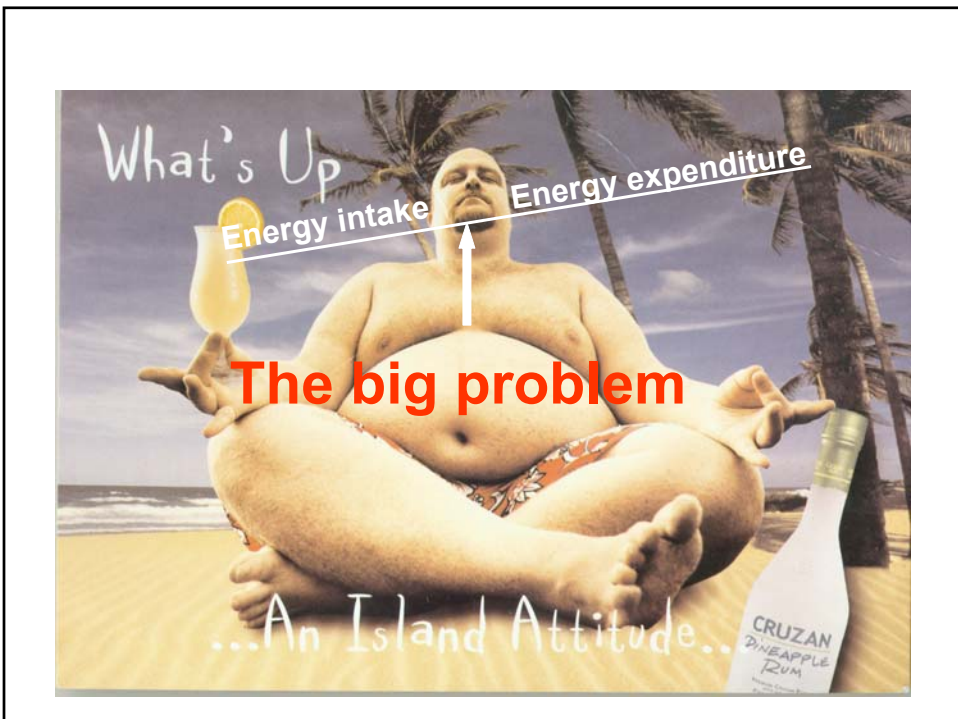


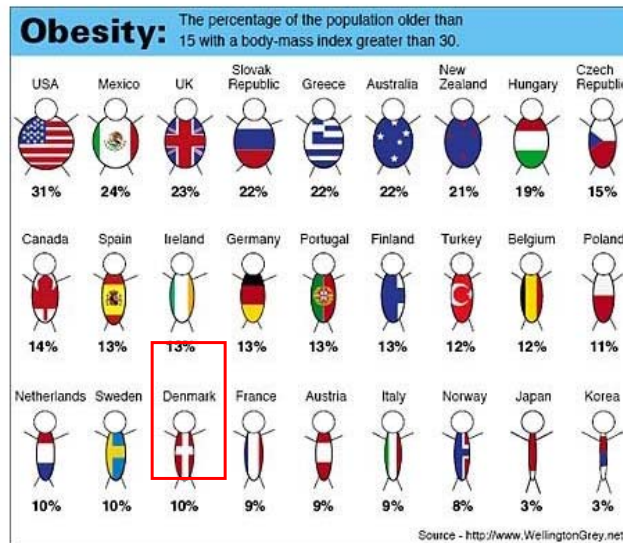
## Plant bioactives for the regulation of metabolism and energy expenditure.



Karsten Kristiansen  
Professor of Molecular Biology  
Department of Biology  
University of Copenhagen  
and  
BGI-Shenzhen



