2009) *Phytother. Res*
## Effect on adipocyte differentiation and glucose uptake

<table>
<thead>
<tr>
<th>DMSO</th>
<th>Rosi</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="DMSO.png" alt="Image" /></td>
<td><img src="Rosi.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Dichloromethane**

<table>
<thead>
<tr>
<th>MeOH</th>
<th>DMSO 1 mM Rosi</th>
<th>$10^3$</th>
<th>$10^4$</th>
<th>$10^5$</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="DMSO.png" alt="Image" /></td>
<td><img src="Rosi.png" alt="Image" /></td>
<td><img src="DMSO.png" alt="Image" /></td>
<td><img src="Rosi.png" alt="Image" /></td>
<td><img src="DMSO.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Beneficial effect on insulin-stimulated glucose uptake by a DCM extract of elderflowers


---

## Isolation of bioactive metabolites

**Methanolic extract of elderflowers**

<table>
<thead>
<tr>
<th>Phenolic Acids</th>
<th>Flavonoids</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="Phenolic.png" alt="Image" /></td>
<td><img src="Flavonoids.png" alt="Image" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fraction I + J</th>
<th>Fraction K</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="Fraction_I_J.png" alt="Image" /></td>
<td><img src="Fraction_K.png" alt="Image" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fraction L</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="Fraction_L.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**(-)-Naringenin**

- Linoleic acid 18:2 (9c, 12c)
- α-Linolenic acid 18:3 (9c, 12c, 15c)

Isolation of bioactive metabolites

Karsten Kristiansen, KU Bio
Winter School December 1 2009
Activation of PPARγ by naringenin

Proposed structural requirements for isoflavane skeleton for PPARγ ligand binding activity

Genistein: R₁ = OH, R₂ = H, R₃ = OH, R₄ = OH
Daidzein: R₁ = R₂ = H, R₃ = OH, R₄ = OH
Biochanin A: R₁ = OH, R₂ = H, R₃ = OH, R₄ = OCH₃

• Two types of fat:
  White fat stores energy as fat
  Brown fat burn fat producing heat

• Humans like other mammals, except pigs, have brown fat at birth, but brown fat has generally been thought to disappear rapidly after birth

• Recent findings, however, have shown that adults do contain brown fat, and that the amount of brown fat can be regulated

• Perspective:

  • 1 g brown fat can dissipates 6 kcal per day
  • 333 g brown fat can dissipates 2000 kcal per day

---

Brown fat in humans

Novel evidence from positron emission tomography

Functional brown fat in humans
Novel evidence from positron emission tomography

Brown fat in humans
Energy expenditure and brown fat

Virtanen et al., NEJM 360, 1518-1525, 2009

Van Marken Lichtenbelt et al. NEJM 360, 1500-1508, 2009
The healthy Mediterranean diet: 
Red wine and olive oil

An example of a unusual fatty acid present in sage
2-hydroxyoleic acids
A feeding experiment in mice
Body weight and body weight change

Liver and Muscle Weight

- **Body weight and body weight change**
  - Graph showing body weight changes over time for different treatments.
  - Treatments include Control, Vehicle, 2-hydroxyoleic acid, and Sibutramine.
  - Body weight change expressed as a percentage.

- **Liver and Muscle Weight**
  - Graphs showing liver and muscle weights for different treatments.
  - Treatments include Vehicle, 2-hydroxyoleic acid, and Sibutramine.
  - Muscle types include Soleus and Gastrocnemius.
  - Liver weight also shown.

**Key Points**
- The study involves evaluating the effects of different treatments on body weight and liver/muscle weights.
- Treatments include a control group, Vehicle, and 2-hydroxyoleic acid at different salt forms (free acid, ammonium salt, sodium salt).
- Sibutramine at 10mg/kg is also compared.
- Statistical significance is indicated by different letters in the graphs.
Fat depot weight

Effects in part due to conversion of white to brown adipose tissue

An alternative view on the heathy Mediterranean diet

- The healthy red wine and olives
Oleanolic acids: A bioactive compound form olive in the combat against obesity: Activation of a GPCR

Red wine in the struggle against obesity: Resveratrol
Red wine in the struggle against obesity: Resveratrol

Karsten Kristiansen, KU Bio
Winter School December 1 2009

Red wine in the struggle against obesity: Resveratrol improves endurance
Global warming, the ageing population and the obesity epidemic

Cypress et al., NEJM 360, 1509-1517, 2009

Acknowledgments

Kathrine B. Christensen
Lars Porskjær Christensen
Institute of Chemical Engineering, Biotechnology and Environmental Technology
University of Southern Denmark

Rasmus Koefoed Petersen
Lone Møller Pedersen
Qin Hao
Tao Ma
Ariane Minet
Dorota Kotowska
Lise Madsen
Department of Biologi
University of Copenhagen
Denmark

Thank you