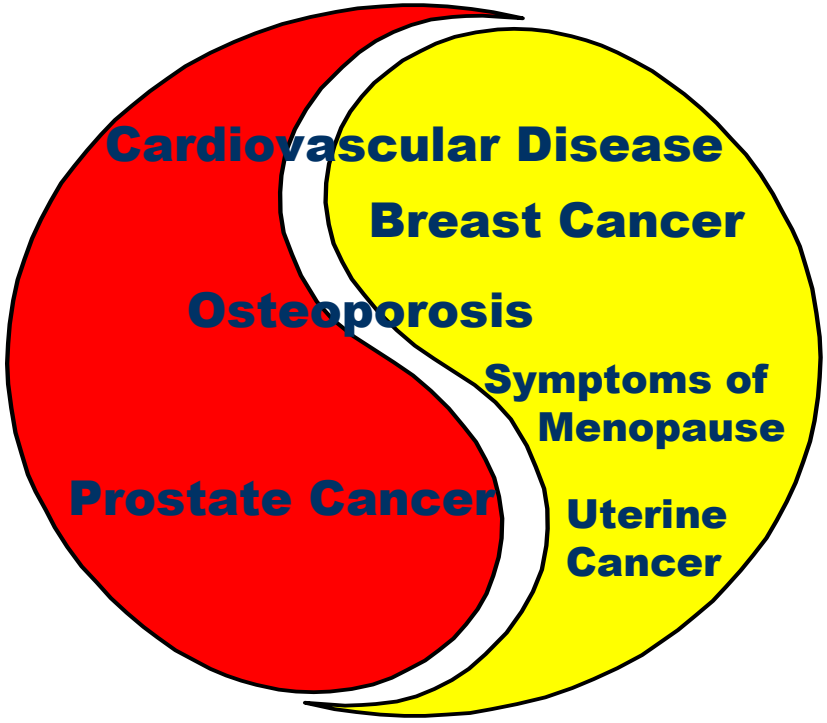
Think SOY!

Bioavailability and Biological Effects of Soy Isoflavones

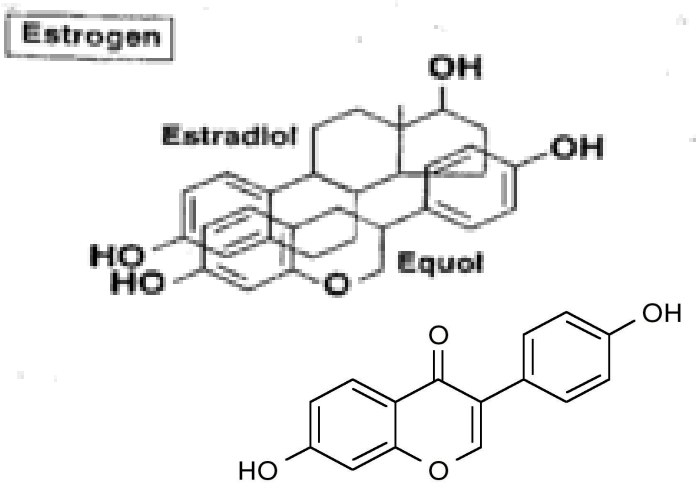
Health benefits from soy isoflavones

Epidemiological studies indicate that dietary isoflavones provide health benefits for men and women.



What are isoflavones ?

- Bioactive plant chemicals in dietary sources, have structure similar to endogenous estrogen
- Important non-hormonal properties (antioxidant, Protease Inhibitor, protein synthesis, etc.)
- Three major families, Genistein, Daidzein, Glycitein in 4 possible forms
  - Aglycone
  - β-glucoside
  - malonylglucoside
  - acetylglucoside