## The global obesity map

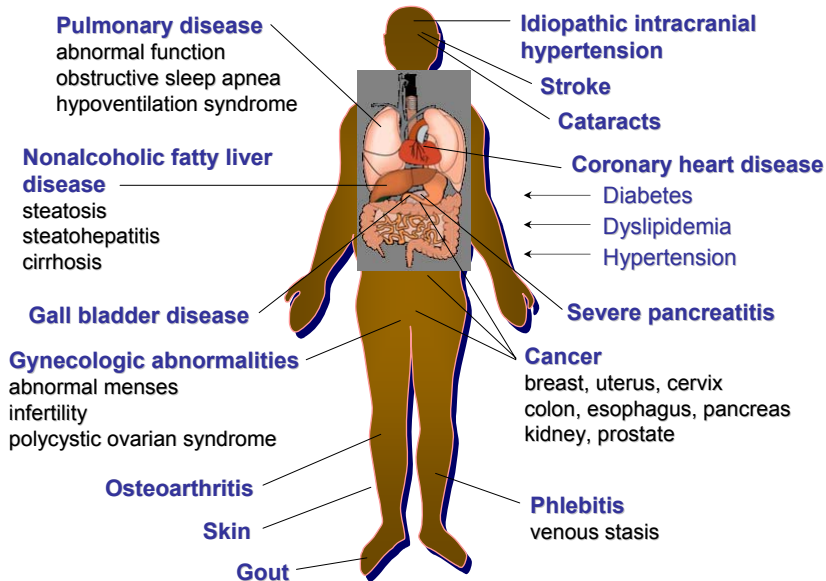


<http://www.neatorama.com/2007/05/15/the-world-fatness-chart/>

## Eventually, the problem will solve itself



## Medical Complications of Obesity

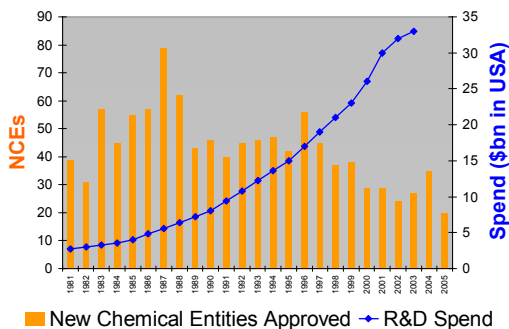


## The problems of modern Pharma

**Very inefficient R&D paradigm**  
 250 man-years/compound that enters clinic

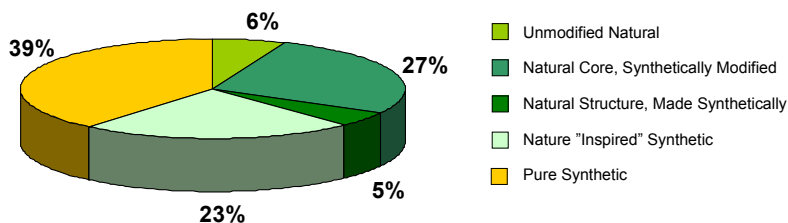
9 in 10 clinical entrants then fail

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



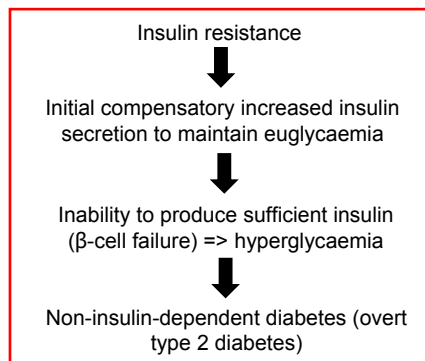
- Nature provides different structures than organic chemists
  - Rigid structures with complex motifs, useful structural blueprints
  - Often outside "rules" of structure guided drug design

## Type 2 diabetes

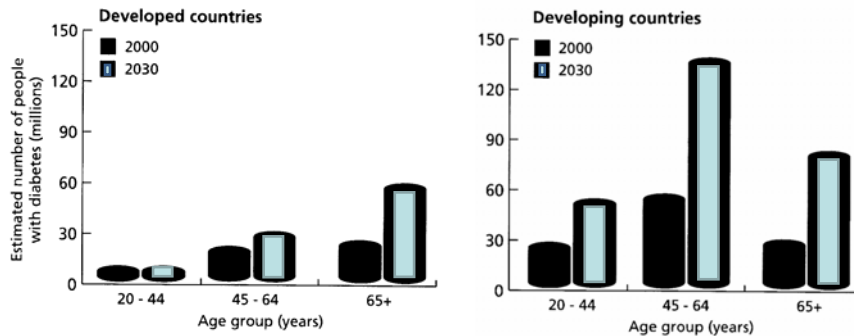
- Type 2 Diabetes (T2D) = *"Inability to effectively use the insulin that the body produces"*

**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**

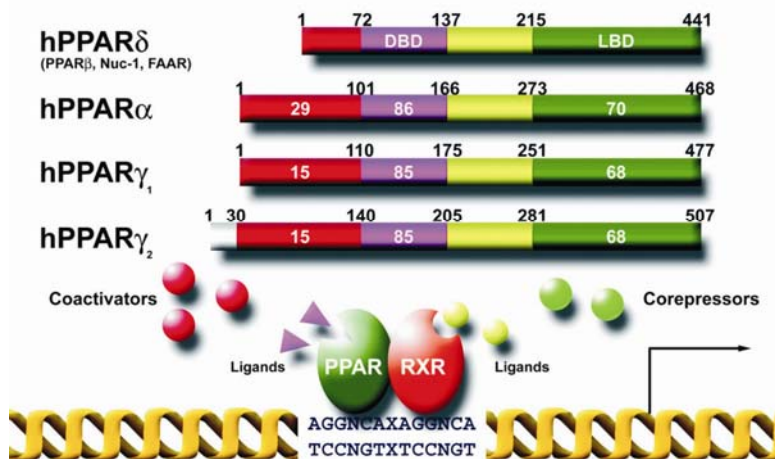


## Prevalence of T2D



\*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:
- PPAR $\gamma$  agonists: improve insulin sensitivity and lower blood glucose
- PPAR $\delta$  agonists:
  - Increase fatty acid  $\beta$ -oxidation and enhances energy expenditure in muscle and fat
  - Depletes fat stores
  - Improve HDL/LDL ratio in primates
- But: Concern that PPAR $\delta$  agonists may promote cellular proliferation and cause cancers

## The glitazones as insulin sensitizers and antidiabetic drugs

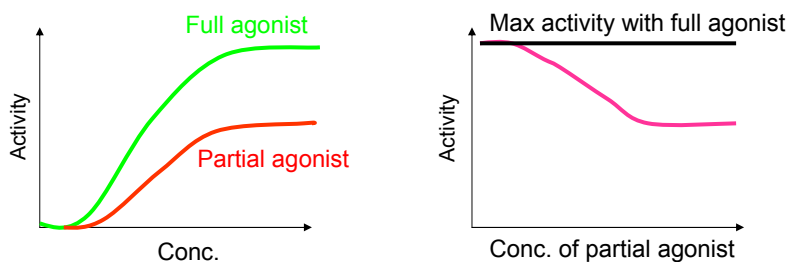
- Bind PPAR $\gamma$  with very high affinity
- Enhance insulin sensitivity and lower blood glucose  
but
- Promotes adipogenesis, i.e. patients gain weight
- Heart enlargement and liver failure have been observed
- Enhance fluid retention - edema

***Still a serious need for novel activators of PPAR $\gamma$  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*)

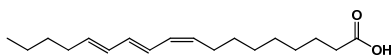


Sage (*Salvia officinalis*)

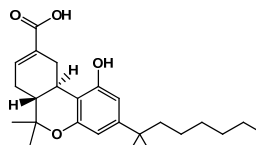
Marles & Farnsworth (1995) *Phytomed.* 2, p. 137-89

## Natural products as modulators of nuclear receptors

### • PPAR modulators (FAs and FA-like compounds)

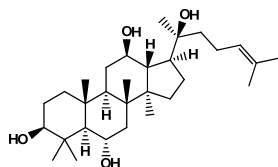


**9Z,11E,13E conj. linolenic acid**  
PPAR $\alpha$  agonist from bitter melon

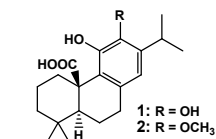


**Ajulemic acid**  
Selective PPAR $\gamma$  agonist related to cannabinoids

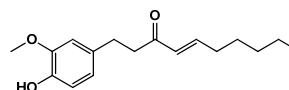
### • PPAR modulators (Terpenoids)



