Phytoestrogens in food

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‘Avoid soya if you want a baby’

- Last weeks headline

Presentation content

- A historical overview of the impact of phytoestrogens
- CSL clinical research (underway)
- CSL food research (completed)

Silphium

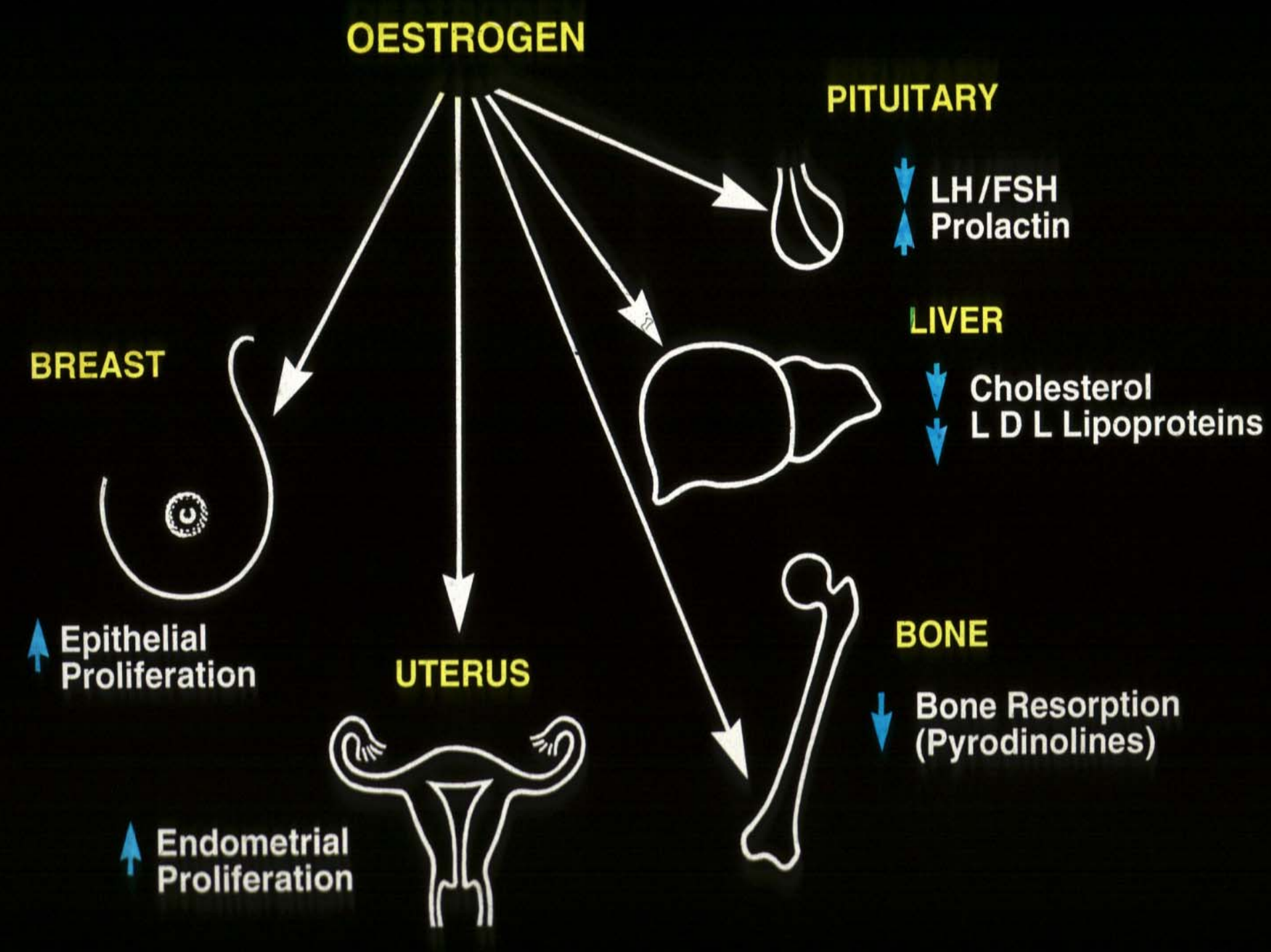
Silphium

- Greeks - 5th or 4th century B.C.
- Related to giant fennel
- Juice - the most effective birth-control methods known at the time - Abortifacient
- Grew on the coastal plateaus near the ancient city of Cyrene (Libya)
- To useful - Now extinct

Queen Anne's Lace
Daucus carota - Wild Carrot

Queen Anne's Lace
Daucus carota - Wild Carrot

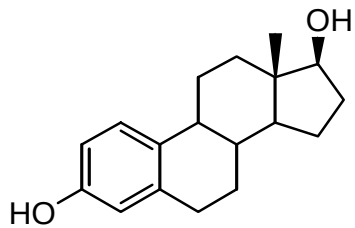
- Animal studies have shown extracts disrupt implantation
- A fertilized egg that has implanted will be released
- Terpenoids in seed block progesterone synthesis in pregnant animals


OESTROGEN
PITUITARY
LH/FSH
Prolactin
LIVER
Cholesterol
L D L Lipoproteins
BREAST
Epithelial
Proliferation
UTERUS
Endometrial
Proliferation
BONE
Bone Resorption
(Pyrodinolines)

Phytoestrogen effects in Humans

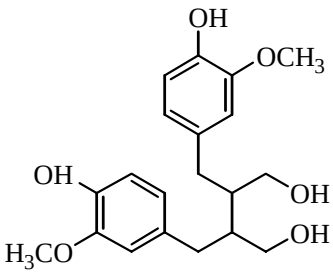
- Cardiovascular disease
- Cancer – Breast/Prostate
- Menopause -HRT
- Osteoporosis – bone density
- Inhibits Aldehyde DH, <EtOH consumption

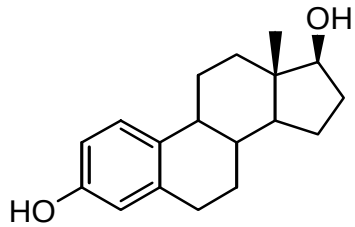
Sources and Classification of Dietary Estrogens



- Dietary Estrogens
  - Naturally occurring
    - Ovarian Steroids (Estradiol) Meat
    - Phytoestrogens
      - Isoflavonoids
        - Isoflavones (Genistein) Soya
        - Coumestans (Coumestrol) Alfalfa
      - Lignans (Seco) Flax
      - Chromene (Miroestrol) Kwao Keur
      - Prenylnaringenins (8-PNG) Hops/Beer
      - Phytosterols (sitosterol) Vegetable oils
      - Stilbenes (Resveretrol) Red wine/Grapes
      - Flavanoids (Apigenin) Orange juice
      - Terpene Glycosides (Actein) Black Cohosh
    - Mycoestrogens (Zearelenone) Grain
  - Synthetic Contaminants
    - Xenoestrogens Endocrine Disruptors (DDT/Phthalates) Packaging
    - Growth Promoters (Diethylstilbesterol) American Beef

Sources and Classification of Dietary Estrogens





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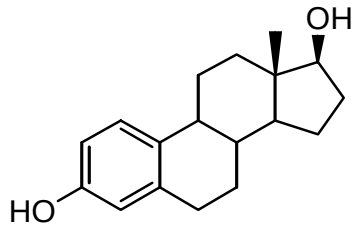

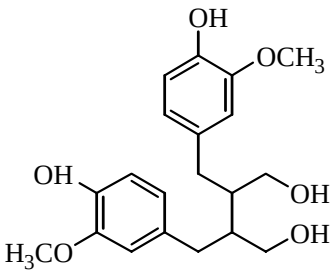

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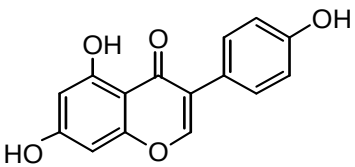

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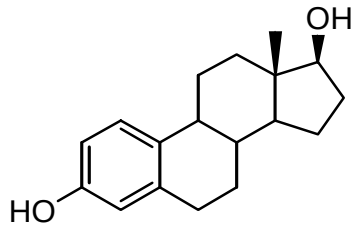

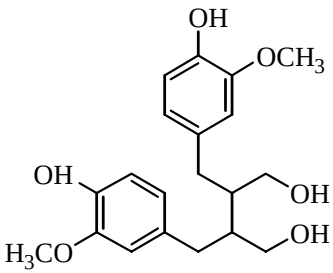

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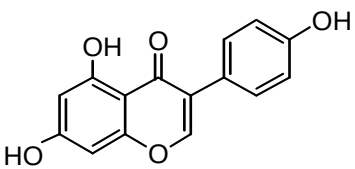
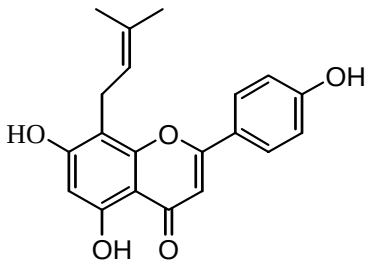
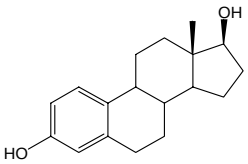


