Plant bioactives for the regulation of metabolism and energy expenditure.



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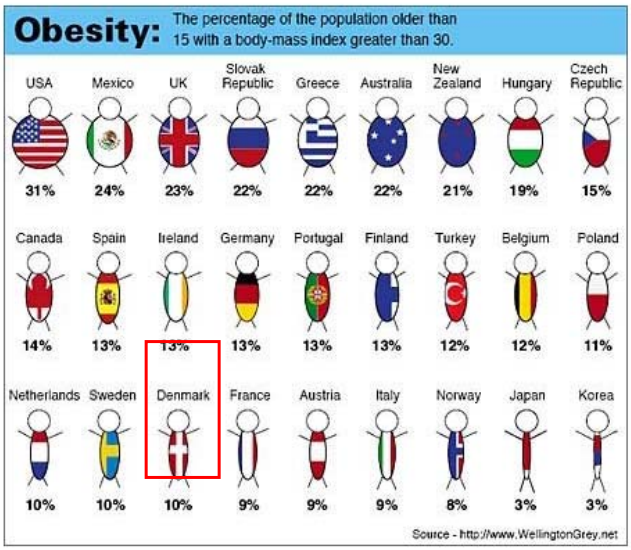
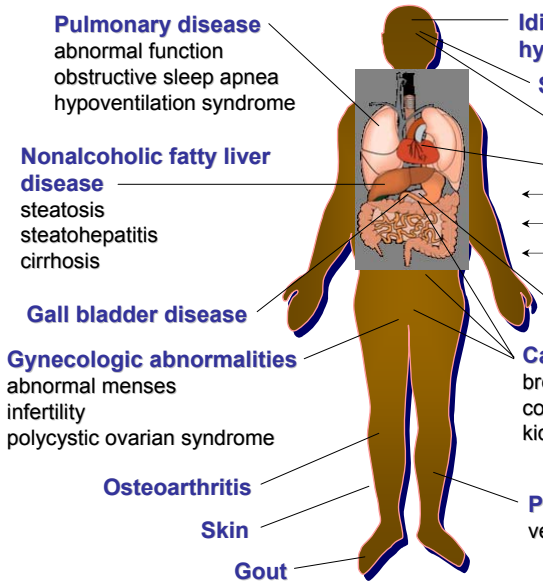
The global obesity map



