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
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

Mycoestrogens (Zearelenone) Grain

Xenoestrogens Endocrine Disruptors (DDT/Phthalates) Packaging

Growth Promoters (Diethylstilbesterol) American Beef

Isoflavonoids

Lignans (Seco) Flax

Chromene (Miroestrol) Kwao Keur

Prenynaringenins (8-PNG) Hops/Beer

Phytosterols (sitosterol) Vegetable oils

Stilbenes (Resveretrol) Red wine/Grapes

Flavanoids (Apigenin) Orange juice

Terpene Glycosides (Actein) Black Cohosh

Isoflavones (Genistein) Soya

Coumestans (Coumestrol) Alfalfa

Naturally occurring

Synthetic Contaminants

Endocrine Disruptors (DDT/Phthalates) Packaging

Growth Promoters (Diethylstilbesterol) American Beef

Synthetic Contaminants

Xenoestrogens

DDT/Phthalates Packaging

Growth Promoters (Diethylstilbesterol) American Beef

OH

OH

OH
Sources and Classification of Dietary Estrogens

- Ovarian Steroids (Estradiol) in Meat
- Isoflavones (Estrone, Genistein) in Soya
- Chromene (Miroestrol) in Kwao Keur
- Prenylnaringenins (8-PNG) in Hops/Beer
- Phytoestrogens
- Phytoestrogens (Estradiol) in Meat
- Coumestans (Coumestrol) in Alfalfa
- Flavonoids (Apigenin) in Orange juice
- Terpenoid Glycosides (Actein) in Black Cohosh
- Mycoestrogens (Zearealenone) in Grain
- Xenoestrogens (DDT/Phthalates) in Packaging
- Growth Promoters (Diethylstilbesterol) in American Beef
- Naturally occurring
- Synthetic Contaminants

Naturally occurring

Synthetic Contaminants
Sources and Classification of Dietary Estrogens

- **Naturally occurring**
  - Ovarian Steroids (Estradiol)
  - Meat
  - Isoflavones (Genistein)
  - Soya
  - Lignans (Secc)
  - Flax
  - Chromene (Miroestrol)
  - Kwao Keur
  - Phytoestrogens
  - Prenynaringenins (8-PNG)
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  - Terpene Glycosides (Actein)
  - Black Cohosh

- **Synthetic Contaminants**
  - Mycoestrogens (Zearalenone)
  - Grain
  - Xenoestrogens
  - Endocrine Disruptors (DDT/Phthalates)
  - Packaging
  - Growth Promoters (Diethylstilbesterol)
  - American Beef

**Herbs and Botanicals**
- Alfalfa
- Kwao Keur
- Hops/Beer
- Red wine
- Orange juice
- Black Cohosh

**Chemical Structures**
- Oestradiol
- Myo-inositol
- Zeaeneolone
- Phthalates
- Diethylstilbesterol
- Black Cohosh
Sources and Classification of Dietary Estrogens

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- Naturally occurring
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Isoflavonoids
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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 disruptors

o,p-DDT
Pesticides

Dibutyl phthalate
Phthalates

Bisphenol A
Packaging

Tetrachlorodibenzodioxin (TCDD)
Dioxins
Pueraria miricica
Pueraria miricica

- 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

Genistein  Coumestrol  Mirificoumestan  Kawakhurin  Deoxymiroestrol
Time line

- 1923  Doisey bioassay
  - A spayed mouse is injected, cornified vaginal cells
- 1926  Estrogenic compounds in plants
  - Several hundred plant species
- 1930’s Sand + Sub + Super = Pasture
Soay Sheep
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
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.