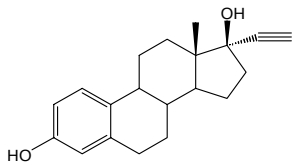


Estrogens

Steroidal estrogens

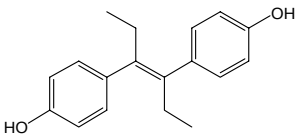


B-Estradiol

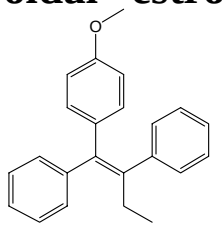


17A-ethynylestradiol

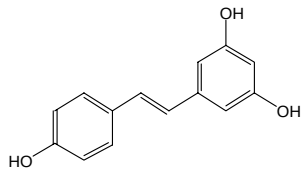
Non - steroidal estrogens (Stilbenes)



Diethylstilbestrol
Veterinary residues

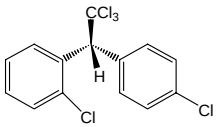


Tamoxifen
Cancer drug

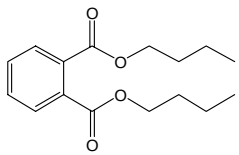


trans-resveratrol
Wine

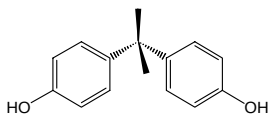
Estrogen distruptors



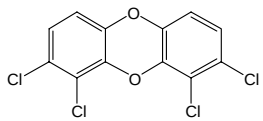
o,p-DDT
Pesticides



Dibutyl phthalate
Phthalates



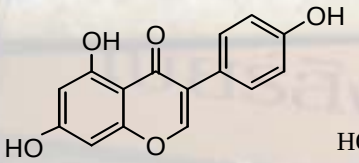
Bisphenol A
Packaging



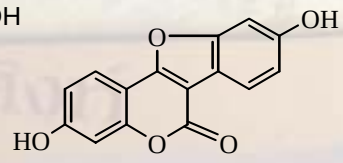
Tetrachlorodibenzodioxin (TCDD)
Dioxins

Pueraria miricica

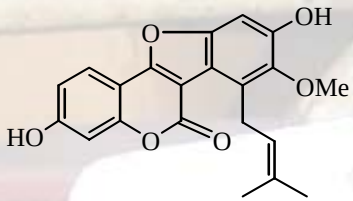
Pueraria miricica



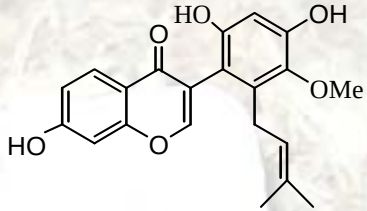
Genistein



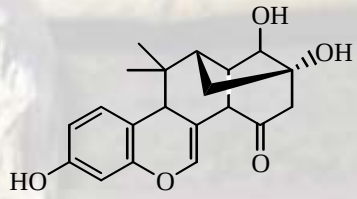
Coumestrol



Mirificoumestan



Kawakhurin



Deoxymiroestrol

- **Rejuvenation wonder drug**
- **“Fountain of youth”**
- **Alleviate/Reverse symptoms of aging**
  - **Lowering of estrogen levels**
- **Sagging breast, wrinkled skin, bone loss, grey hair, boost memory**
- **Increase energy, vigor, body movement**

Time line

- 1923 Doisey bioassay
  - A spayed mouse is injected, cornified vaginal cells
- 1926 Estrogenic compounds n plants
  - Several hundred plant species
- 1930's **Sand + Sub + Super = Pasture**

Soay Sheep

Time line

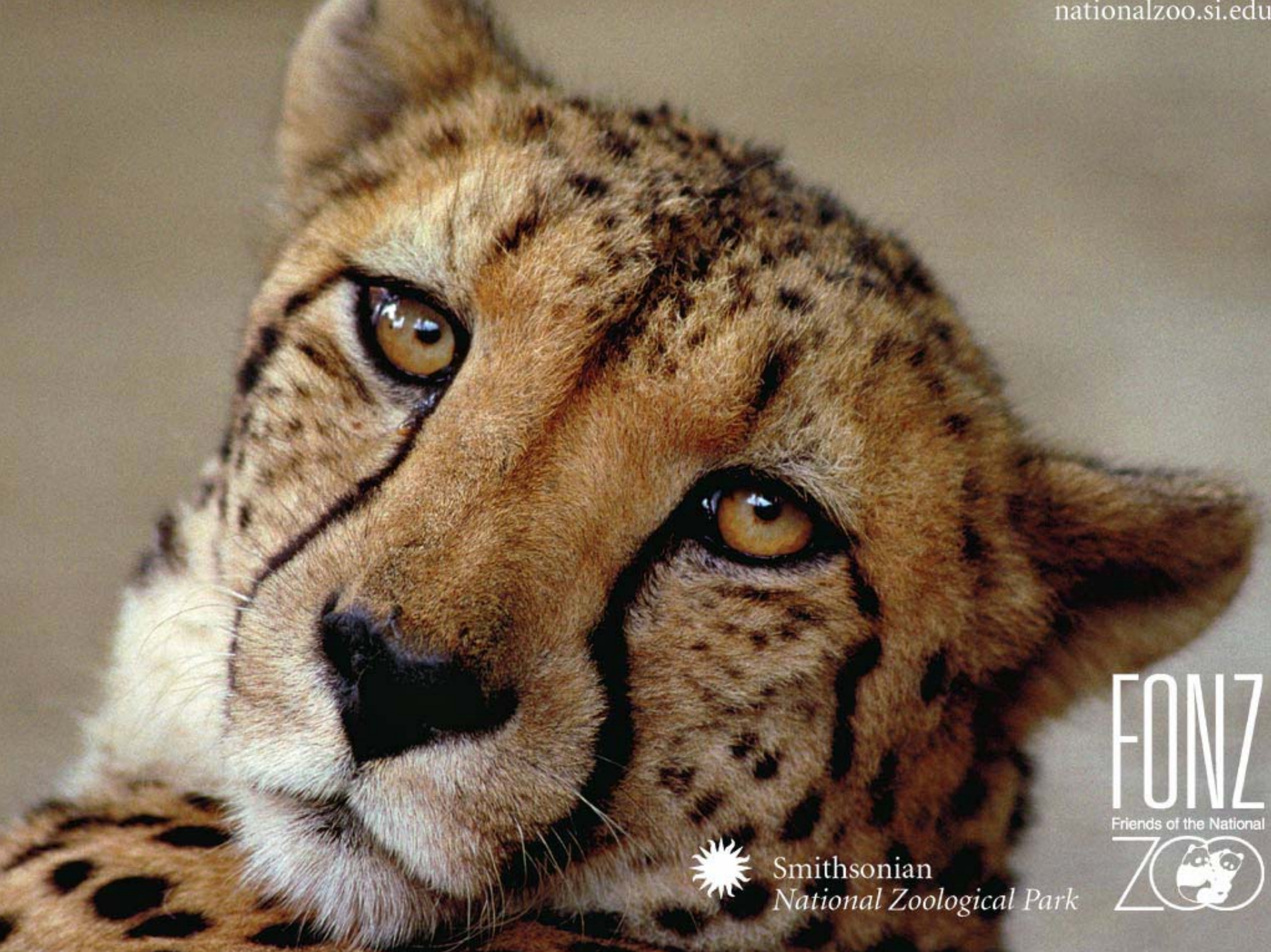
- 1930's Sand + Sub + Super = Pasture
- 1940's Scientific curiosity -until infertility of sheep

  -Strains of Mediterranean subterranean clover estrogenic -"Clover disease" lambing rate of 15%
- 1954 Genistein (isoflavone) - clover
- 1963 Genistein, daidzein, formononetin, coumestrol

Californian Quail Reproductive Cycle

- Desert - legumes and Subterranean clover = phytoestrogens
- Drought, more dry matter >conc phyto's -Reduced fertility
- Rains <conc phyto's
- Variety of non-phyto food
- Increased fertility
- Biological feedback loop

-the plants controlling the herbivores


nationalzoo.si.edu
Smithsonian
National Zoological Park
FONZ
Friends of the National
ZOO

Cheetah "racing towards extinction“

- Its survival will probably depend on accelerated captive breeding
- Reproductive failure and liver disease threaten North American captive cheetah population
- 1987 Setchell K. D. R.
- Daidzein and genistein ca 50 mg/day from a soybean product in cheetah diet
- Withdraw of “soya feline diet” & substitution with chicken resulted in an improvement in liver function

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‘Avoid soya if you want a baby’