http://www.neatorama.com/2007/05/15/the-world-fatness-chart/

Eventually, the problem will solve itself

Medical Complications of Obesity



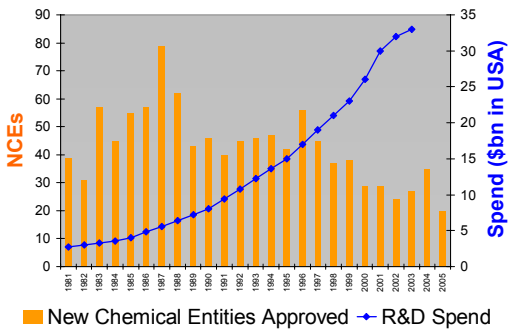
The problems of modern Pharma

Very inefficient R&D paradigm

250 man-years/compound that enters clinic

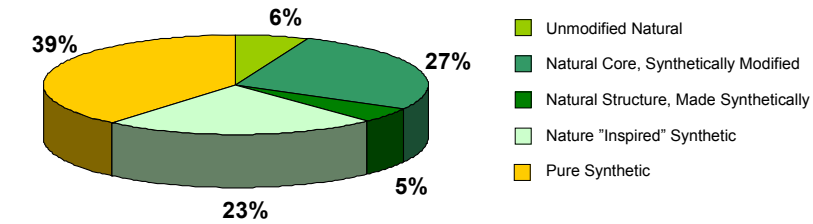
9 in 10 clinical entrants then fail

No real improvements, despite massive investment



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

Insulin resistance

↓

Initial compensatory increased insulin secretion to maintain euglycaemia

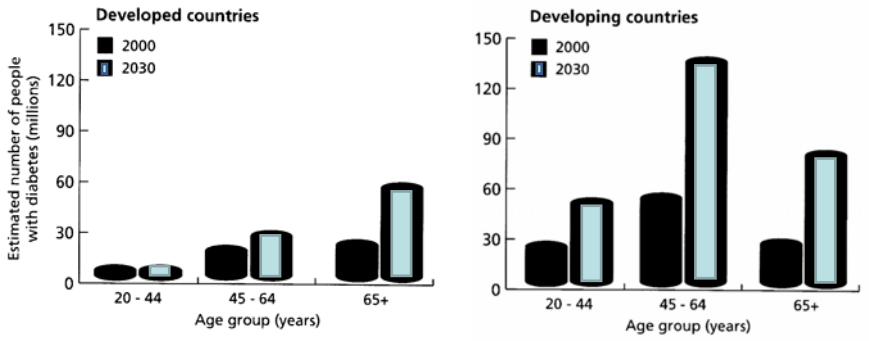
↓

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

↓

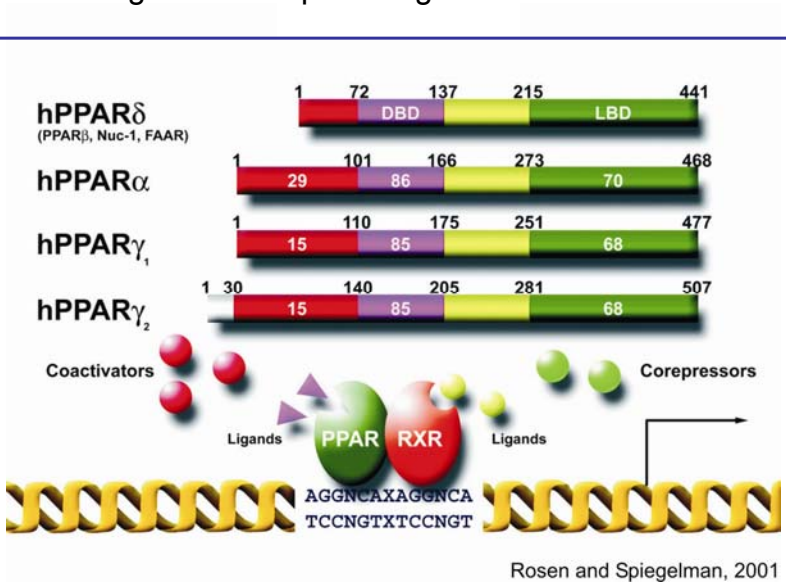
Non-insulin-dependent diabetes (overt type 2 diabetes)

Prevalence of T2D



*Adapted from www.who.int

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



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

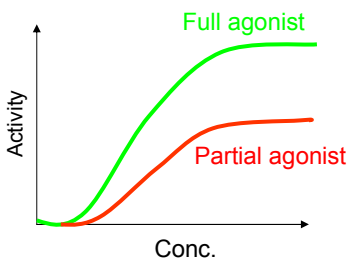
- Promotes adipogensis, i.e. patients gain weigth
- Heart enlargement and liver failure have been observed
- Enhance fluid retention - edema

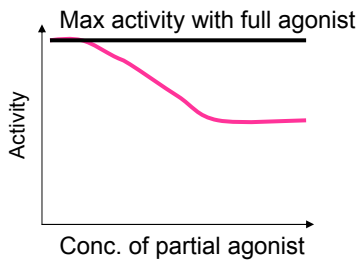
Still a serious need for novel activators of PPARγ for the treatment of type 2 diabetes

Desirable PPAR profiles

- Insulin sensitizers with better safety profiles
- **Partial PPARγ or dual partial PPARγ/PPARδ 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 PPARγ agonists and what is a partial PPARγ agonist





Available evidence indicate that partial PPARγ agonists cause less:

- fat deposition
- Plasma volume expansion
- Myalgia
- Hepatotoxicity

Natural products are promising candidates as partial PPARγ 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*)

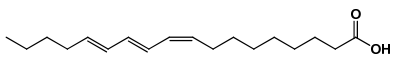
Stinging nettle (*Urtica dioica*)

Sage (*Salvia officinalis*)

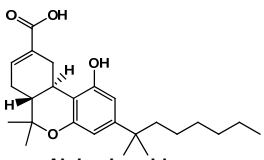
Marles & Farnsworth (**1995**) *Phytomed.* 2, p. 137-89

Natural products as modulators of nuclear receptors

• PPAR modulators (FAs and FA-like compounds)

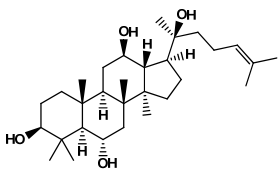


9*Z*,11*E*,13*E* conj. linolenic acid
PPARα agonist from bitter melon

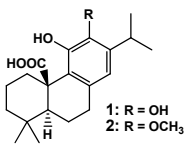


Ajulemic acid
Selective PPARγ agonist related to cannabinoids

• PPAR modulators (Terpenoids)

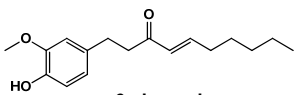


20(*S*)-protopanaxatriol
PPARγ activator from ginseng



1: R = OH
2: R = OCH_3

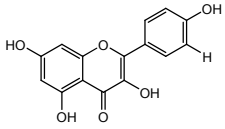
Carnosic acid (**1**) and
12-*O*-methyl carnosic acid (**2**)
PPARγ activators from sage



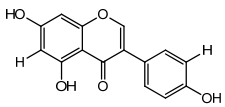
6-shogaol
PPARγ agonist from ginger

Natural products as modulators of nuclear receptors

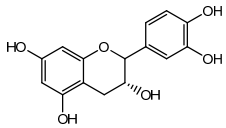
- **PPAR modulators (Flavonoid derivatives)**



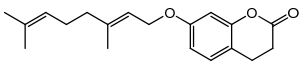
Kaempferol
PPARγ activator



Genistein
PPARγ and α + ERα and β agonist

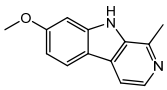


(–)-Catechin
PPARγ activator from green tea



Auraptene
PPARα and γ activator from *Citrus* sp.

- **PPAR modulators (Alkaloids)**



Harmine
PPARγ
modulators

Harmine is one of the most well-studied PPARγ moduators. It is not a true agonist but has *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

Selected plants

Plants	Indications from literature and own preliminary results, prevention/treatment	Classes of compounds
1. Food plants		
Cabbage, many kinds (*Brassica oleracea* ssp.)	Cancer	Glucosinolates (e.g., glucotropaeolin, glucobrassicin) and degradation products (e.g., indole-3- carbinol, benzyl isothiocyanate)
Carrot (*Daucus carota*)	Cancer, cardiovascular disorders, inflammation	carotenes, polyacetylenes (e.g., falcarinol, falcarindiol)
Buckwheat (*Fagopyrum esculentum/tartaricum*)	Cardiovascular disorders, obesity, insulin resistance,	Flavonoids (e.g. rutin, quercetrin)
2. Herbs and spices		
Savory (*Satureja hortensis/montana*)	Diabetes, inflammation,	Essential oils/ flavonoids, phenolic acids, polyacetylenes
Sage (*Salvia officinalis*)	Insulin resistance, Inflammation, Alzheimer	Essential oils/ flavonoids, phenolic acids
Oregano (*Origanum vulgare*)	Inflammation	Essential oils/ flavonoids, phenolic acids
Rosemary (*Rosmarinus officinalis*)	Inflammation, Alzheimer	Essential oils/ flavonoids, phenolic acids