**20(S)-protopanaxatriol**  
PPAR $\gamma$  activator from ginseng



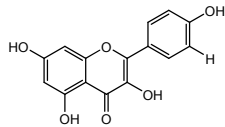
**Carnosic acid (1) and 12-O-methyl carnosic acid (2)**  
PPAR $\gamma$  activators from sage  
1: R = OH  
2: R = OCH<sub>3</sub>



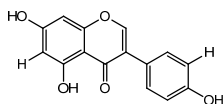
**6-shogaol**  
PPAR $\gamma$  agonist from ginger

# Natural products as modulators of nuclear receptors

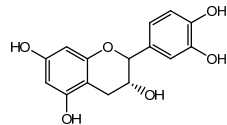
## • PPAR modulators (Flavonoid derivatives)



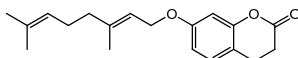
**Kaempferol**  
PPAR $\gamma$  activator



**Genistein**  
PPAR $\gamma$  and  $\alpha$  + ER $\alpha$  and  $\beta$  agonist

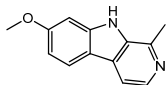


**(-)-Catechin**  
PPAR $\gamma$  activator from green tea



**Auraptene**  
PPAR $\alpha$  and  $\gamma$  activator from *Citrus* sp.

## • PPAR modulators (Alkaloids)



**Harmine**  
PPAR $\gamma$   
modulators

Harmine is one of the most well-studied PPAR $\gamma$  modulators. 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
<b>1. Food plants</b>		
Cabbage, many kinds ( <i>Brassica oleracea</i> ssp.)	Cancer	Glucosinolates (e.g., glucotropaeolin, glucobrassicin) and degradation products (e.g., indole-3- carbinol, benzyl isothiocyanate)
Carrot ( <i>Daucus carota</i> )	Cancer, cardiovascular disorders, inflammation	carotenes, polyacetylenes (e.g., falcarinol, falcarindiol)
Buckwheat ( <i>Fagopyrum esculentum/tartaricum</i> )	Cardiovascular disorders, obesity, insulin resistance,	Flavonoids (e.g. rutin, quercetrin)
<b>2. Herbs and spices</b>		
Savory ( <i>Satureja hortensis/montana</i> )	Diabetes, inflammation,	Essential oils/ flavonoids, phenolic acids, polyacetylenes
Sage ( <i>Salvia officinalis</i> )	Insulin resistance, Inflammation, Alzheimer	Essential oils/ flavonoids, phenolic acids
Oregano ( <i>Origanum vulgare</i> )	Inflammation	Essential oils/ flavonoids, phenolic acids
Rosemary ( <i>Rosmarinus officinalis</i> )	Inflammation, Alzheimer	Essential oils/ flavonoids, phenolic acids



## Selected plants

Plants	Indications from literature and own preliminary results, prevention/treatment	Classes of compounds
<b>3. Medicinal plants</b>		
Purple coneflower ( <i>Echinacea</i> ssp.)	Immunostimulatory, neuroprotective, obesity, insulin resistance	Alkamides, polyacetylenes, phenolic acids (e.g., chicoric acid)
Elderflower, Elderberry ( <i>Sambucus nigra</i> )	Insulin resistance, obesity	Flavonoids (e.g. rutin, quercetrin), phenolic acids (e.g., chlorogenic acid)
Ginseng ( <i>Panax ginseng/quinquefolium</i> )	Insulin resistance, immunomodulatory, cancer, inflammation, CNS modulatory	Ginsenosides, (e.g., Rb <sub>1</sub> , Rg <sub>1</sub> , Re), polyacetylenes (e.g., falcarinol, panaxydol)
Thyme ( <i>Thymus vulgaris</i> )	Inflammation	Essential oils, flavonoids
Rosenrod ( <i>Rhodiola rosea</i> )	Obesity, insulin resistance, cancer	Phenylpropanoid glycosides (e.g., salidroside, rosavin) flavonoids, phenolic acids