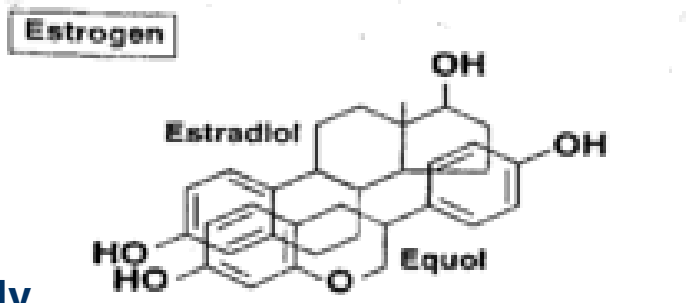


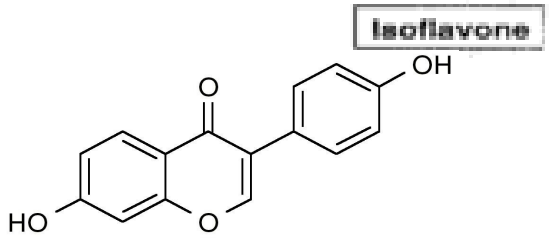
Daidzein - aglycone

What are isoflavones ?

Phytoestrogen

- Can bind to the mammalian estrogen receptor (ER), affinities to ER ranges from 10^{-2} to $2x10^{-5}$ compared to 17β-estradiol (human estrogen)
- Have estrogenic and/or anti-estrogenic effects depending on hormonal environment in the body
- Their precusors in foodstuffs might be converted into actual hormones in the human intestinal tract.
- Three main classes:
  - isoflavones
  - coumestans
  - lignins





Daidzein - aglycone

Isoflavones in Dietary Sources

- Found in fruit, legumes, whole grains, nuts, etc.
- The legumes, soy, has the most concentrated amount
- Typically occur as glycosides in raw soybeans and non-fermented soy foods
- In the range of 0.1-3.0 mg/gram (as is) in soy foods
- Concentrations depend on
  - soybean cultivar
  - environmental conditions
  - the processing procedures

Physical & chemical properties of isoflavones

- **Stability**
  - Aglycones are stable under physiological conditions
  - The acetyl- and malonyl-glucose ester bonds are labile at elevated temperatures and under acidic or basic conditions.
- **Solubility**
  - The aqueous solubility of algycones are low and are pH dependent due to the phenolic groups.
  - Conjugation to glucose residues increase the solubility, while acetylation or malonylation of the aglucones reduces solubility

Isoflavone biological activity

- **Bioavailability Studies**
  - Tissue distribution study
  - Pharmacokinetics after a single challenge
  - Impact of isoflavone structure
- **Mechanism**
  - Postulated metabolism pathway
  - A cellular transport model
- **Biological Effects**
  - hormonal effects
  - cancer modification

Bioavailability - (Yueh & Chu, 1977)

Tissue Distribution Studies

- **Study Design**

  Rats 15 mins after intravenous injection of 40mg of daidzein / B.W.

- **Results**
  - High in plasma, liver, lung, kidney (30μg/g, w.w.)
  - Modest in skeletal muscle, spleen and heart (15-20 μg/g w.w.)
  - Low in brain and testis (2-5 μg/g w.w.)

Bioavailability - Pharmacokinectic studies (King and Bursill, 1998)

Plasma and urinary kinetics after a single challenge

Study Design___

- 6 healthy men (ave. 37y)
- soy-free diet for 1 wk
- Two phases

Phase 1: To determine the time required to reach Max. plasma concentration

Phase 2: To determine the elimination half-life

Bioavailability - Pharmacokinectic studies (King and Bursill, 1998)

Plasma and urinary kinetics after a single challenge

Phase 1: To determine the time required to reach Max. plasma concentration

- Soy flour meal (0.84g/kg body wt)
  ~ 2.7μmol daidzein, 3.6 μmol genistein /kg body wt
- 10ml blood sample before and 1,2,4,6 and 8h after the meal

Observations:

- Isoflavone began to appear in plasma within 30min after consumption

- Isoflavone concns rose slowly and reached max. values of ~ 3.14 μmol/L at ~7h for daidzein and ~4mol/L at ~ 8h for genistein.