The expert's opinions

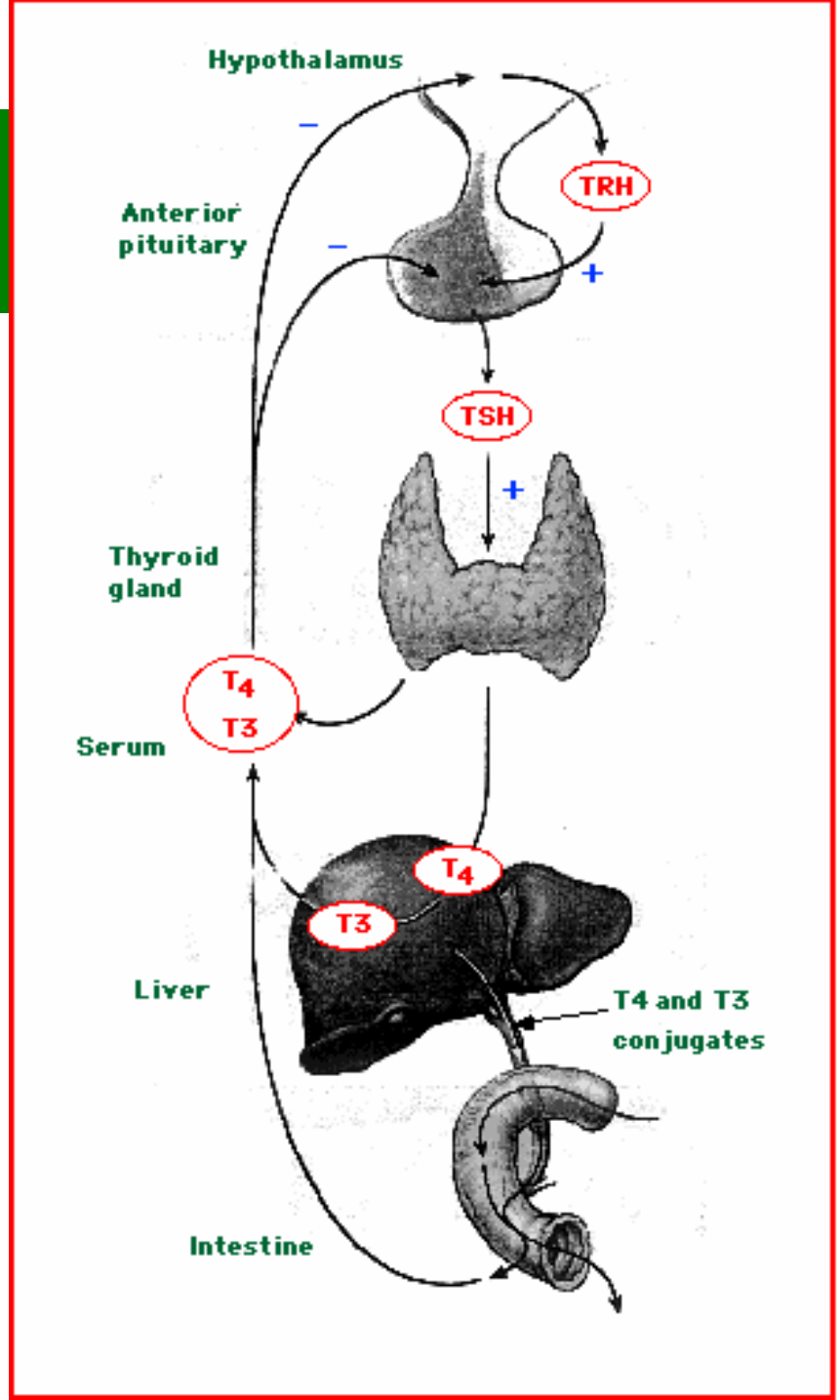
- ….. there is no direct evidence for the beneficial effects of phytoestrogens in humans. ... All information is based on consumption of phytoestrogen-rich diets, and the causal relationship and the mechanisms of phytoestrogen action in humans still remain to be demonstrated ... In addition, the possible adverse effects of phytoestrogens have not been evaluated ... It is plausible that phytoestrogens, as any exogenous hormonally active agent, might also exhibit adverse effects on the endocrine system, i.e. act as endocrine disrupters ....

  [FDA soy protein health claim 1999]

- UK Expert Committee COT 2003

  …..Recommends research to monitor plasma thyroxine levels of children and adults with hypothyroidism who consume large quantities of dietary phytoestrogens

Thyroid Function



Pathways of thyroid hormone metabolism

Thyrotropin-releasing hormone (TRH) increases the secretion of thyrotropin (TSH), which stimulates the synthesis and secretion of trioiodothyronine (T3) and thyroxine (T4) by the thyroid gland. T3 and T4 inhibit the secretion of TSH, both directly and indirectly by suppressing the release of TRH. T4 is converted to T3 in the liver and many other tissues by the action of T4 monodeiodinases. Some of the T4 and T3 is conjugated with glucuronide and sulfate in the liver, excreted in the bile, and partially hydrolyzed in the intestine. Some of the T4 and T3 formed in the intestine may be reabsorbed. + = stimulatory pathway; – = inhibitory pathway. Drug interactions can occur at any of these sites. (Reprinted with permission from Surks, MI, Sievert, R, N Engl J Med 1995; 333:1688. Copyright 1995 Massachusetts Medical Society. All rights reserved)

Soy Hypothryoidism
UK FSA, CSL, University of Hull

- Soy phytoestrogens affect thyroid function in both animals and in humans
- Changes considered too small in magnitude to be physiologically important in subjects with normal thyroid function
- The effect of soy in patients whose thyroid function is already compromised may be clinically important
- This project will determine if isoflavones have an effect on subjects with compensated hypothyroidism.

  -TSH is elevated, but thyroxine and tri-iodothyronine levels are normal

  -This will be achieved by a cross over, double blind, placebo controlled trial involving 134 patients

Phytoestrogens and Food

Total Diet Survey (TDS)

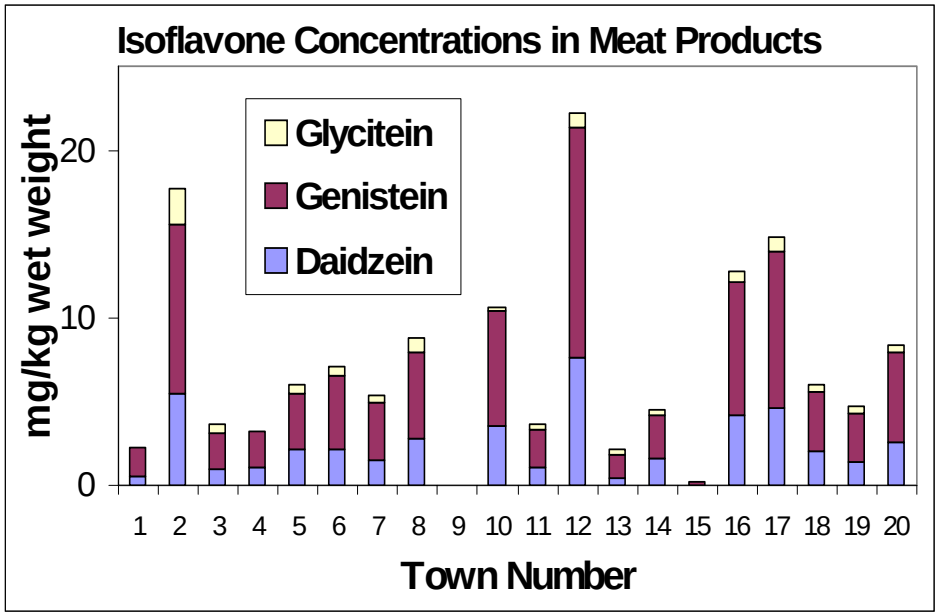
Food Group	Food Items Sampled
1	Bread
2	Miscellaneous cereals
3	Carcass meat
4	Offal
5	Meat products
6	Poultry
7	Fish
8	Oils and fats
9	Eggs
10	Sugars and preserves
11	Green vegetables
12	Potatoes
13	Other vegetables
14	Canned vegetables
15	Fresh fruit
16	Fruit products
17	Beverages
18	Milk
19	Dairy products
20	Nuts

Isoflavone Daily Intake Calculation

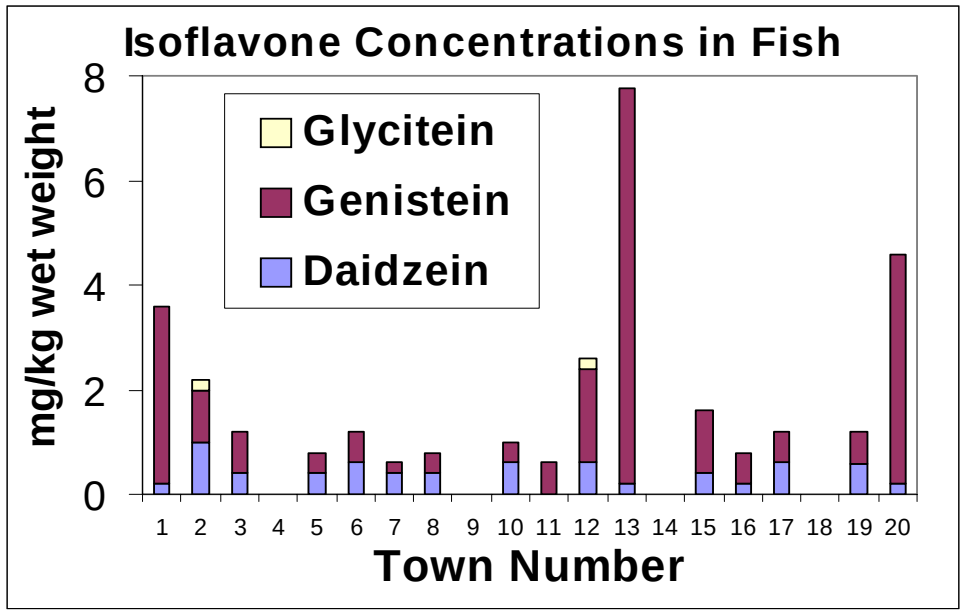
Food Group	Food Items Sampled	Food Intake g day^{-1}	Mean Level in Food mg kg^{-1}	Total in Food mg	Daily Intake mg
			Genistein	Genistein	
1	**Bread**	**108**	**17.1**	**1.85**	**2.42**
5	**Meat products**	**47**	**4.4**	**0.21**	**0.34**
2	Cereals	106	0.2	0.02	0.06
13	Other vegetables	77	0.5	0.04	0.05
16	Fruit products	45	0.2	0.01	0.05
18	Milk	278	0.05	0.01	0.03
7	Fish	14	1.2	0.02	0.02
Total Daily Intake of These Isoflavones					**3.00 mg**