## Plants of current particular interest

Purple coneflower

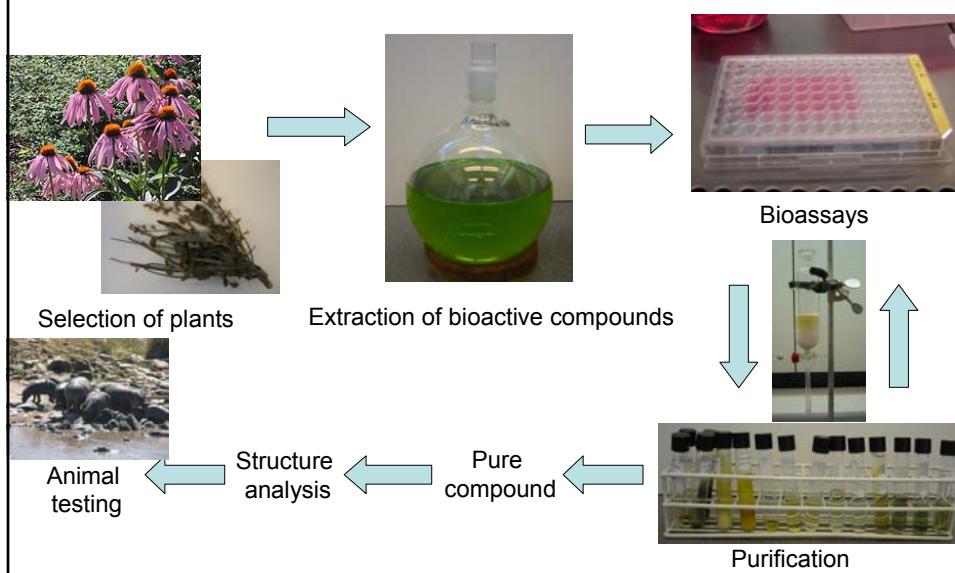


Elder flower



Vinter savory

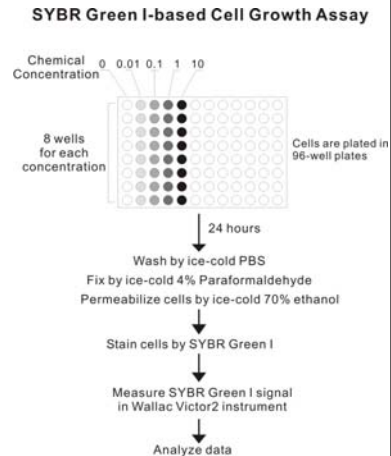
## From plants to bioactive compounds



## 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.

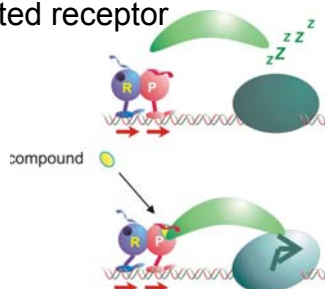


## Activation of nuclear receptors

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

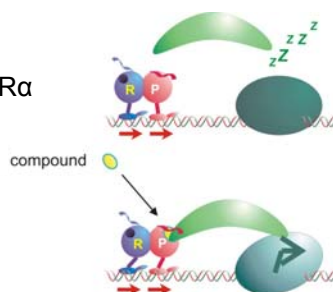


## Recruitment of specific co-activators to nuclear receptors

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

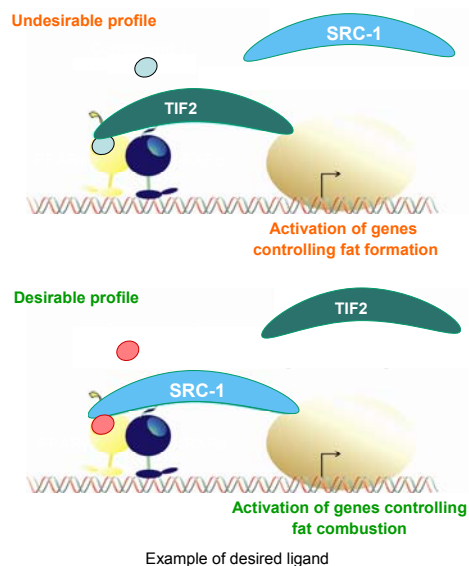
Examples:

- Recruitment of TIF-2/GRIP-1 to PPAR $\gamma$ /RXR $\alpha$
- Recruitment of SRC-1 to PPAR $\gamma$ /RXR $\alpha$
- Recruitment of PGC-1 $\alpha$  to PPAR $\gamma$ /RXR $\alpha$
- Recruitment of TRAP220 to PPAR $\gamma$ /RXR $\alpha$
- Recruitment of RIP140 to PPAR $\gamma$ /RXR $\alpha$



## Desired partial PPAR $\gamma$ agonist profile

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

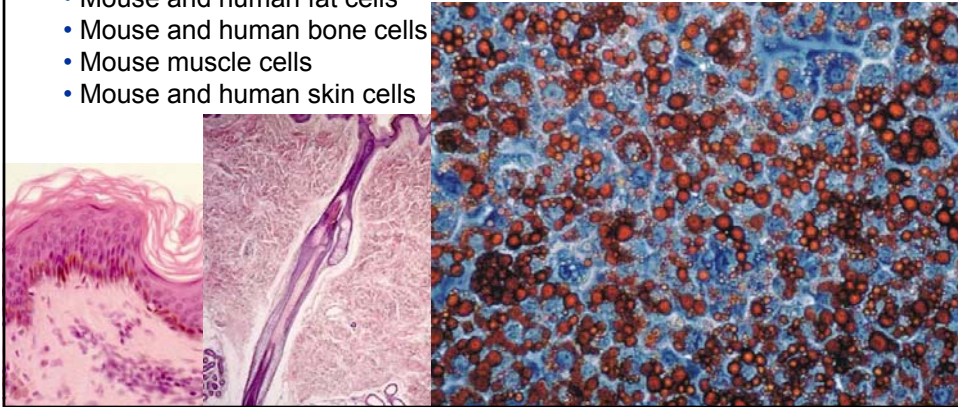


## Differentiation profiling

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

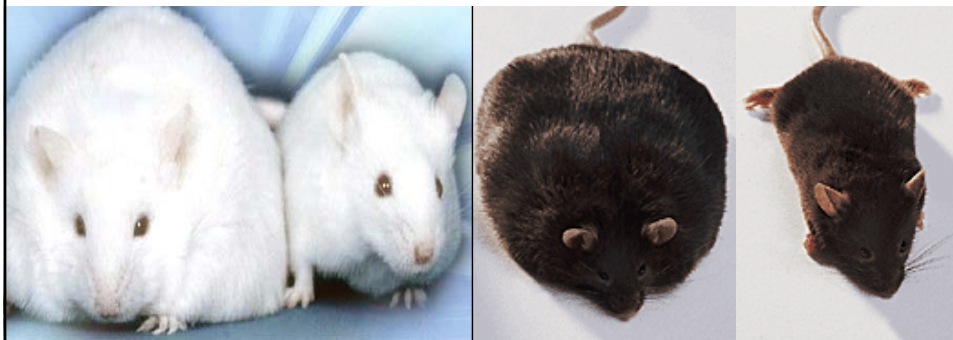


## Determination of insulin-dependent glucose uptake in adipocytes and muscle

- 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

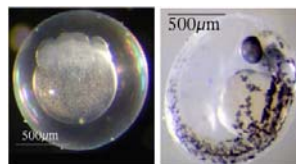


## Alternative test systems for evaluation of bioactivity

- *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



K. Ashrafi (2007) [www.wormbook.org](http://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

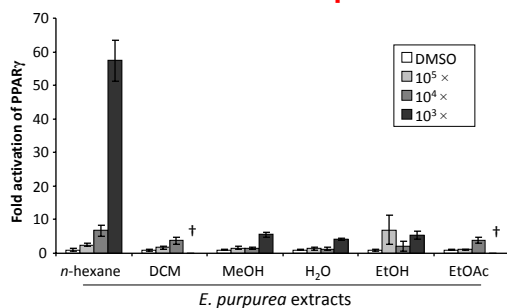


Purple coneflower (*Echinacea purpurea*)