Bioavailability - Pharmacokinectic studies (King and Bursill, 1998)

Plasma and urinary kinetics after a single challenge

Phase 2: To determine the elimination half-life

- Urine samples collected 24h before and over the periods 0-11, 11-12, 12-14, 14-16, 16-18, and 18-35h after the meal
- Plasma collected 11,12,14,16,18 and 35h after the meal

Observations:

- Elimination half-lives: 5-9 hrs, returning to baseline by about 48 hrs after a meal
- Excreted in the urine as glucuronides and sulphates.
- Much greater excretion of daidzein than genistein (mean recoveries of ~ 62% and ~ 22% of the dose)
- A wide range of the percentage of ingested dose that is excreted by urine, however, the extent to which genistein is absorbed following ingestion is about the same or somewhat more than daidzein

Bioavailability - Pharmacokinectic studies (King and Bursill, 1998)

Plasma and urinary kinetics after a single challenge

Conclusions

- Higher urinary excretion of daidzein compared with genistein suggests a greater fractional excretion of the latter via the bile.
- Interindividual differences may reflect differences in gut microflora populations
- The nature of soy food may influence bioavailability through differences in the nature of isofavone conjugates
- The difference in conjugation pattern may influence the ease of hydrolysis of the glycosidic bond or bacterial degradation, and hence, bioavailability.

Bioavailability - Aglycone or glucosides ?

A human study vs. an animal study

(Hutchins A M, et al., 1995)

Study Design_
- 17 male consuming either:
  - (F)112g of fermented soy tempeh, or
  - (UF)125g of unfermented soybean
- for nine days

Observation_
Urinary recovery of daidzein and genistein:
(F) 9.7% and 1.9%
(UF) 5.7% and 1.3%

Suggesting:

Isoflavone aglycones may be more bioavailable their glucosides.

?

(King et al., 1996)

Study Design_
Rats were given a single dose of
(G)genistein (20mg/kg b.d.), or
(C)an equivalent dose of its conjugated forms in soy extract

Observations on plasma, urinary and fecal excretion at 2h,8h, and 48h after dosing.

Suggesting:

The extent of absorption of genistein is similar for the aglycone and conjugated forms.

Although higher initial plasma concn. May be achieved with the aglycone, by 8h the concentrations are similar.

Bioavailability – (Franke at al, 1998)