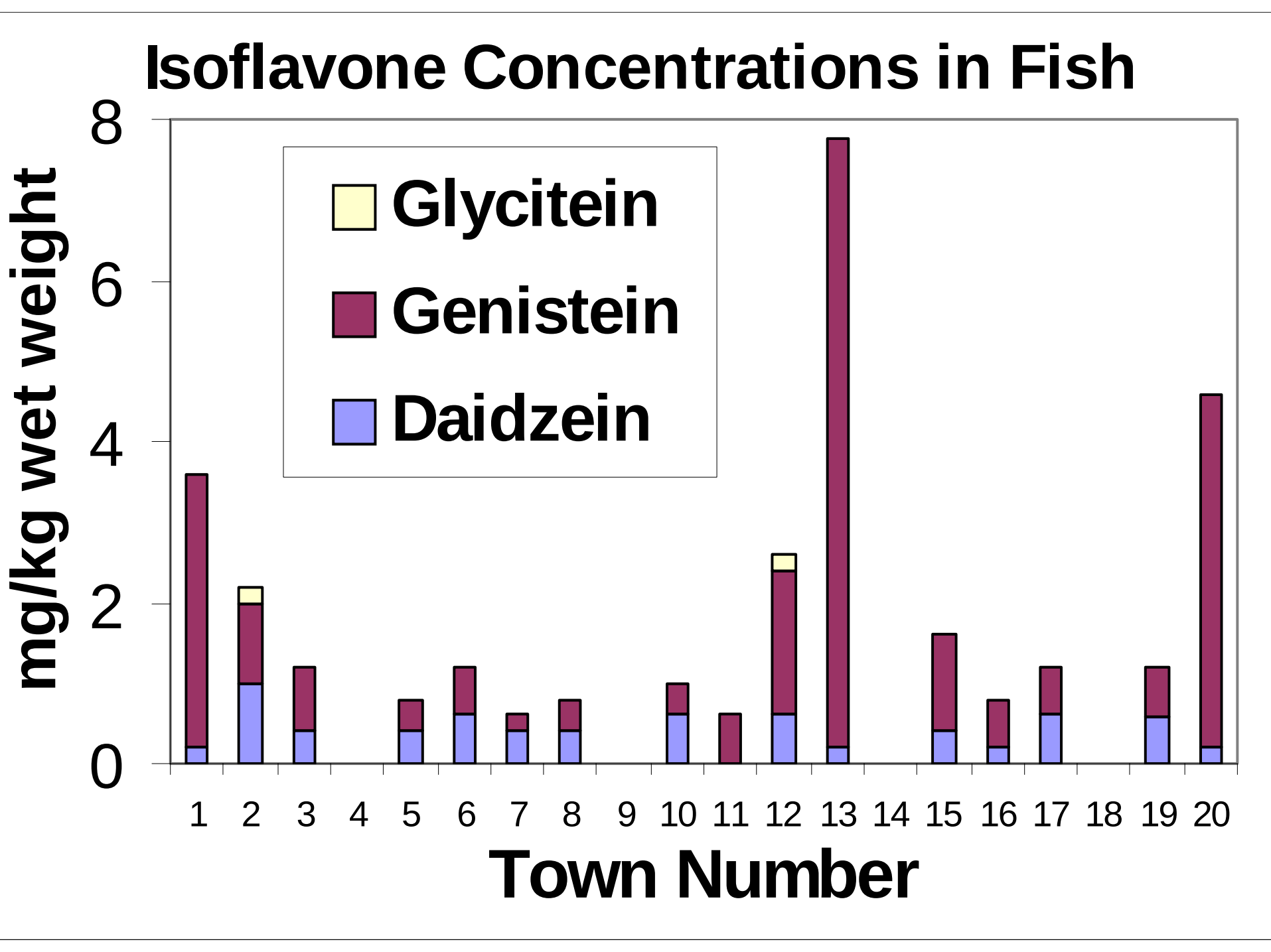
Distribution of Isoflavones

Group 1, Processed Meat Products

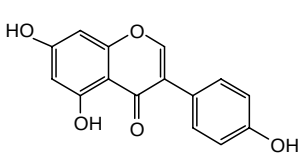


Group 5, Fish

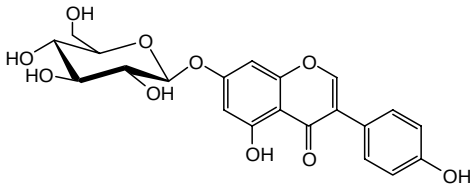



Isoflavone Concentrations in Fish
mg/kg wet weight
0
2
4
6
8
Glycitein
Genistein
Daidzein
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Town Number

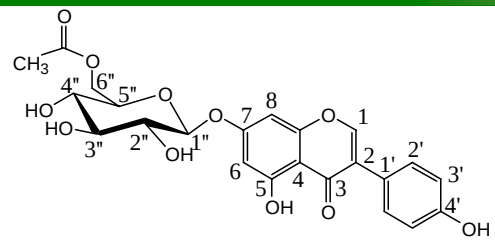
Soya - Food Conjugates



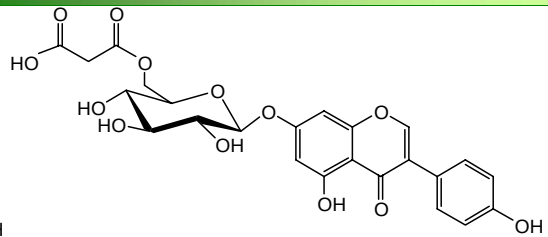
Genistein



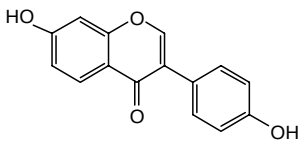
Genistin (genistein-7-*O*-glucoside)



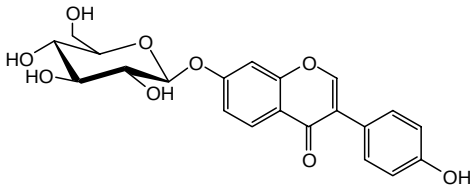
6"-*O*-Acetylgenistin



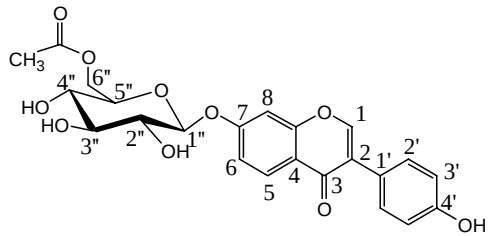
6"-*O*-Malonylgenistin



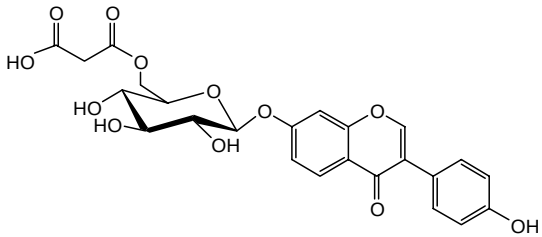
Daidzein



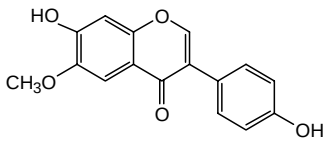
Daidzin (daidzein-7-*O*-glucoside)



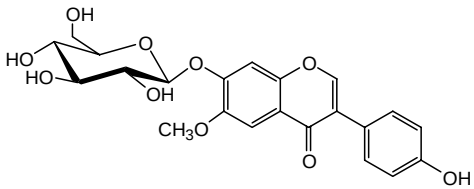
6"-*O*-Acetyldaidzin



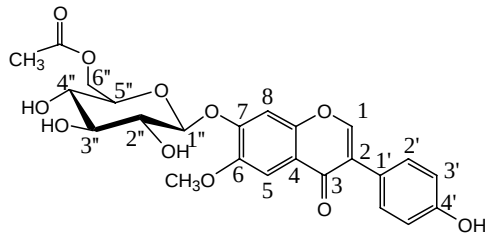
6"-*O*-Malonyldaidzin



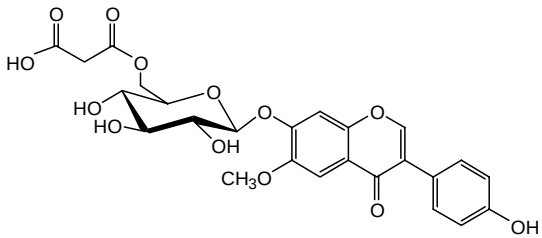
Glycitein



Glycitin (glycitein-7-*O*-glucoside)