Selected plants

3.Medicinal plants	Indications from literature and own preliminary results, prevention/treatment	Classes of compounds
Purple coneflower (*Echinacea* ssp.)	Immunostimulatory, neuroprotective, obesity, insulin resistance	Alkamides, polyacetylenes, phenolic acids (e.g., chicoric acid)
Elderflower, Elderberry (*Sambucus nigra*)	Insulin resistance, obesity	Flavonoids (e.g. rutin, quercetrin), phenolic acids (e.g., chlorogenic acid)
Ginseng (*Panax ginseng/quinquefolium*)	Insulin resistance, immunomodulatory, cancer, inflammation, CNS modulatory	Ginsenosides, (e.g., Rb_1, Rg_1, Re), polyacetylenes (e.g., falcarinol, panaxydol)
Thyme (*Thymus vulgaris*)	Inflammation	Essential oils, flavonoids
Rosenrod (*Rhodiola rosea*)	Obesity, insulin resistance, cancer	Phenylpropanoid glycosides (e.g., salidroside, rosavin) flavonoids, phenolic acids

Plants of current particular interest

Purple coneflower

Elder flower

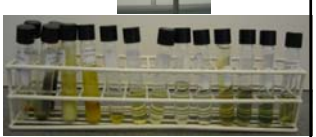
Vinter savory

From plants to bioactive compounds

Selection of plants

Extraction of bioactive compounds

Bioassays



Purification

Pure compound

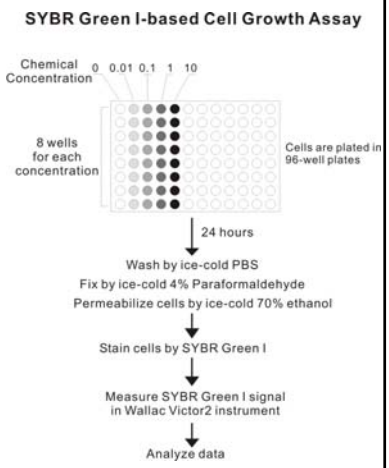
Structure analysis

Animal testing

High-throughput analysis of cellular growth

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

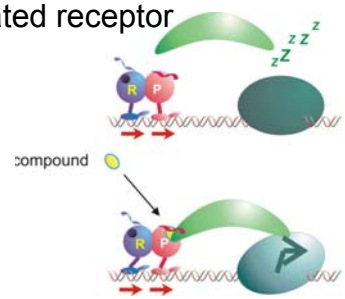
High-throughput fluorometric determination of DNA accumulation.
Microtiterplate format.



Activation of nuclear receptors

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

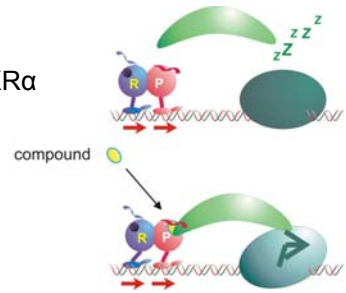
Examples:
- Peroxisome proliferator-activated receptor
- Retinoic acid receptors
- Estrogen receptors



Recruitment of specific co-activators to nuclear receptors

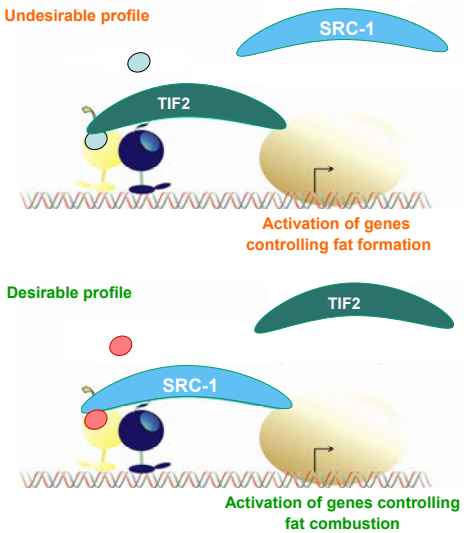
Medium-througput microtiterplate-based assays.
Determines the ability of compounds to
recruit subsets of co-activators to nuclear receptors.

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



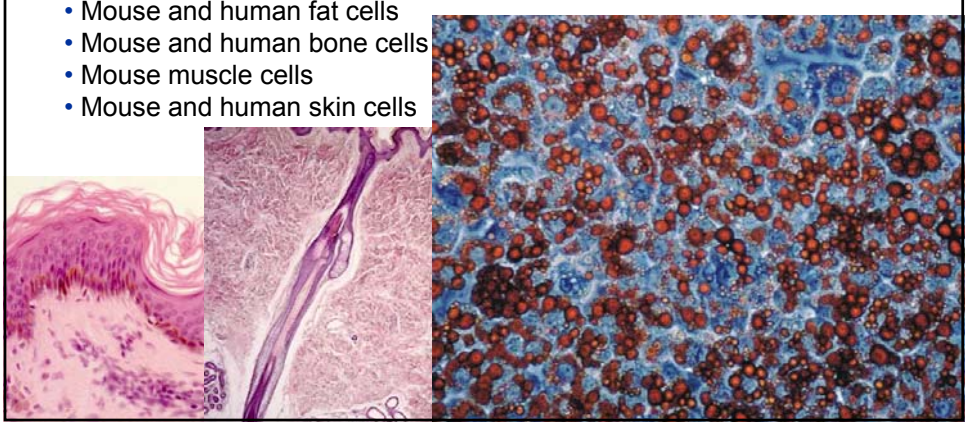
Example of desired ligand

Differentiation profiling

Determining the effect on cellular differentiation and function.