Isoflavones in human breast milk and other biological fluids

Study Design___

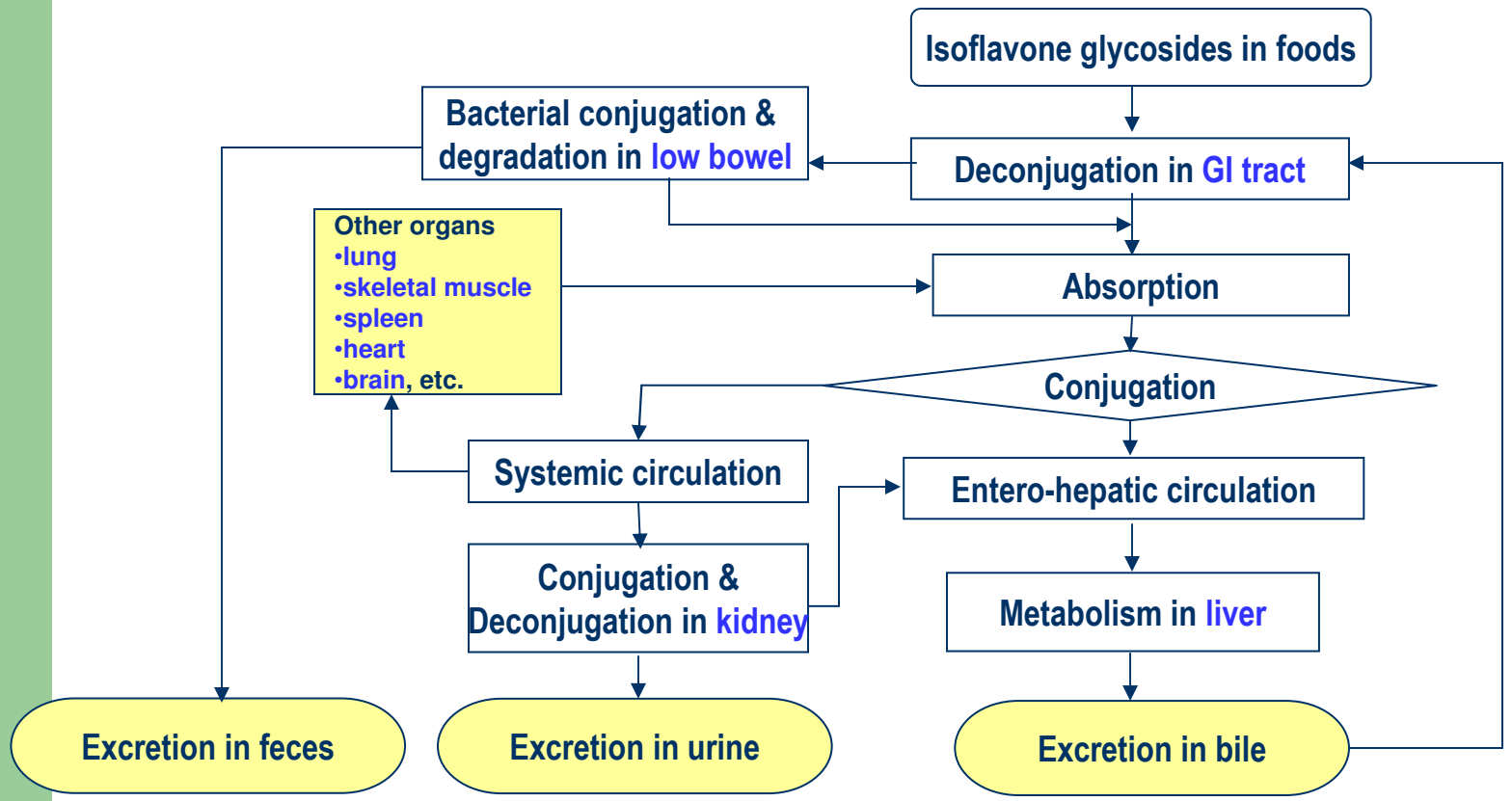
- single challenge with 20g roasted soybeans (37mg isoflaovnes)
- detect isoflavones in plasma, breast milk, and urine

Observations___

- mean total isoflavones:
  - 2.0 μmol/L in plasma
  - 0.2 μmol/L in breast milk
  - 3.0 μmol/L in urine