6"-*O*-Acetylglycitin



6"-*O*-Malonylglycitin

Aglycones

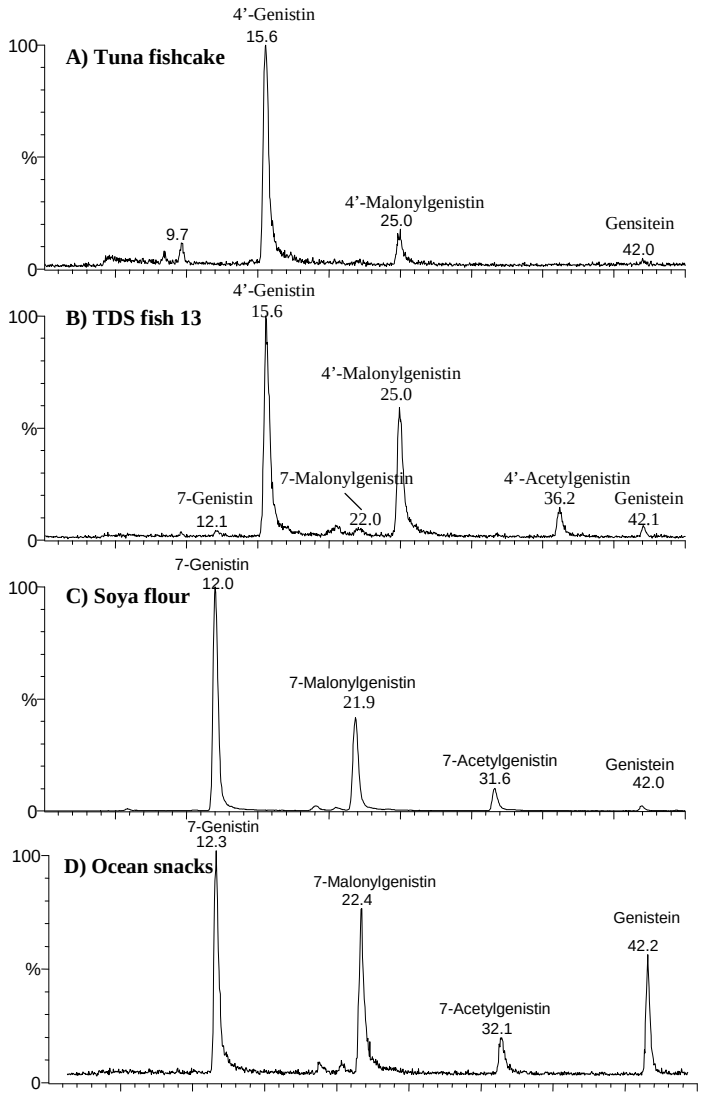
Glucosides

Acetylglucosides

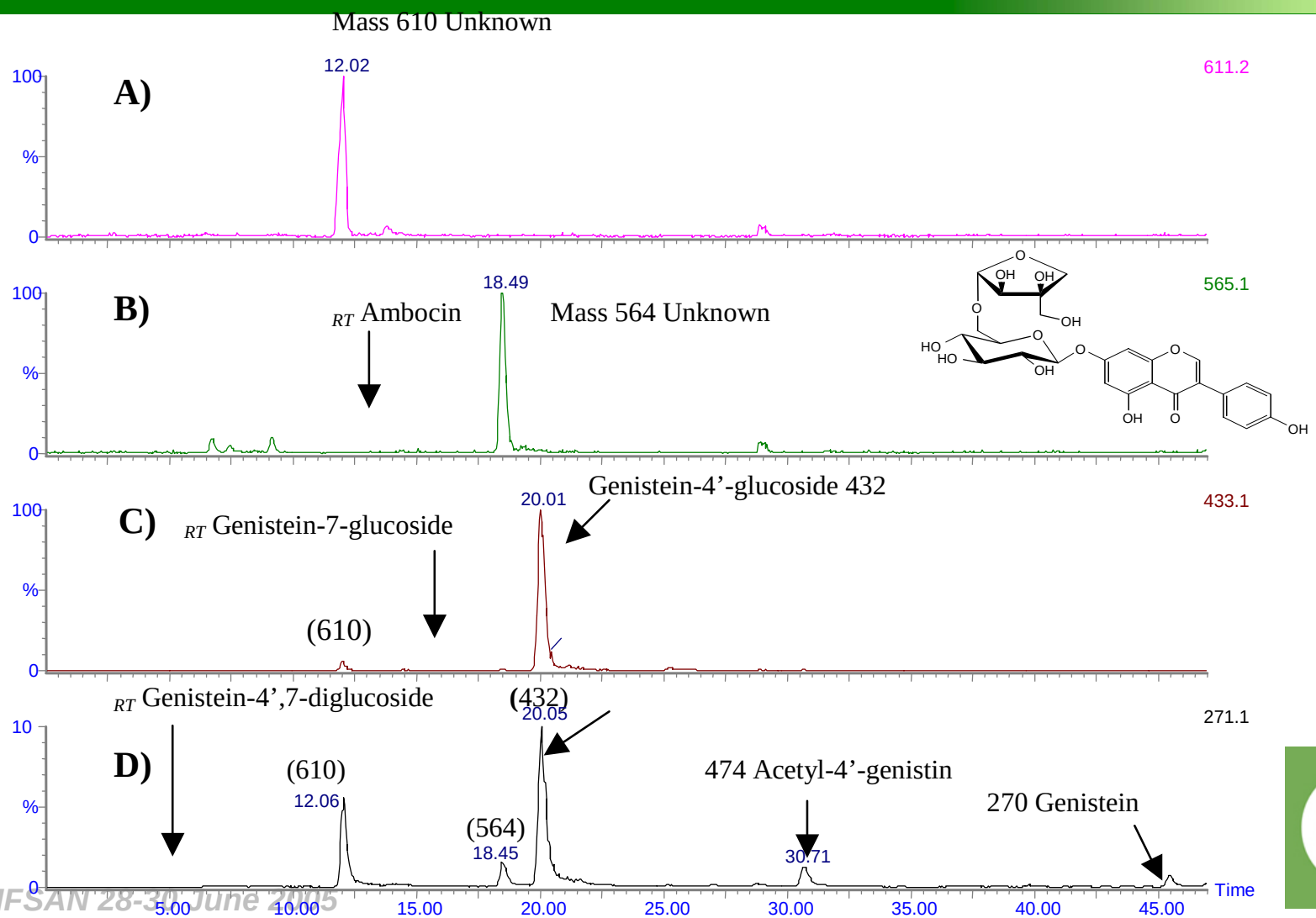
Malonylglucosides

Non-soya genistein profiles of Fish

- Only traces of soya incorporation
- Majority of components are 4’-glycoside esters
- Not from known phytoestrogen sources