Available cell systems:

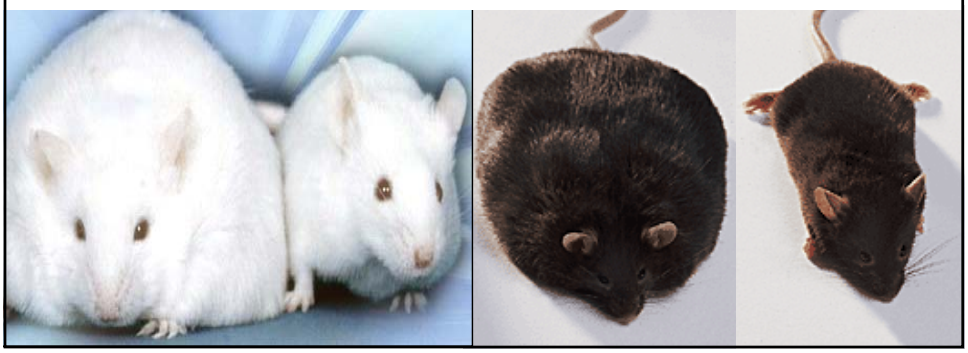
- Mouse and human fat cells
- Mouse and human bone cells
- Mouse muscle cells
- Mouse and human skin cells



Determination of insulin-dependent glucose uptake in adipocytes and muscle

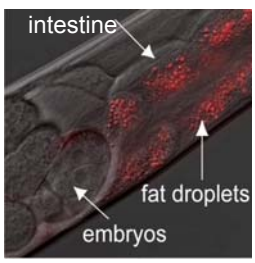
- Medium-througput microtiterplate-based assays.
- Determines the ability of compounds to stimulate glucose uptake in adipocytes or pig muscle cells

Mouse and rat models of obesity

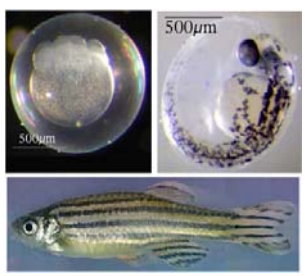


Alternative test systems for evaluation of bioactivity

- *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



K. Ashrafi (**2007**) www.wormbook.org



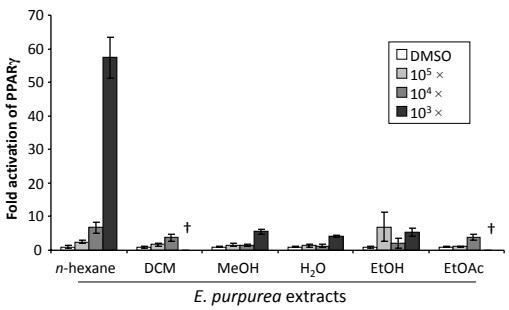
Identification of bioactive compounds from purple coneflower

- 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

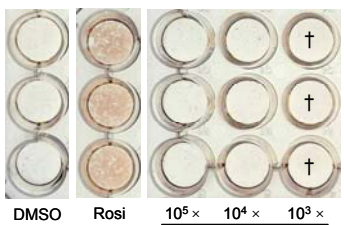
Purple coneflower (*Echinacea purpurea*)

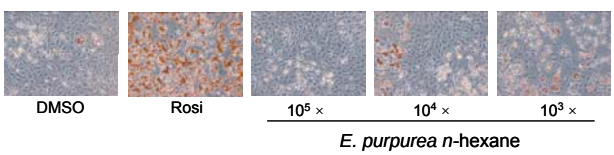
Results from general screening

Activation of PPARγ



Effect on adipocyte differentiation



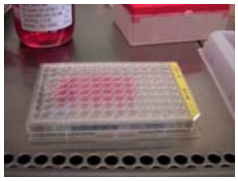


KB Christensen *et al.* (**2009**) *J. Nat. Prod.* 72,

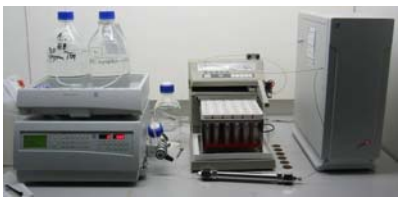
Bioassay-guided set-up for identification