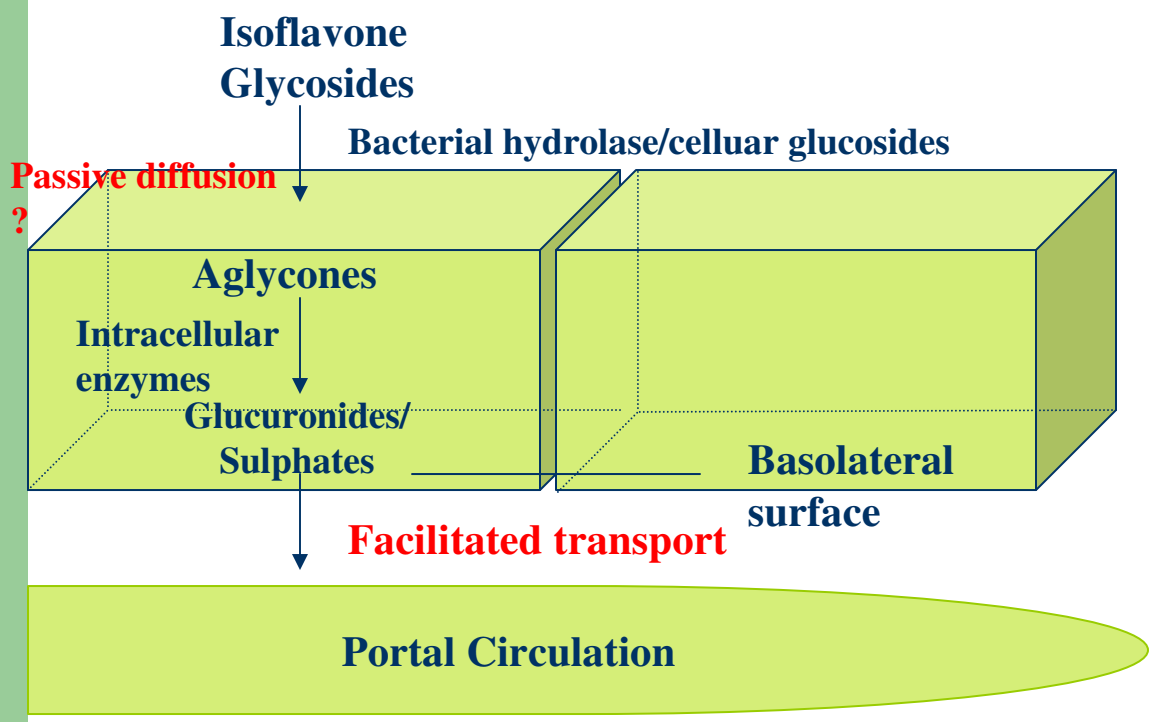
Possible Metabolic Pathway

Conjugation and Deconjugation — the major route of metabolism



Possible Metabolic Pathway

A Cellular Intestinal Transport Model on Caco-2 cells

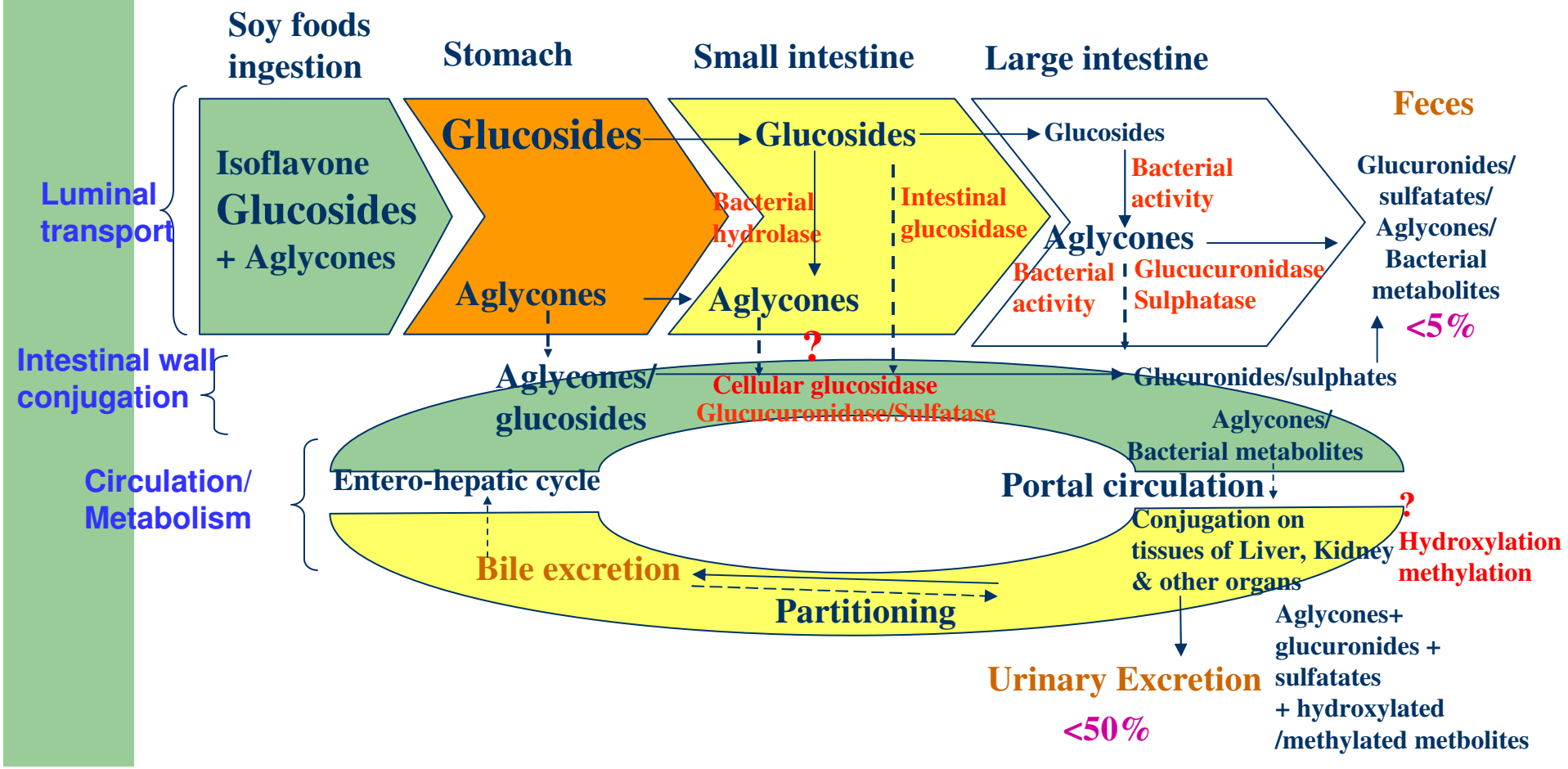


- Isoflavone aglycones are more rapidly transported than the glucoside

- The aglycone probably enter the cells by passive diffusion, but the mechanism of uptake of isoflavone glucosides has not been established clearly.

Possible Metabolic Pathway

Conjugation — the major route of metabolism



Possible Metabolic Pathway

Unknown/Unclear

Transport mechanism thru intestinal cell wall, passive diffusion, facilitated transport, or active transport ?

Whether glycosides can be absorbed as is?

Whether conjugation (e.g. glucuronidation) take places exclusively in the liver, or in the intestine ?

Bioavailability

HOT TOPICS —Soy- based Infant formula

Subject: Soy-based milk replacers to the newborn