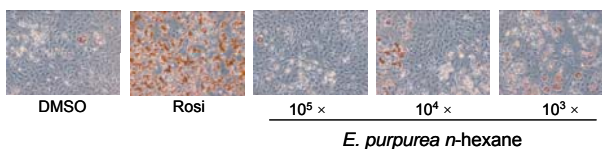
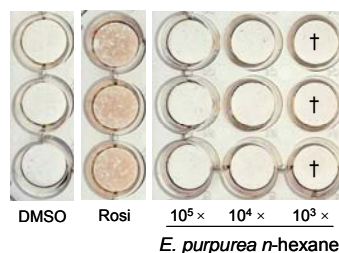


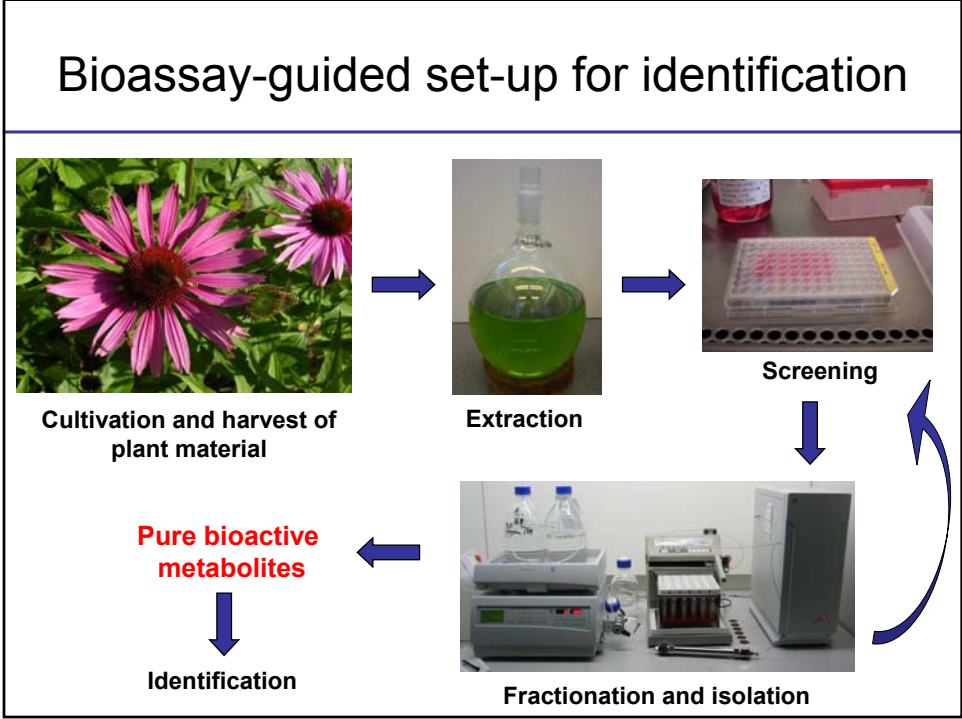
## Results from general screening

### Activation of PPAR $\gamma$



### Effect on adipocyte differentiation





## Isolation of bioactive metabolites

*E. purpurea* (Hexane)

Fractions A-H    Fractions I+J    Fraction K    Fractions L-N

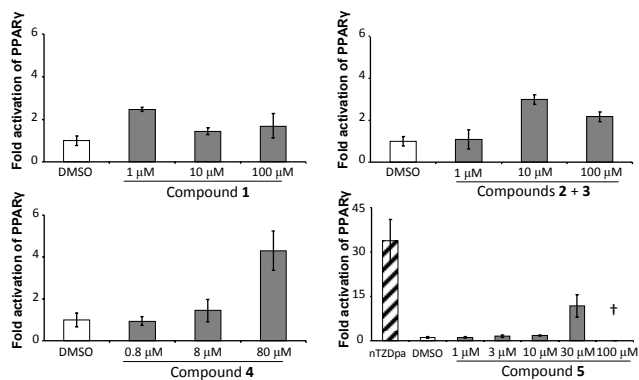
I1 I2 I5 I6    K1 K2 K3

Alkarnides    Fatty acids

**I1**  
**I2**  
**I3**  
**I5**  
**I6**

KB Christensen *et al.* (2009) *J. Nat. Prod.* 72, 933-37 23/33

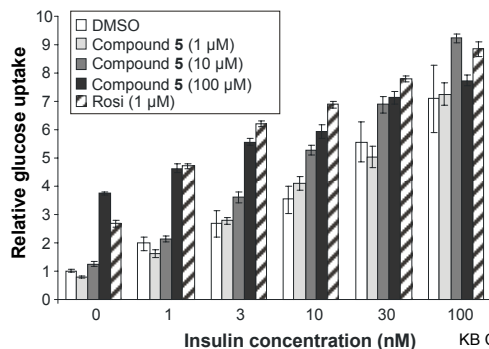
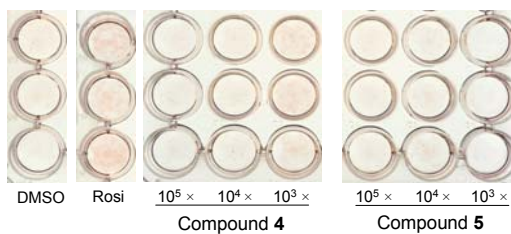
## Activation of PPAR $\gamma$ by alkamides



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

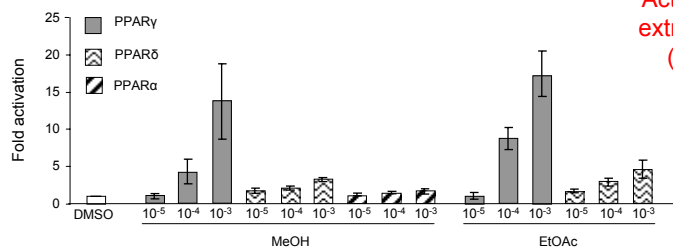
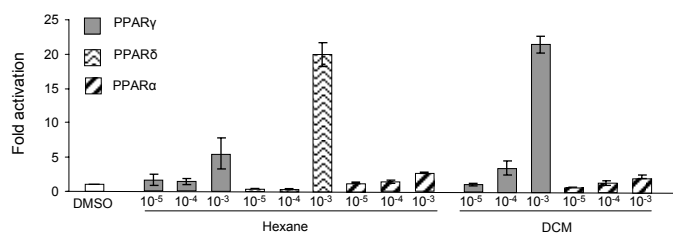
## Identification of bioactive compounds from elderflowers