Cultivation and harvest of plant material

Extraction



Screening

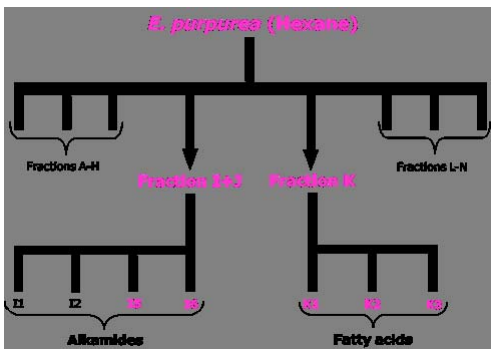


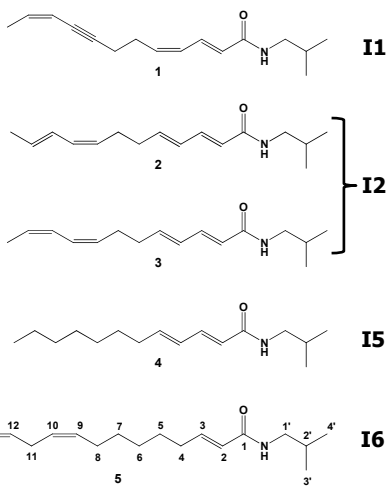
Fractionation and isolation

Pure bioactive metabolites

Identification

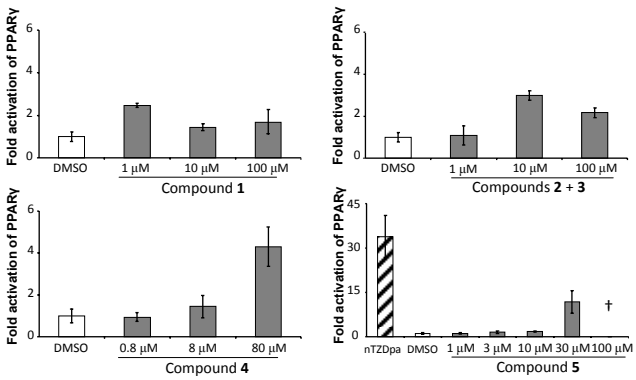
Isolation of bioactive metabolites





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

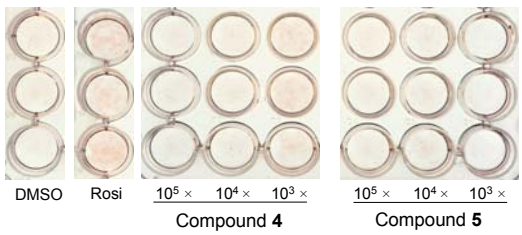
Activation of PPARγ by alkamides

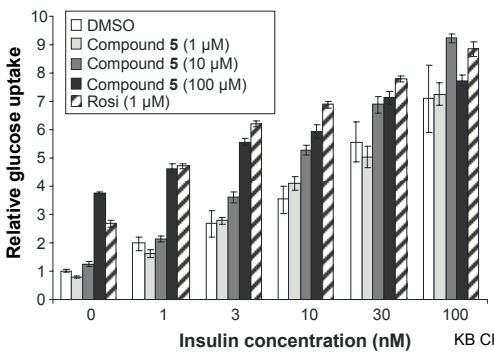


KB Christensen *et al.* (**2009**) *J. Nat. Prod*. 72, 933-37

Effect on adipocyte differentiation and glucose uptake

Effect on adipocyte differentiation





Effect on insulin-stimulated glucose uptake

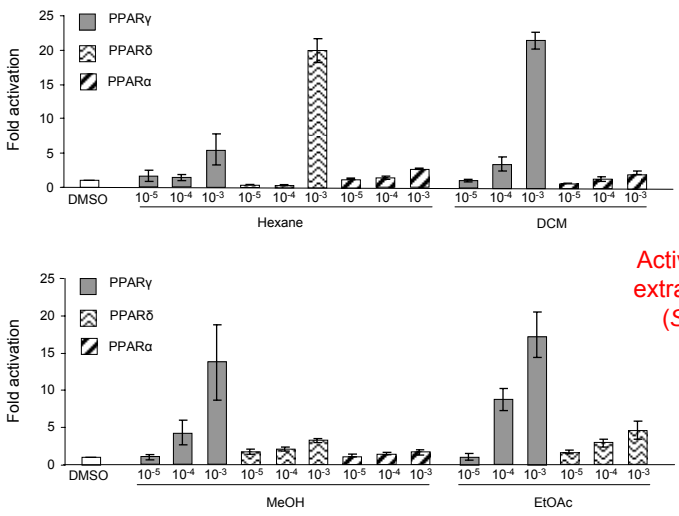
KB Christensen *et al.* (**2009**) *J. Nat. Prod*. 72, 933-37

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.

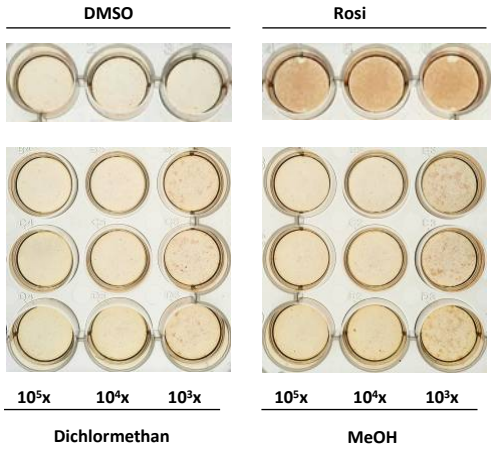
Activation of PPARs



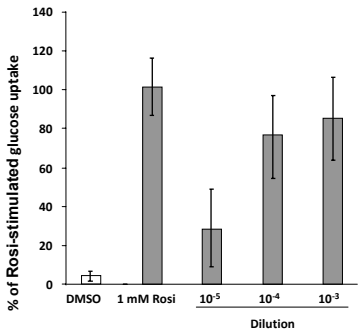
Activation of PPARs by extracts of elderflowers (*Sambucus nigra)*