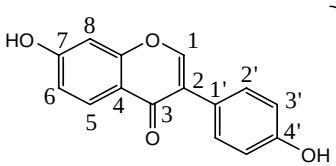


Garam Masala Extract



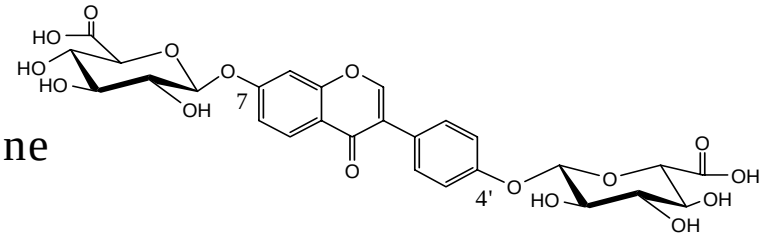
Human conjugates

Human conjugates



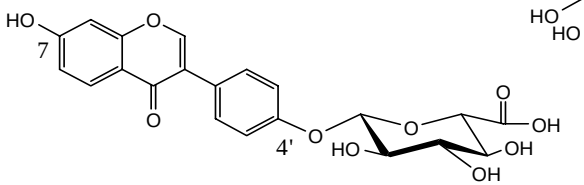
Daidzein

Aglycone

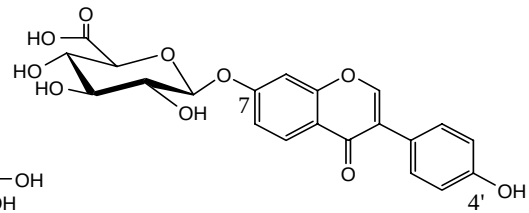


Daidzein-7,4'-diglucuronide

Diglucuronide

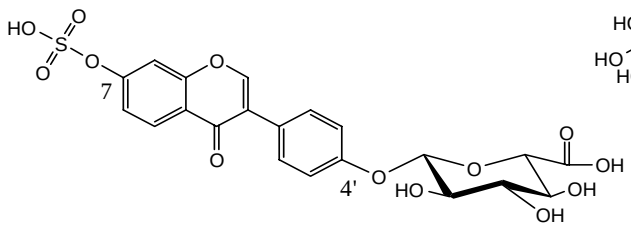


Daidzein-4'-glucuronide

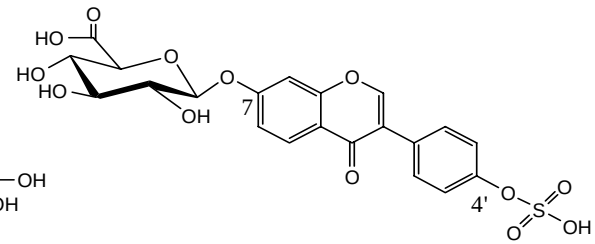


Daidzein-7-glucuronide

Glucuronides



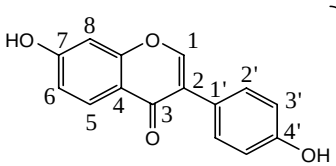
Daidzein-7-sulpho-4'-glucuronide



Daidzein-4'-sulpho-7-glucuronide

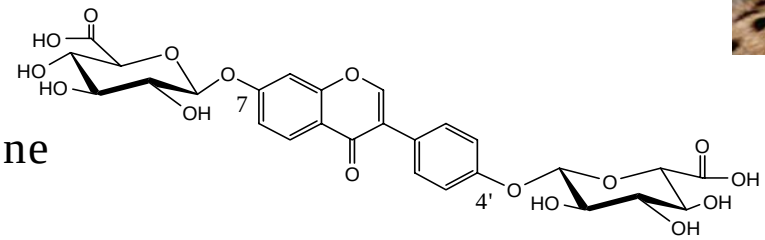
Sulphoglucuronides

Human conjugates