- 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



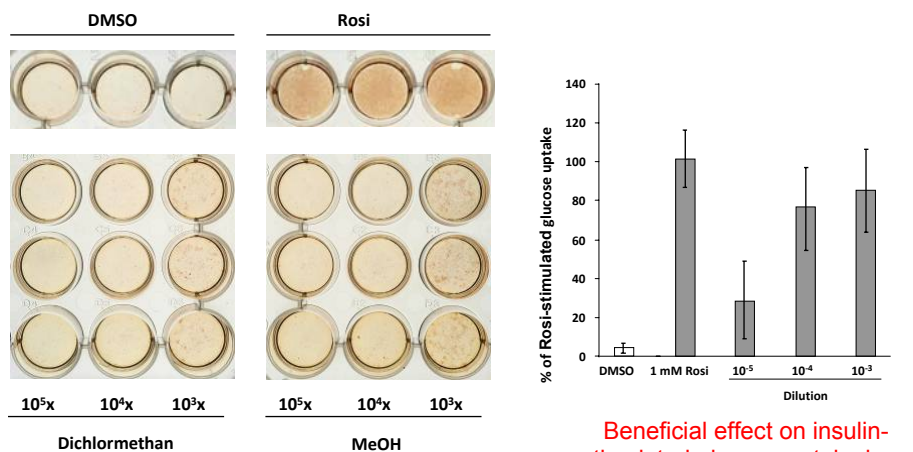
A study on aq. extracts of elderflowers showed that they exhibit insulin-like and insulin-releasing actions *in vitro*. However, the bioactive metabolites were not identified.

## Activation of PPARs



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

## Effect on adipocyte differentiation and glucose uptake



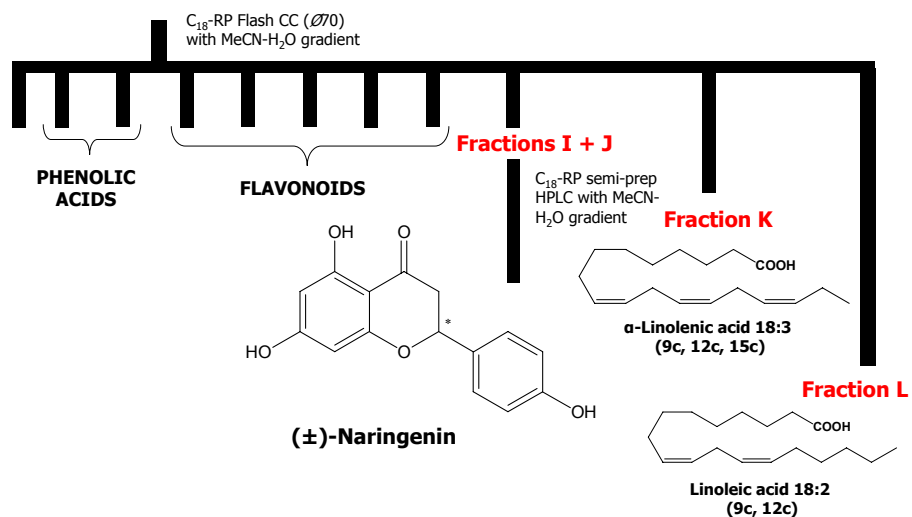
Slight stimulation of adipocyte differentiation by extracts of elderflowers

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

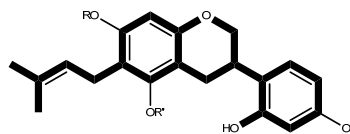
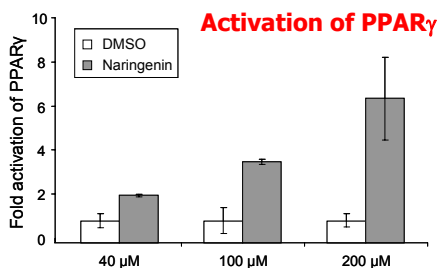
KB Christensen *et al.* (2009) *Phytother. Res.*

## Isolation of bioactive metabolites

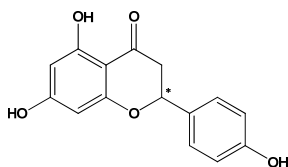
### Methanolic extract of elderflowers



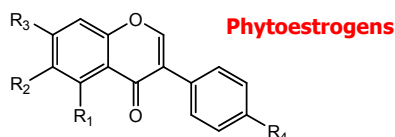
## Activation of PPAR $\gamma$ by naringenin