- **Concerns:**
  - isoflavone supply may amount to 3mg/kg b.m.(exceed the amount needed to alter reproductive hormones in adults
  - the glucuronidation potency of infant liver is not fully developed, may lead to higher bioavailability of isoflavoe

- **On the other hand:**
  - phytoestrogens are discussed as having positive effects on newborns (e.g. Improved resistence against some chronic diseases in adulthood)
  - no adverse clinical effects have been observed in infant fed with soy products.

Biological effects - Case Study III
(Cassidy and Bingham, 1995)

Biological effects in young women

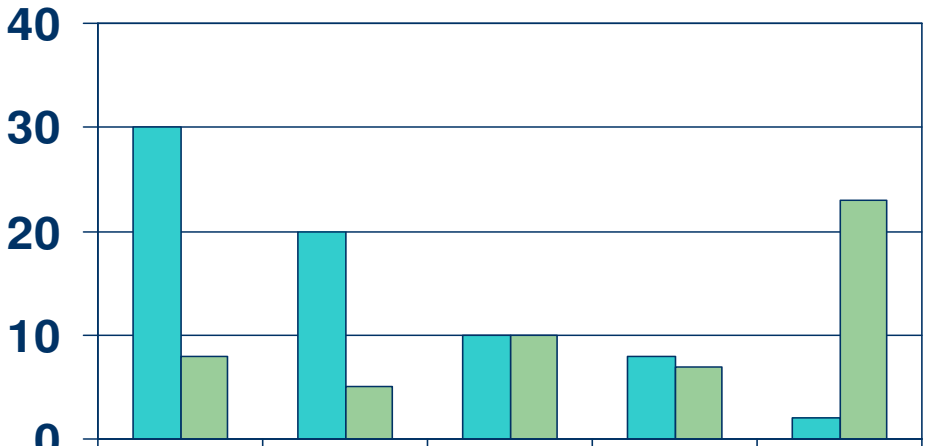
Study Design _____

- 15 healthy **premenopausal women**
- 4-6 month in **controlled metabolic** suites)
- **Hormonal status** measured over menstrual cycles
- **60g/day soy protein** (**45mg/day Isolfavone** in different conjugated forms)
- Similar non-soy diet as control