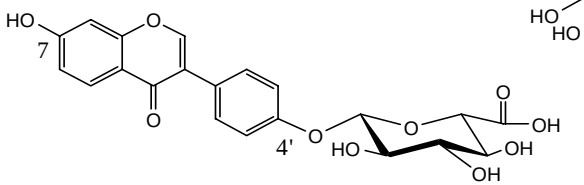
Daidzein

Aglycone

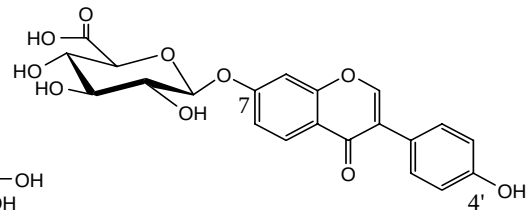


Daidzein-7,4'-diglucuronide

Diglucuronide

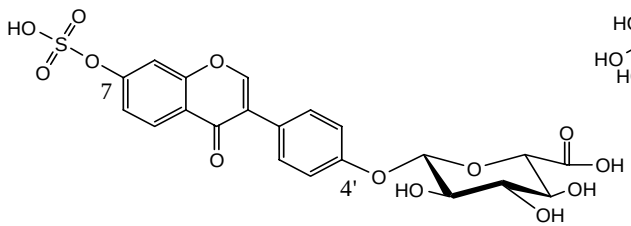


Daidzein-4'-glucuronide

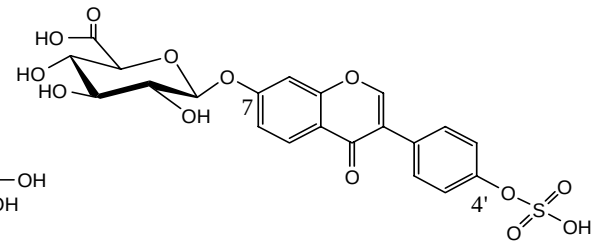


Daidzein-7-glucuronide

Glucuronides



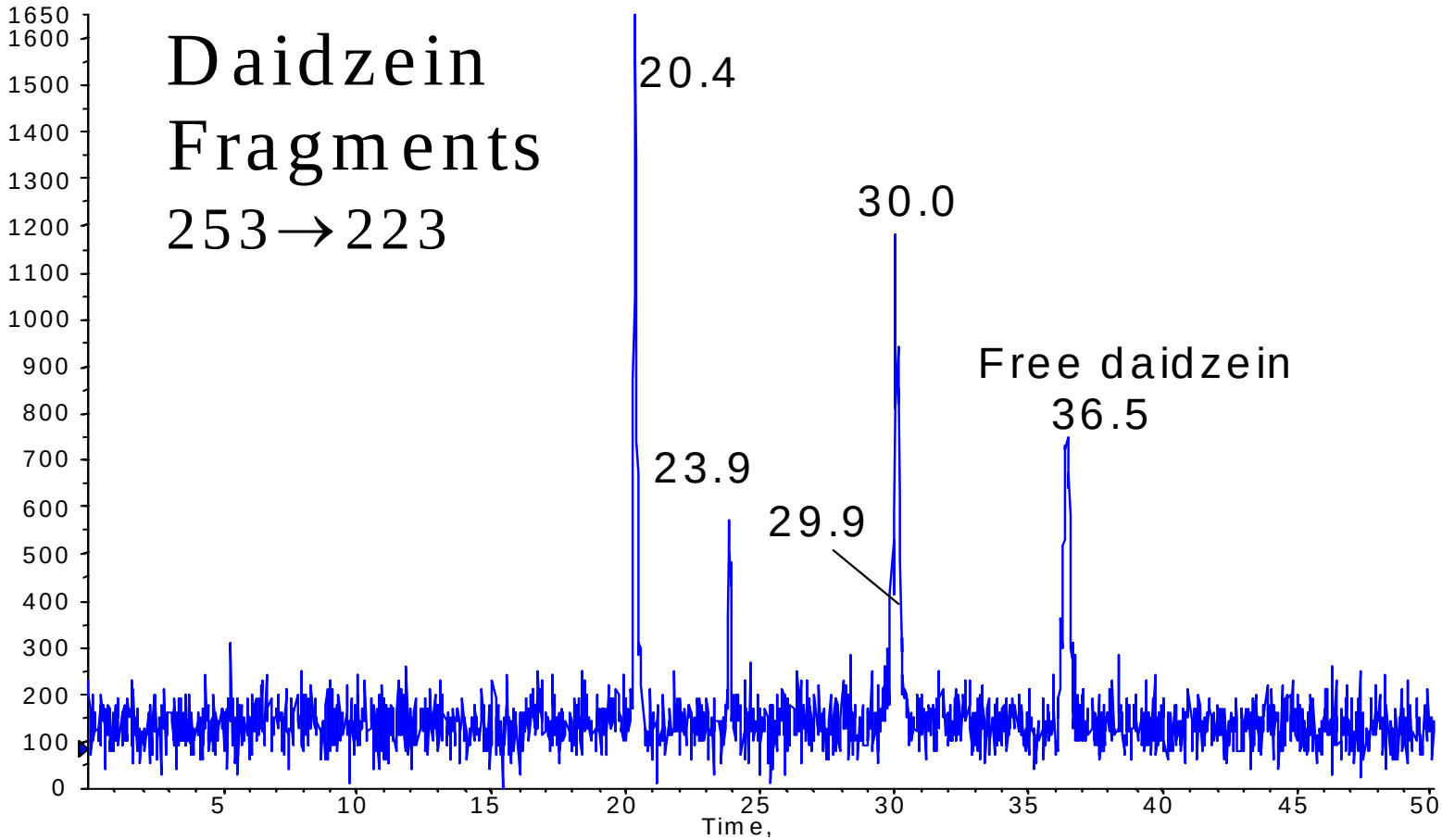
Daidzein-7-sulpho-4'-glucuronide

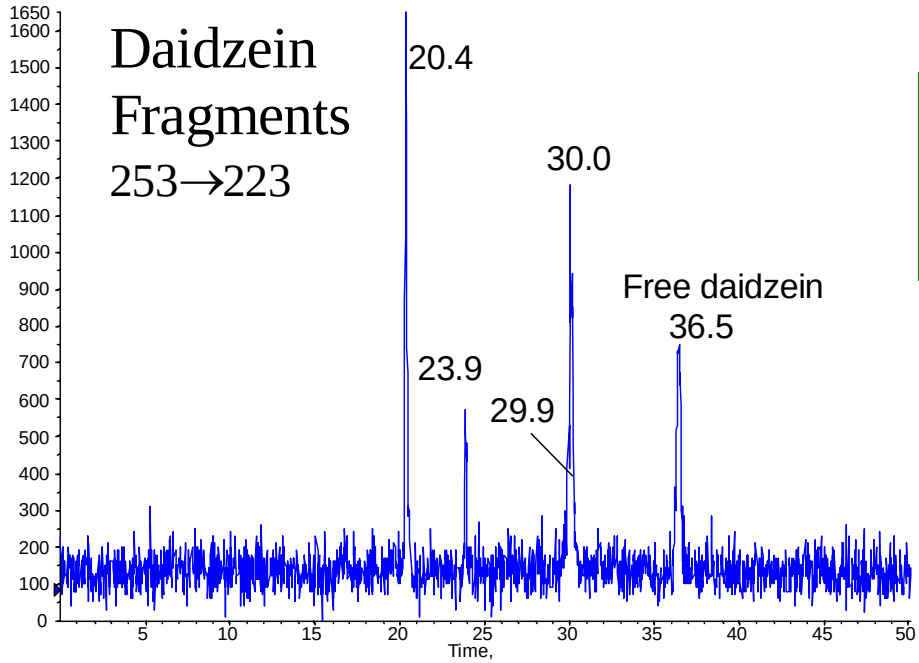


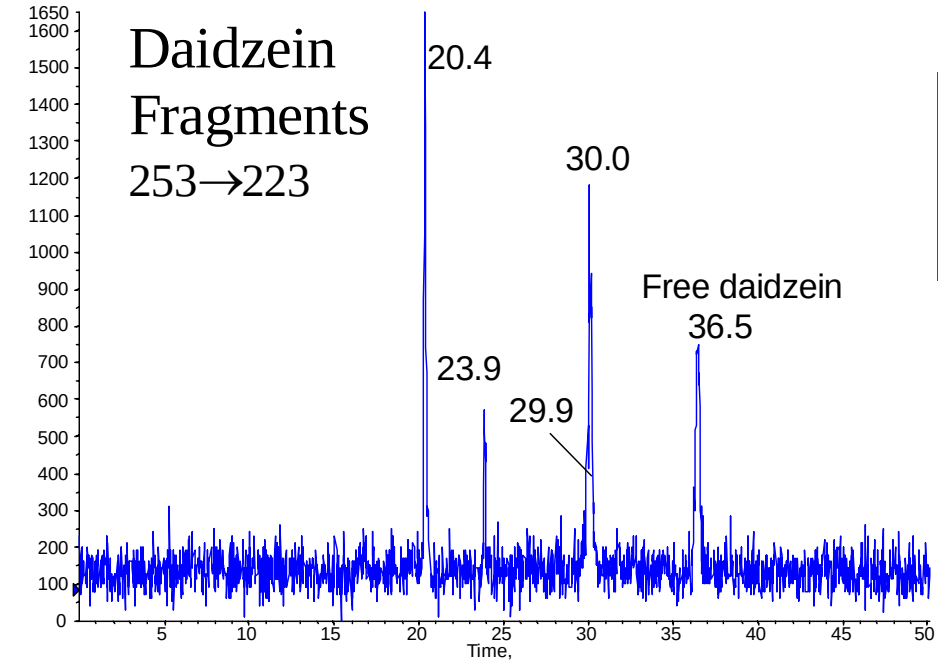
Daidzein-4'-sulpho-7-glucuronide

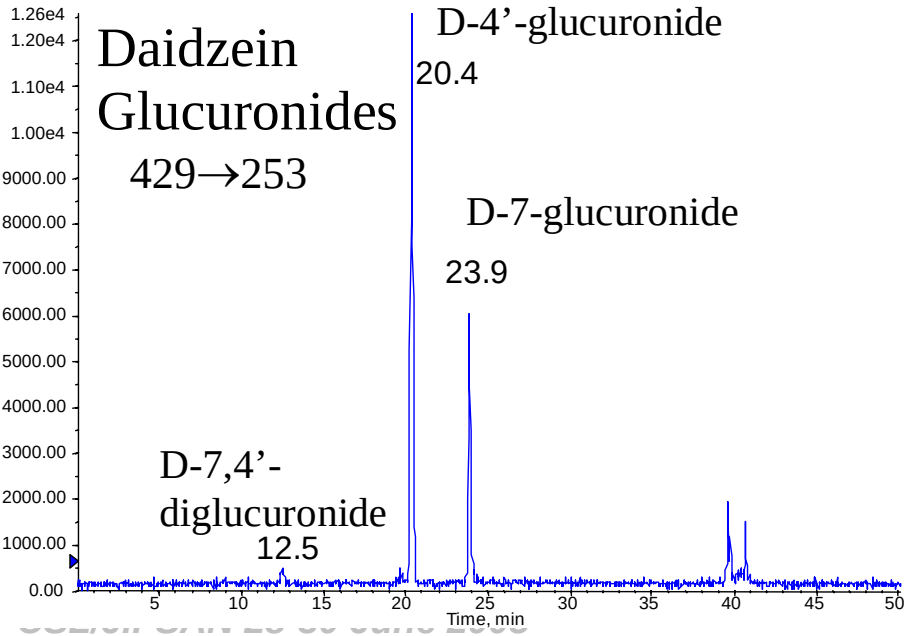
Sulphoglucuronides

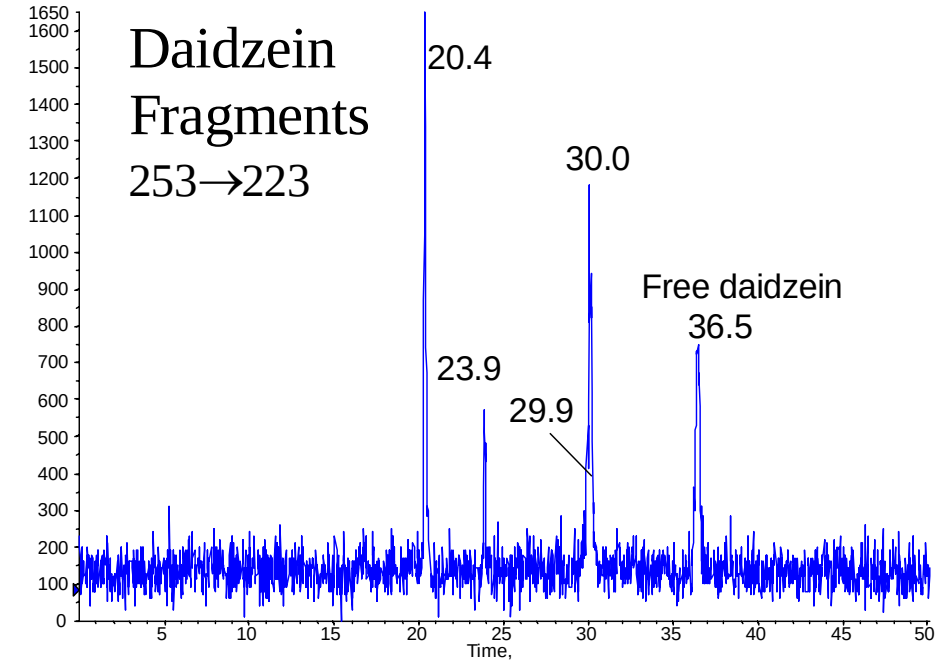
LC/MS-MS of daidzein conjugates in human urine