KB Christensen *et al.* (**2009**) *Phytother. Res*

Effect on adipocyte differentiation and glucose uptake



Slight stimulation of adipocyte differentiation by extracts of elderflowers

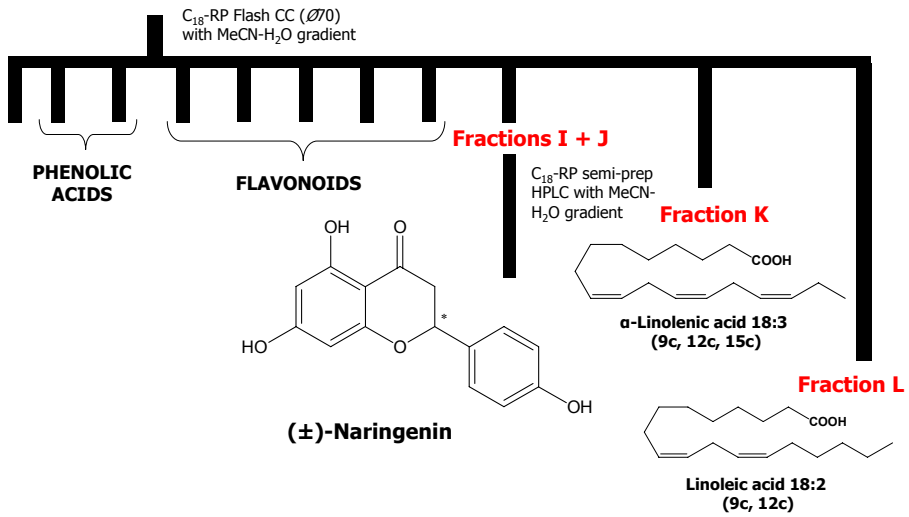


Beneficial effect on insulin-stimulated glucose uptake by a DCM extract of elderflowers

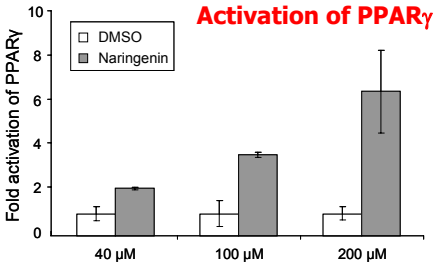
KB Christensen *et al.* (**2009**) *Phytother. Res.*

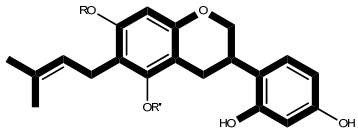
Isolation of bioactive metabolites

Methanolic extract of elderflowers

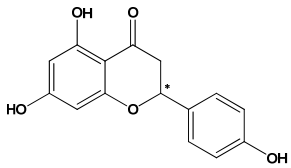


Activation of PPARγ by naringenin

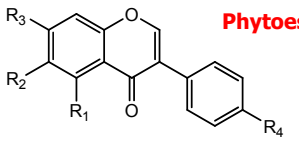




Proposed structural requirements for isoflavane skeleton for PPARγ ligand binding activity
(Kuroda *et al.* (**2003**) *Bioorg. Med. Chem. Lett.* 13, 4267-72.



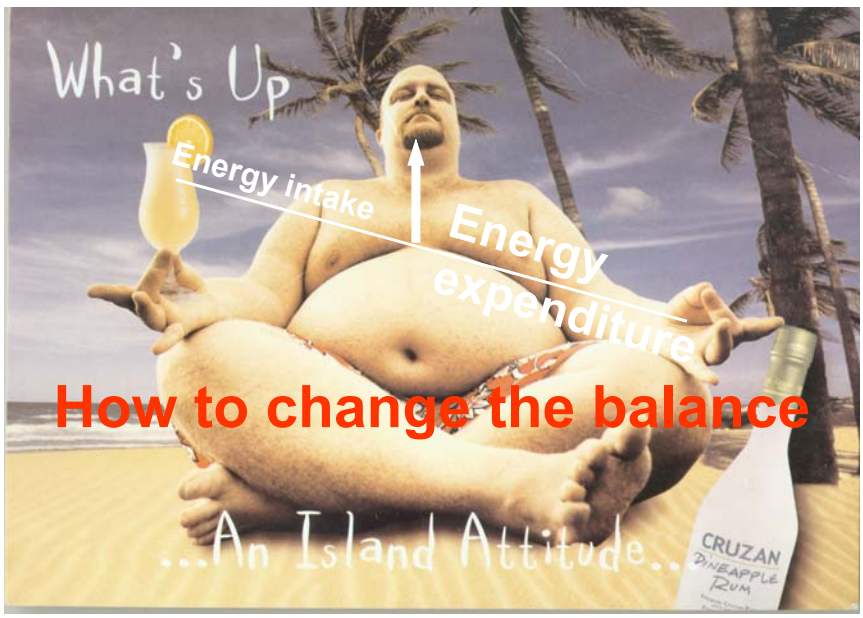
Naringenin



Phytoestrogens

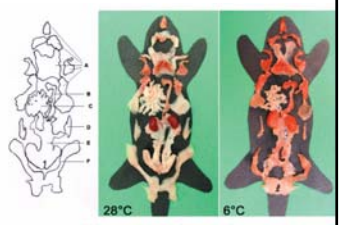
Genistein: R_1 = OH, R_2 = H, R_3= OH, R_4 = OH
Daidzein: R_1 = R_2 = H, R_3 = OH, R_4 = OH
Biochanin A: R_1 = OH, R_2 = H, R_3 = OH, R_4 = OCH_3