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

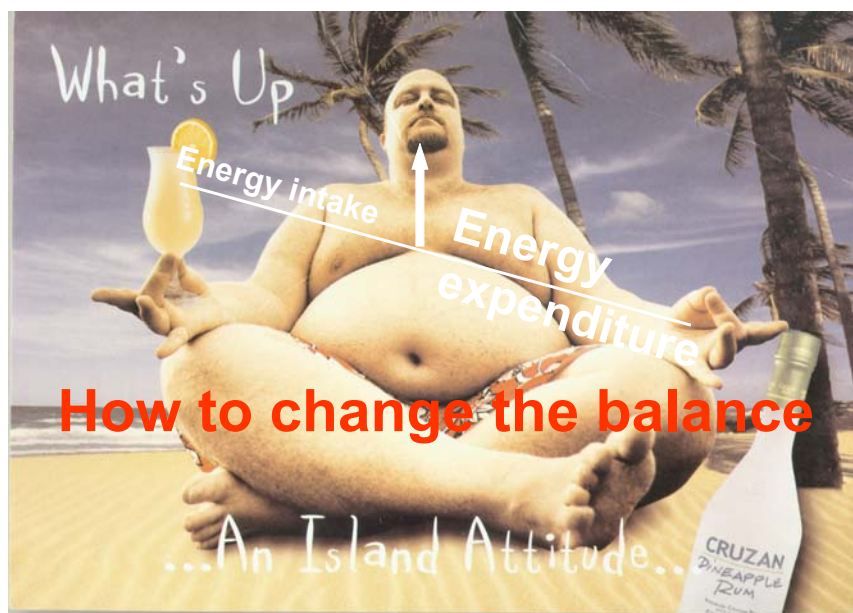


**Naringenin**



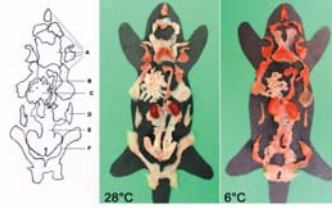
**Genistein:** R<sub>1</sub> = OH, R<sub>2</sub> = H, R<sub>3</sub> = OH, R<sub>4</sub> = OH  
**Daidzein:** R<sub>1</sub> = R<sub>2</sub> = H, R<sub>3</sub> = OH, R<sub>4</sub> = OH  
**Biochanin A:** R<sub>1</sub> = OH, R<sub>2</sub> = H, R<sub>3</sub> = OH, R<sub>4</sub> = OCH<sub>3</sub>

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



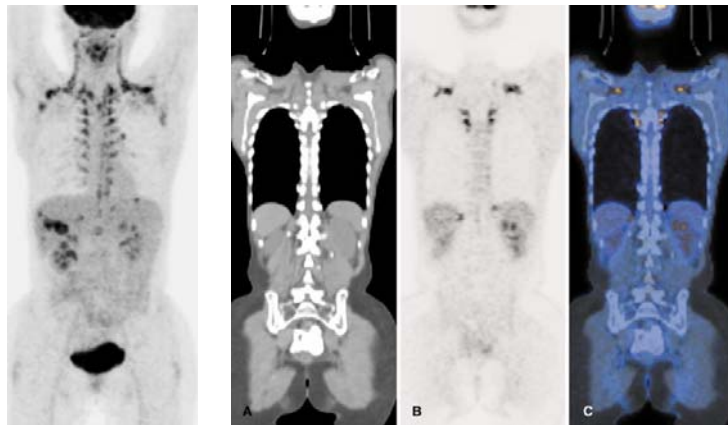
## Brown fat as a fat burner

- 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

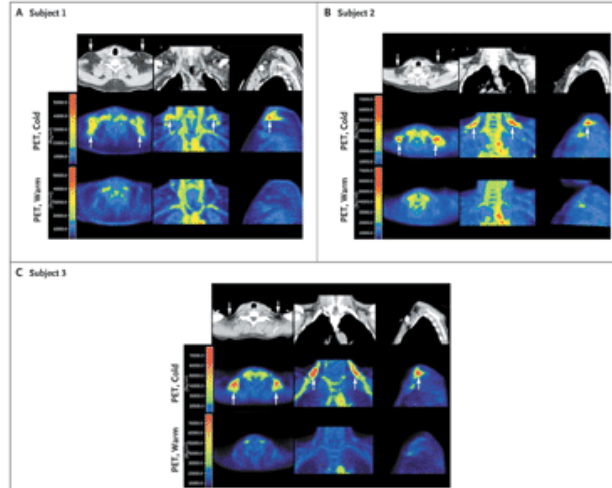
Novel evidence from positron emission tomography



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

## Functional brown fat in humans

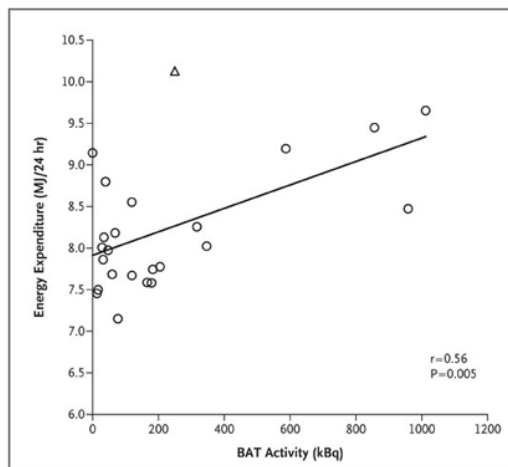
### Novel evidence from positron emission tomography



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

## Brown fat in humans

### Energy expenditure and brown fat



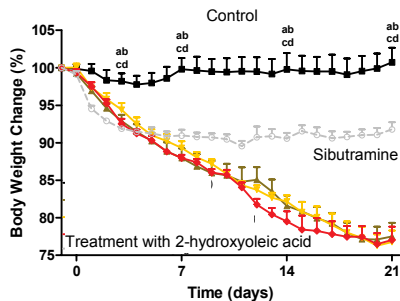
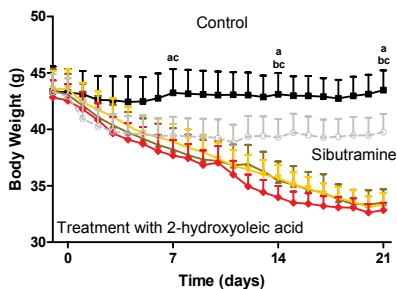
Van Marken Lichtenbelt et al. NEJM 360, 1500-1508, 2009

## The healthy Mediterranean diet: Red wine and olive oil

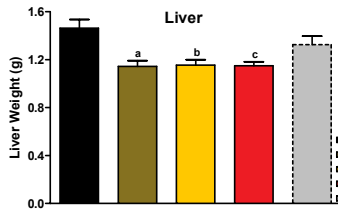
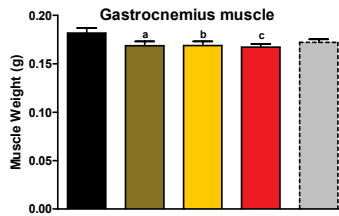
**An example of a unusual fatty acid present in sage  
2-hydroxyoleic acids  
A feeding experiment in mice**