CSL/JIFSAN 28-30 June 2005
Thyroid Function

Pathways of thyroid hormone metabolism
Thyrotropin-releasing hormone (TRH) increases the secretion of thyrotropin (TSH), which stimulates the synthesis and secretion of triiodothyronine (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 Hypothyroidism
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
<table>
<thead>
<tr>
<th>Food Group</th>
<th>Food Items</th>
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<tbody>
<tr>
<td>1</td>
<td>Bread</td>
</tr>
<tr>
<td>2</td>
<td>Miscellaneous cereals</td>
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<tr>
<td>7</td>
<td>Fish</td>
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<tr>
<td>8</td>
<td>Oils and fats</td>
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<td>Beverages</td>
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<td>Milk</td>
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<td>19</td>
<td>Dairy products</td>
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<td>20</td>
<td>Nuts</td>
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## Isoflavone Daily Intake Calculation

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Food Items Sampled</th>
<th>Food Intake g day⁻¹</th>
<th>Mean Level in Food mg kg⁻¹</th>
<th>Total in Food mg</th>
<th>Daily Intake mg</th>
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<td>14</td>
<td>1.2</td>
<td>0.02</td>
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**Total Daily Intake of These Isoflavones** 3.00 mg
Isoflavone Concentrations in Meat Products

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<th>Town Number</th>
<th>Glycitein</th>
<th>Genistein</th>
<th>Daidzein</th>
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Isoflavone Concentrations in Fish

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</table>

Distribution of Isoflavones

Group 1, Processed Meat Products

Group 5, Fish
Soya - Food Conjugates

Genistein

Genistin (genistein-7-O-glucoside)

Genistein-7-O-glucoside

6''-O-Acetylgenistin

6''-O-Malonylgenistin

Daidzein

Daidzin (daidzein-7-O-glucoside)

Daidzein-7-O-glucoside

6''-O-Acetyldaidzin

6''-O-Malonyldaidzin

Glycitein

Glycitin (glycitein-7-O-glucoside)

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

A) Mass 610 Unknown

B) RT Ambocin Mass 564 Unknown

C) RT Genistein-7-glucoside (610)

D) RT Genistein-4’,7-diglucoside (432)

Acetyl-4’-genistin (564)

270 Genistein (564)
Human conjugates
Human conjugates

Daidzein

Daidzein-7,4'-diglucuronide

Daidzein-4'-glucuronide

Daidzein-7-glucuronide

Daidzein-7-sulpho-4'-glucuronide

Daidzein-4'-sulpho-7-glucuronide
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

Free daidzein
36.5

23.9
29.9
30.0
20.4
Daidzein Fragments
253→223

Free daidzein

23.9
29.9
30.0
36.5
Daidzein
Fragments
253→223

Daidzein
Glucuronides
429→253

Free daidzein
20.4

D-4’-glucuronide
20.4

D-7-glucuronide
23.9

D-7,4’-diglucuronide
12.5
Daidzein Fragments
253→223

Daidzein Fragments
253→223

Free daidzein
36.5

Daidzein Glucuronides
429→253

D-4’-glucuronide
20.4

D-7-glucuronide
23.9

D-7,4’-diglucuronide
12.5

D-7-sulphate
30.1

D-4’-sulphate
29.7

D-7,4’-disulphate ??
16.7
Daidzein Fragments
253→223

Free daidzein
36.5

Daidzein Sulphates
333→253

D-7-sulphate
30.1

D-4’-sulphate
29.7

D-7,4’-disulphate ??
16.7

Daidzein Glucuronides
429→253

D-4’-glucuronide
20.4

D-7-glucuronide
23.9

D-7,4’-diglucuronide
12.5

Daidzein Sulphoglucuronides
509→253

D-4’-sulpho-7-glucuronide
16.4

D-7-sulpho-4’-glucuronide
19.8
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

<table>
<thead>
<tr>
<th></th>
<th>Daidzein</th>
<th>Equol</th>
<th>Genistein</th>
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<td>Mid_vs_End.Diet</td>
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<td>Sex.Mid_vs_End.Diet</td>
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**Legend**
- *** Less than 0.1%
- ** Less than 1%
- * Less than 5%
- . Less than 10%
- Blank otherwise

**Difference in O-DMA between sexes**
‘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