KB Christensen *et al.* (**2009**) *Phytother. Res.*



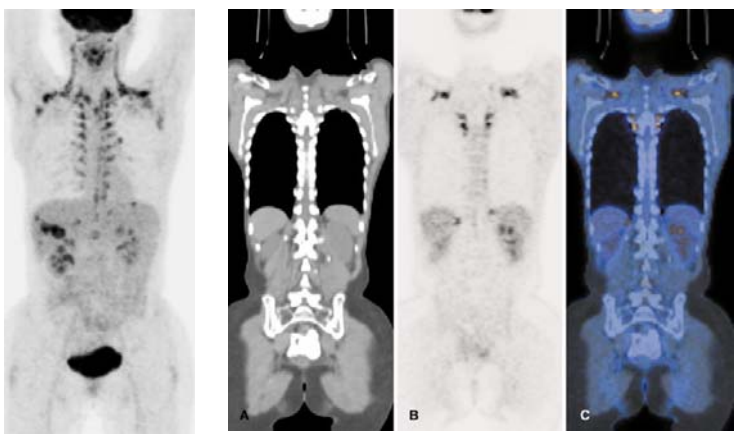
Brown fat as a fat burner

- 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



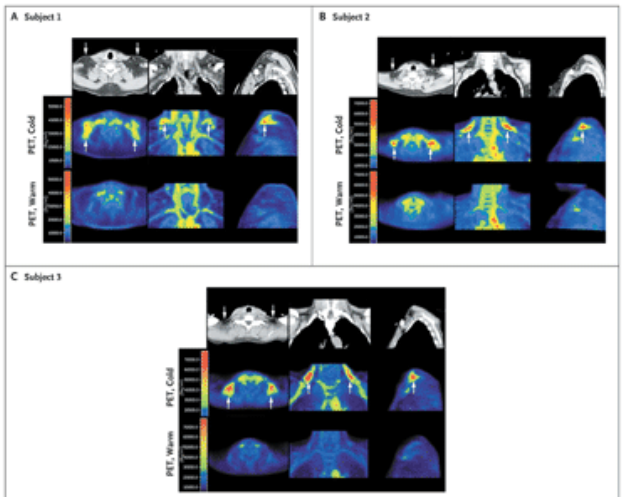
Brown fat in humans

Novel evidence from positron emission tomography



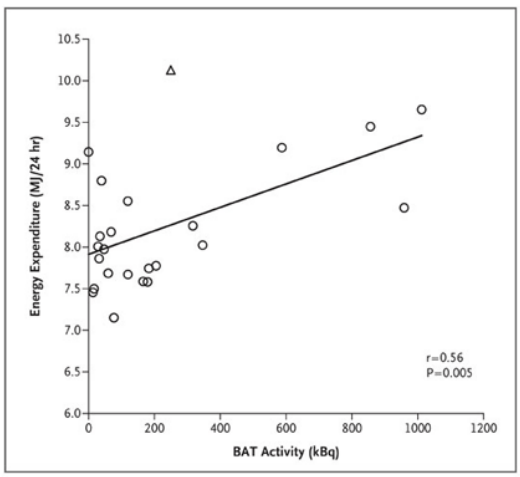
Hany et al., Eur J Nucl Med (2002) 29:1393–1398

Functional brown fat in humans
Novel evidence from positron emission tomography



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

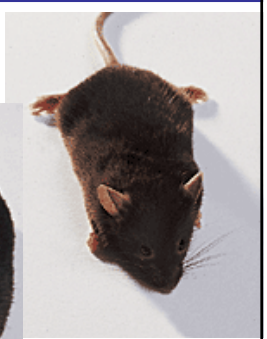
Brown fat in humans
Energy expenditure and brown fat



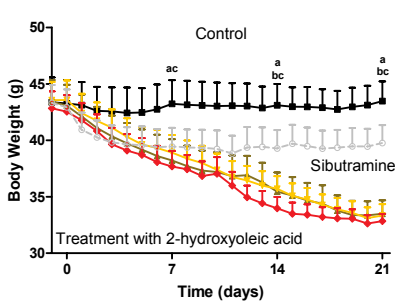
Van Marken Lichtenbelt et al. NEJM 360, 1500-1508, 2009

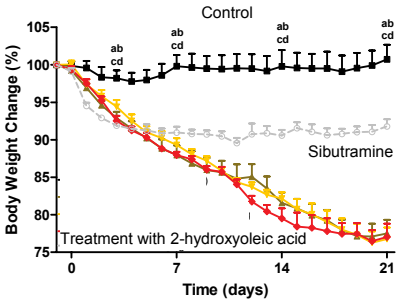
The healthy Mediterranean diet:
Red wine and olive oil

An example of a unusual fatty acid present in sage
2-hydroxyoleic acids
A feeding experiment in mice