## Body weight and body weight change

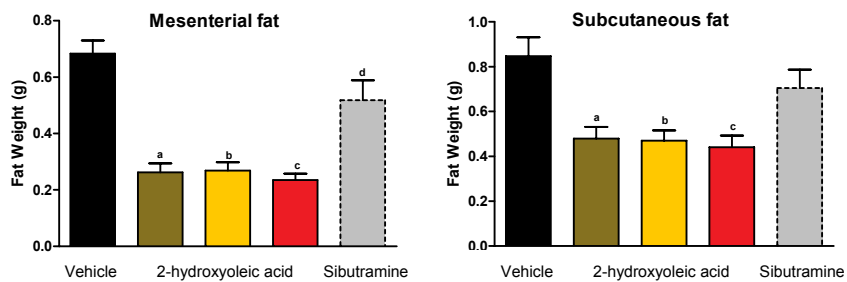


## Liver and Muscle Weight



Vehicle  
 2-hydroxyoleic acid  
 2-hydroxyoleic acid  
 2-hydroxyoleic acid  
 Sibutramine

## Fat depot weight

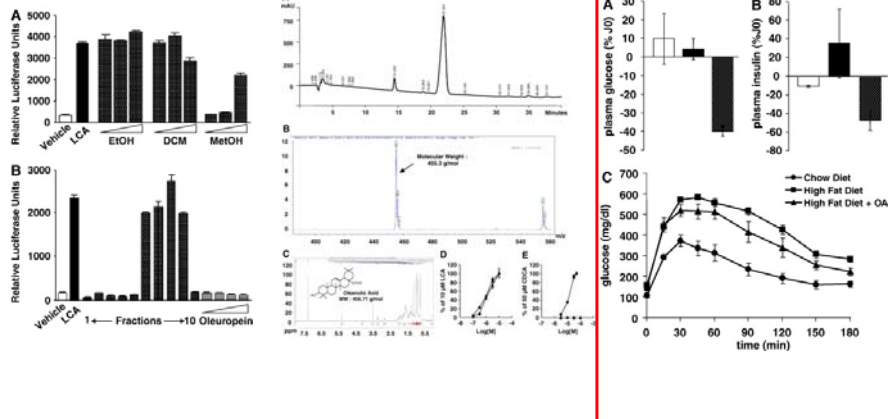


Effects in part due to conversion of white to brown adipose tissue

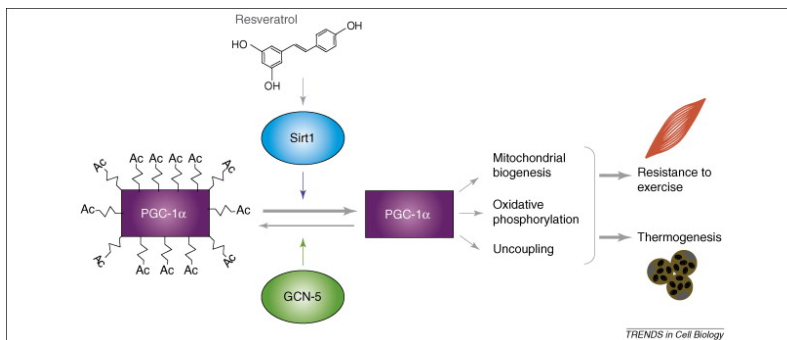
## An alternative view on the healthy Mediterranean diet

- The healthy red wine and olives

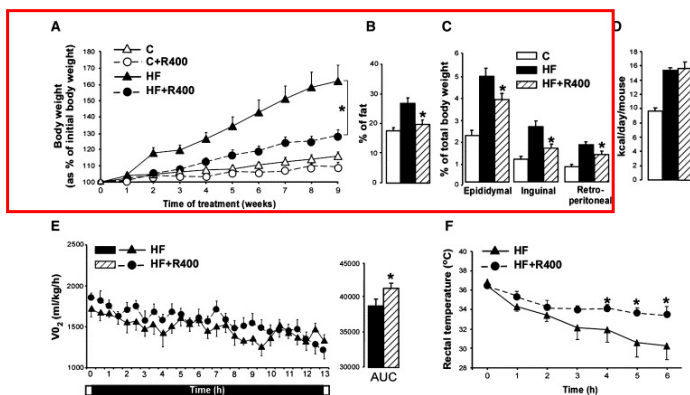
## Oleanolic acids: A bioactive compound from olive in the combat against obesity: Activation of a GPCR



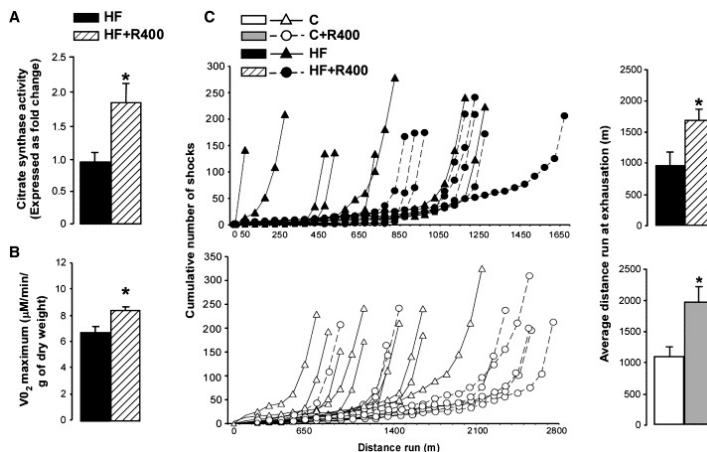
## Red wine in the struggle against obesity: Resveratrol



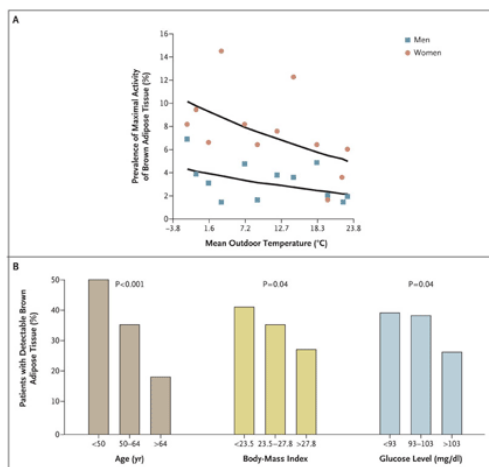
## Red wine in the struggle against obesity: Resveratrol



## 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

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**Thank you**