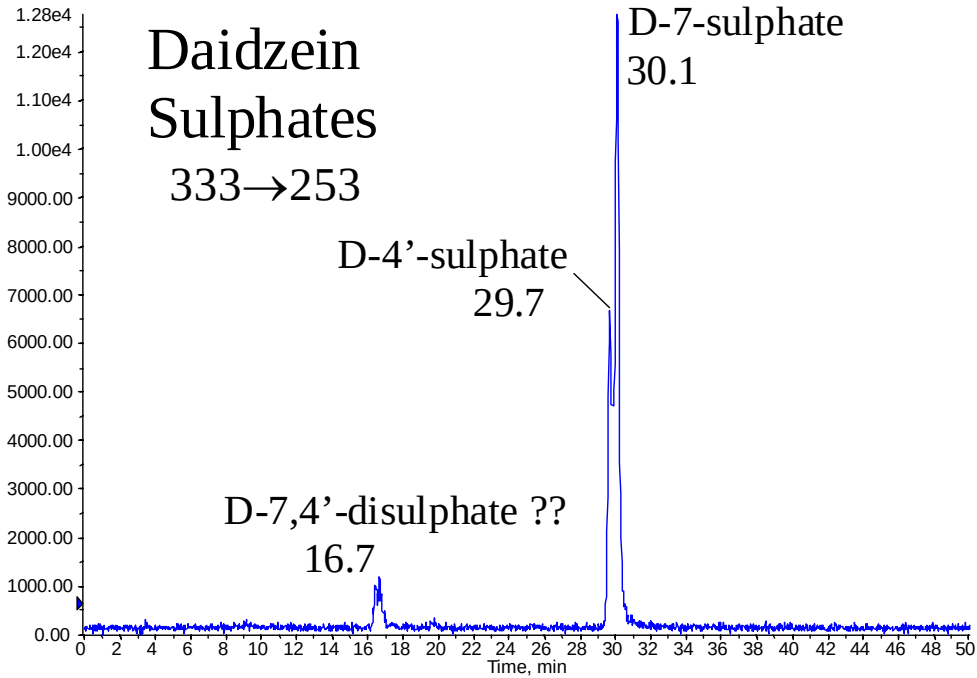


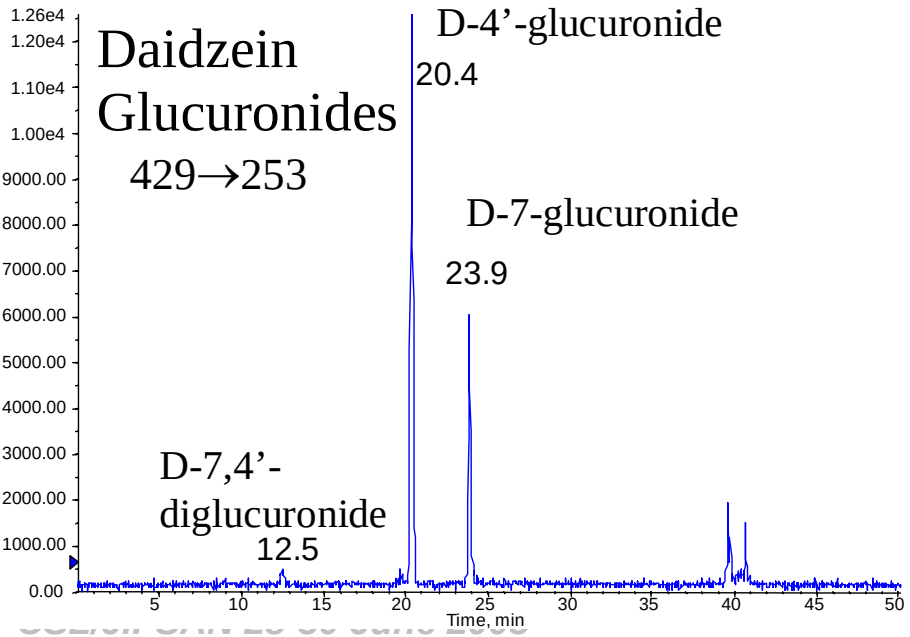

Daidzein
Fragments
253→223
20.4
30.0
Free daidzein
36.5
23.9
29.9
Time,

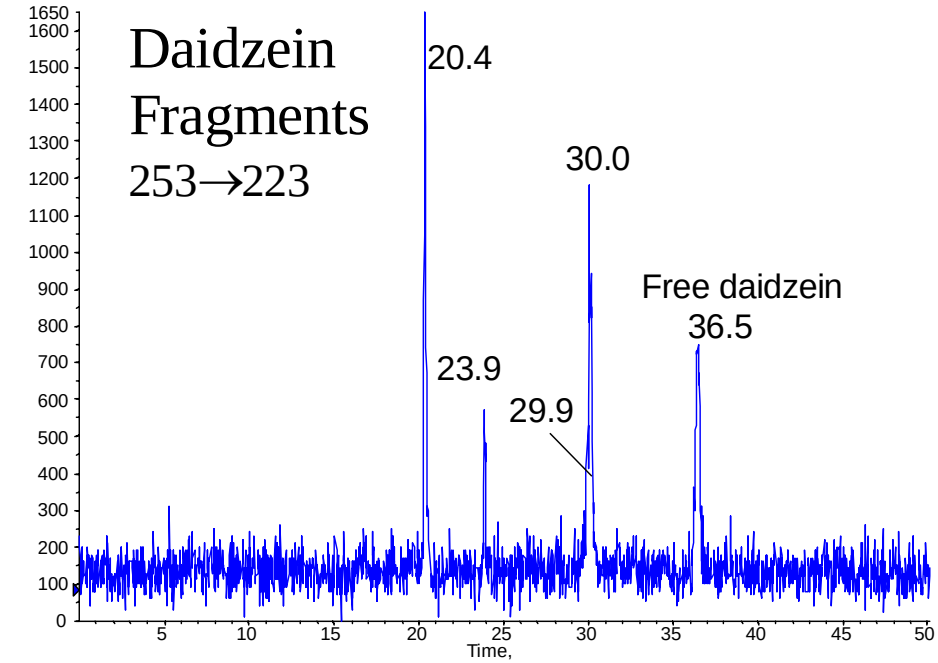

Daidzein
Fragments
253→223
20.4
30.0
Free daidzein
36.5
23.9
29.9
Time,

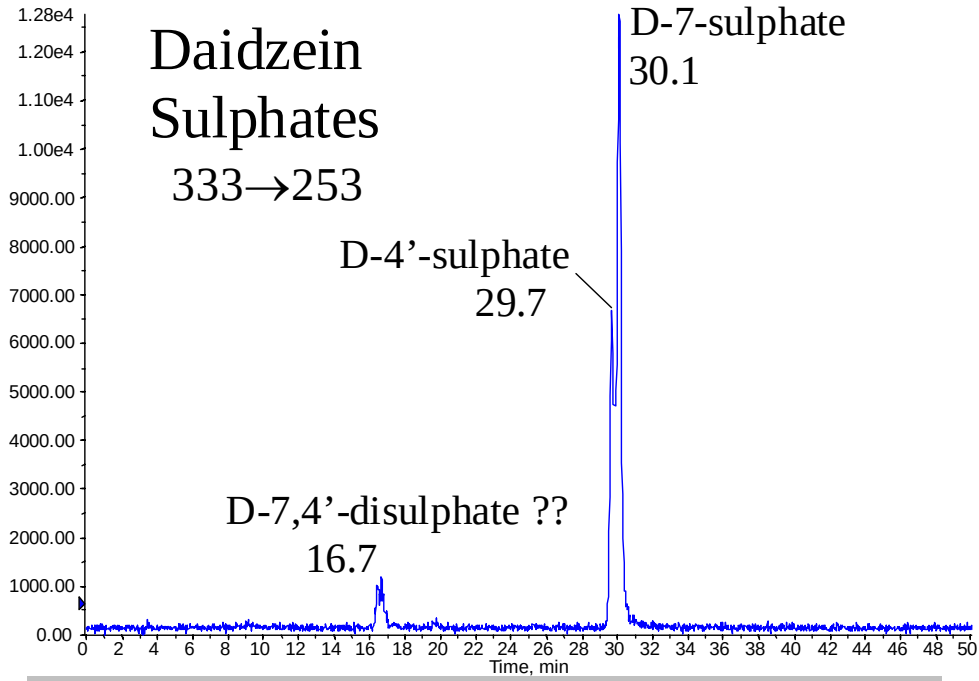

Daidzein
Glucuronides
429→253
D-4'-glucuronide
20.4
D-7-glucuronide
23.9
D-7,4'-
diglucuronide
12.5
Time, min

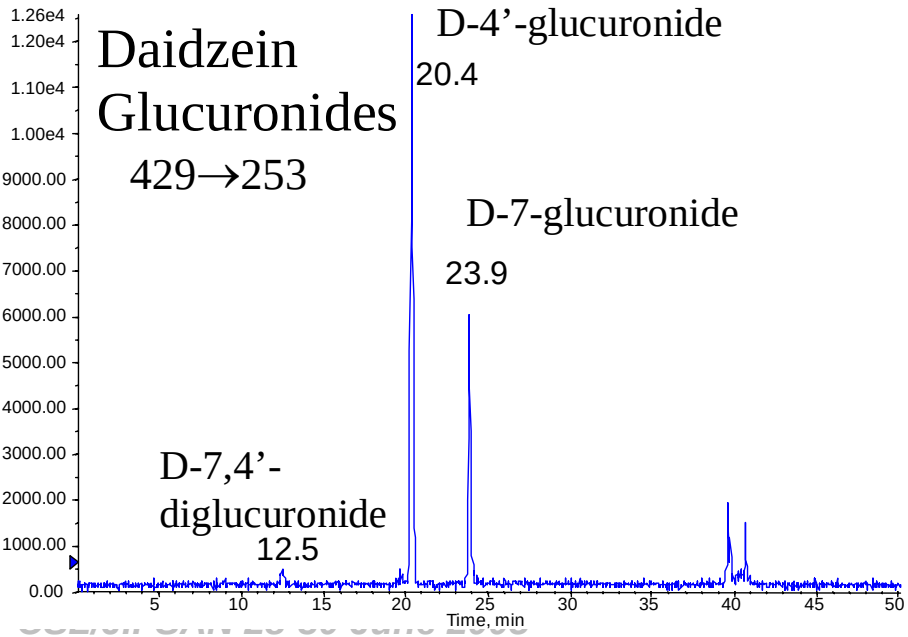

Daidzein
Fragments
253→223
20.4
30.0
Free daidzein
36.5
23.9
29.9
Time,

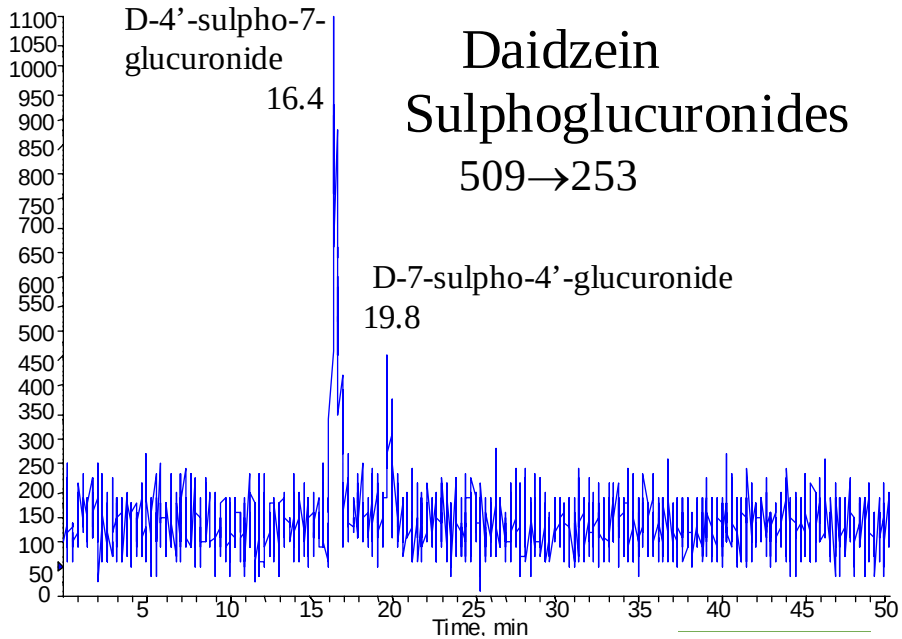

Daidzein
Sulphates
333→253
D-7-sulphate
30.1
D-4'-sulphate
29.7
D-7,4'-disulphate ??
16.7
Time, min


Daidzein
Glucuronides
429→253
D-4'-glucuronide
20.4
D-7-glucuronide
23.9
D-7,4'-
diglucuronide
12.5
Time, min


Daidzein
Fragments
253→223
20.4
30.0
Free daidzein
36.5
23.9
29.9
Time,


Daidzein
Sulphates
333→253
D-7-sulphate
30.1
D-4'-sulphate
29.7
D-7,4'-disulphate ??
16.7
Time, min


Daidzein
Glucuronides
429→253
D-4'-glucuronide
20.4
D-7-glucuronide
23.9
D-7,4'-
diglucuronide
12.5
Time, min


D-4'-sulpho-7-
glucuronide
16.4
Daidzein
Sulphoglucuronides
509→253
D-7-sulpho-4'-glucuronide
19.8
Time, min

Conclusions

- ALL the possible S/G conjugates can be found
- Can be quantified if standards supplied
- Little variation between individuals
- Little substrate competition *vs* dose level
- Same for other isoflavones

Table of Significant Values

	Daidzein			Equol			Genistein			Glycitein			*O*-DMA		
	Plasma	Urine	Faeces	Plasma	Urine	Faeces	Plasma	Urine	Faeces	Plasma	Urine	Faeces	Plasma	Urine	Faeces
Sex			*										**	**	*
Phase	***	***	***	***	**	*	***	***	***		***		***	***	***
Sex.Phase															
Mid_vs_End								.							
Diet	***	***	***	***	***	***	***	***	***		***	*	***	***	***
Sex.Mid_vs_End															
Sex.Diet						*		*		.			**	*	
Mid_vs_End.Diet															
Sex.Mid_vs_End.Diet			*												

Legend	*** Less than 0.1%	** Less than 1%	* Less than 5%	. Less than 10%	Blank otherwise

Difference in *O*-DMA between sexs

‘Avoid soya if you want a baby’

- Last weeks headline
- Genistein in womb could hamper conception
- Sperm sabotaged
- Genistein kickstarts fertilisation step too soon cAMP
- At levels seen in blood
- But is it present?

Conclusions avoid soya