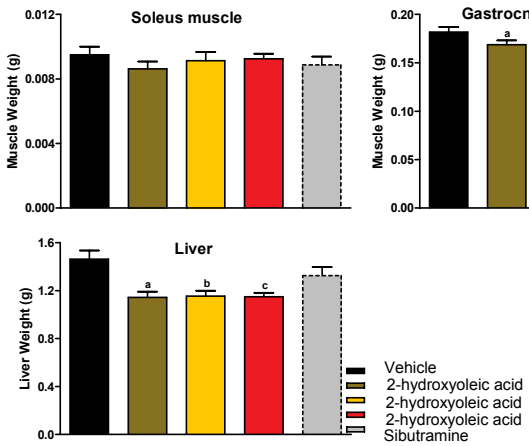


Body weight and body weight change

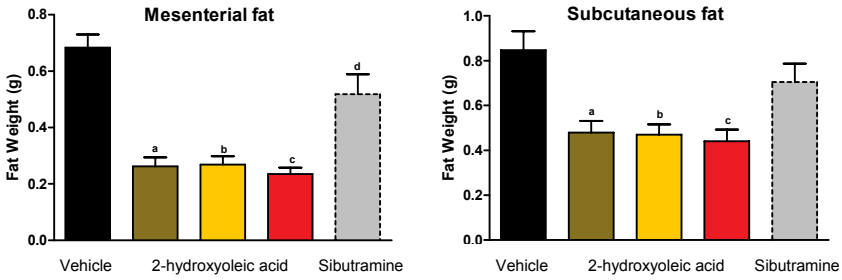




Liver and Muscle Weight



Fat depot weight

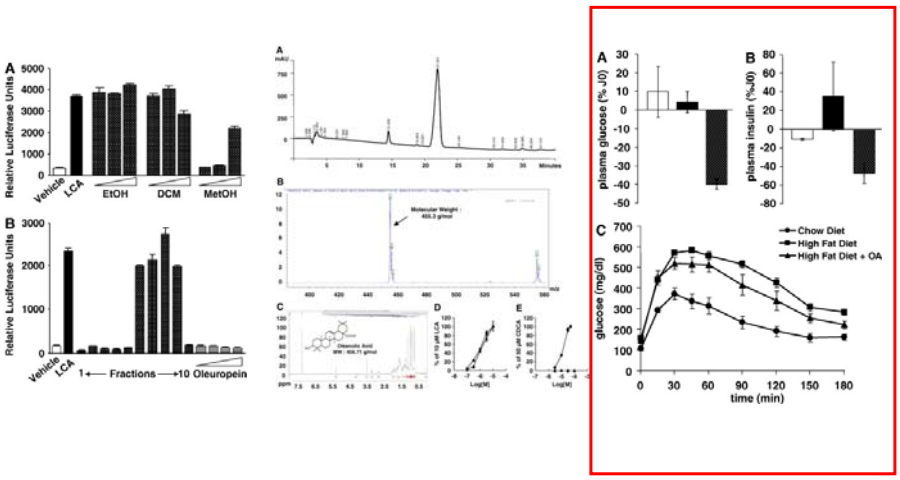


Effects in part due to conversion of white to brown adipose tissue

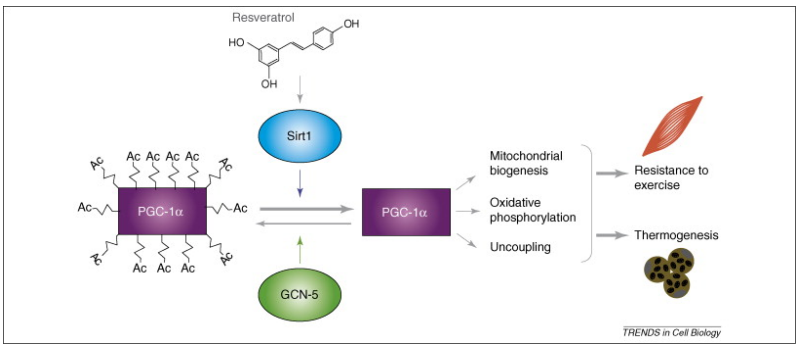
An alternative view on the heathy Mediterranean diet

- The healthy red wine and olives

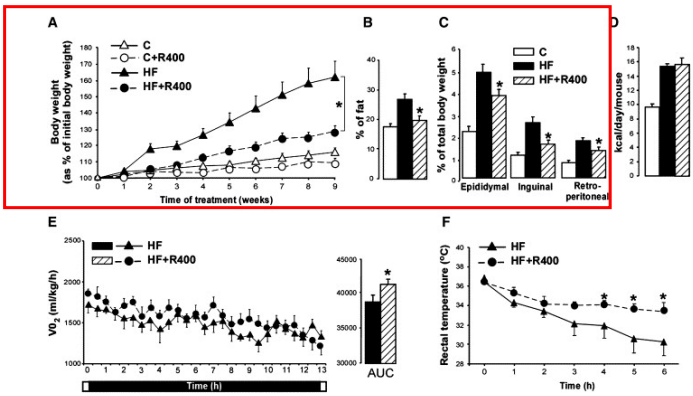
Oleanolic acids: A bioactive compound form olive in the combat against obesity: Activation of a GPCR



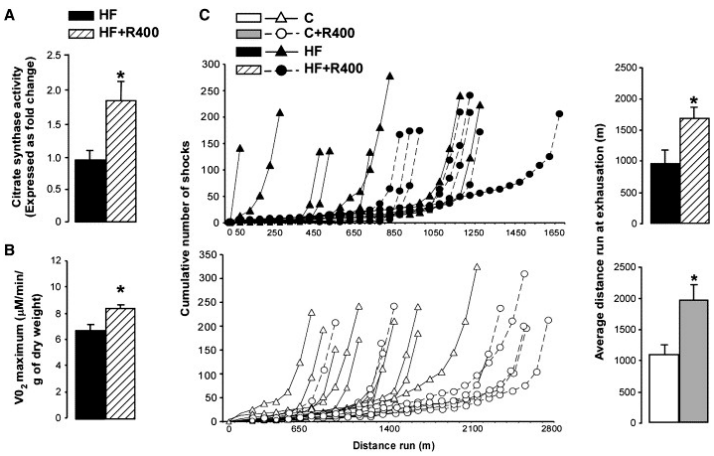
Red wine in the struggle against obesity: Resveratrol



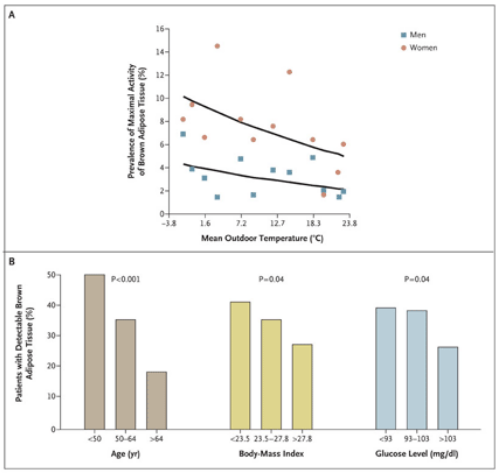
Red wine in the struggle against obesity: Resveratrol



Red wine in the struggle against obesity: Resveratrol improves endurance



Global warming, the ageing population and the obesity epidemic



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

Acknowlegdments

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Biotechnology and Environmental Technology
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Lone Møller Pedersen
Qin Hao
Tao Ma
Ariane Minet
Dorota Kotowska
Lise Madsen
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Thank you