- **Diet modified hormonal status in normal ovulating women.**
- **Dietary composition of isoflavones is probably responsible for the observed biological effects.**
- **Inconjugated isoflavones are potentially more efficient probably due to their amphiphilic properties**
- **Reduction in total cholestroal levels and a trend towards ↓ in LDL: HDL value**

Soy isoflavones and cancer

Breast Cancer Mortality vs. Soy Consumption



	Japan	Korea	Hong Kong	China	United States
Soy Intake (gms/day)	30	20	10	8	2
Breast Cancer Death (per 100,000)	8	5	10	7	23

Soy isoflavones and cancer – a review
(Boersma et al., 2001)

Purified isoflaones shown in vitro to suppress a wide variety of cancer cell lines.

Proposed mechanisms include

- **regulation of estrogen-mediated events**
- **inhibition of tyrosine kinase and DNA topoisomerase activities**
- **synthesis and release of TGFβ**
- **modulation of apoptosis**
- **interaction with oxidants produced by inflammatory cells**

However__

Biological effects in cell culture occur far above the physiological concentrations.

Future studies

- Biotransformation study
- Identification of isoflavone metabolites
- Dose-response study
- Antioxidant effects in oxidation events
- Possible synergism between soy isoflavones and other soy components on cancer cell growth inhibition

Biotransformation

Isoflavones and their precursors in soy foods

FORMONONETIN
(4-methyl ether derivative of daidzein)

↓ Intestinal bacteria

Daidzein

↓

4'-O-methylequol

↓

(more estrogenic) Equol
(7-OH-3-(4'-OH-phenyl)chroman)
or
O-demethylangolensin

BIOCHANIN A
(4-methyl ether derivative of genistein)

↓ Intestinal bacteria

Genistein

↓

P-ethylphenol

- **Further mammalian metabolites of isoflavononoids phytoestrogens have to be expected**
- **Their properties are still unknown**

Relatives to isoflavones

Coumestans

- Structurally and biosynthetically related to the isoflavones
- Occur in legume shoots and sprouts
- Estrogenic activity greater than isoflaones (affinity to ER is ~10% of 17β-estradiol)
- Mainly act as an estrogen agonist
- Stimulate bone-mineralizing activity in animal model

Relatives to isoflavones

Lignans

- Contain a diphenolic ring system and are stereoisomeric
- Two major mammalian lignans, enterodiol and enterolactone
  - The products of colonic bacterial metabolism of the plant lignans secoisolariciresinol and matairesinol
- Weak estrogenic, some have antioxidative properties
- Dietary sources are flax seed, whole grain prodcuts, fruits, vegetables, tea.
- Concn. in human can exceed the endogenous concn.s of steroid hormones up to 1000 times.
- Consumption has been negatively correlated with coronary heart disease.
- Inhibit steroid hormones metabolizing enzymes
- Ingestion of flax seed revealed a cancer preventive roles at early tumor stages in anima models
- Enterolactone can stimulate growth of breast cancer cell lines.

Relatives to isoflavones

Phytosterols

- Daily intake estimated to be 160-360mg/day in Western diets
- Most important phytosterols in nature are β-sitosterol, campesterol, stigmasterol and dihydrobrassicasterol
- Natural constituents of foodstuffs (Bourbon whisky contain β-sitosterol in addition to biochanin A)
- Weak estrogenic
- Seems to reduce steroid biosynthetic capacity through effects on cholesterol availability or the activity of the side-chain cleavage enzyme cytochrome P450
- Reduce plasma total and LDL cholesterol levels (may due to reduced intestinal absorption of cholesterol)