Dr MR. Ritchie

PHYTO-OESTROGENS, HRT AND CANCER WORKSHOP.

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Asssessment of current knowledge about Phyto-oestrogens

True, False or Don't Know

1. PE are ONLY found in plants
2. All flavones are PE
3. All PE are isoflavones
4. PE are oestrogens
5. Beer is oestrogenic
6. Women with BCa must AVOID PE
7. Genistein is a PE
8. Baby milk/infant formula (cows) is a source of PE
9. All PE have the same oestrogenicity

10. British diets contain PE
11. Isoflavones circulate (mainly) as isolated molecules
12. Anti-biotics affect PE metabolism
13. Cimetidine (Tagamet) affect PE metabolism
14. PE are consumed as inactive compounds
15. British Rail sandwiches are a rich source of PE

16. Women metabolise PE better than men
17. Processing does not alter the PE content of foods
18. Rats are a better model of the effects of PE on humans than rabbits
19. PE can be taken safely by women with ER-ve Bca
20. PE should be taken by women at high risk of developing Bca
21. Calm (non-stressed) plants produce more PE

1. PE are ONLY found in plants (T)
2. All flavones are PE (F)
3. All PE are isoflavones (F)
4. PE are oestrogens (F)
5. Beer is oestrogenic (T)
6. Women with BCa must AVOID PE (D/K)
7. Genistein is a PE (T)
8. Baby milk/infant formula (cows) is a source of PE (T)
9. All PE have the same oestrogenicity (F)

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19. PE can be taken safely by women with ER-ve Bca (D/K)
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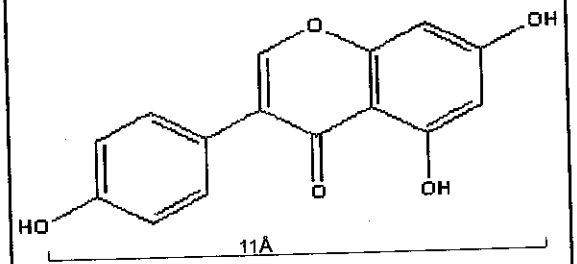
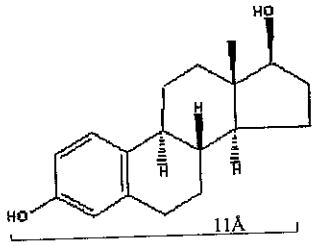
21. Calm (non-stressed) plants produce more PE (F)

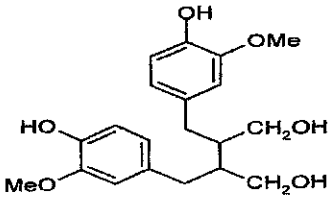
Genistein **(no 6A biophore)**



oestrogen, 17-beta estradiol
(6A biophore present)

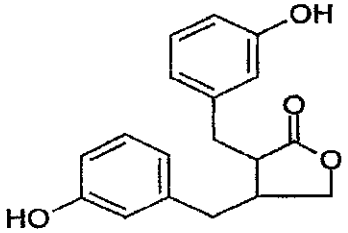


Structure of a lignan precursor



Secoisolariciresinol

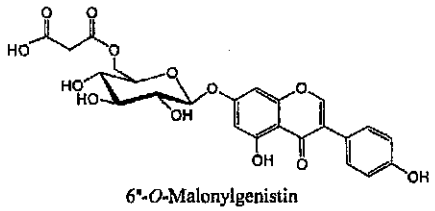
Structure of a lignan



Enterolactone

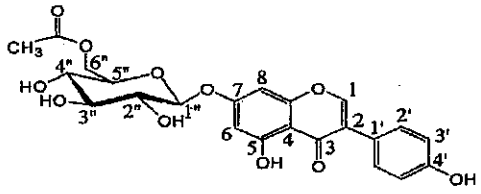
What is the natural form of isoflavones?

Isoflavone glycone structures



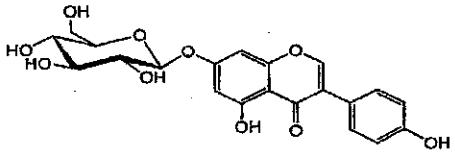
6"-*O*-Malonylgenistin

Isoflavone glycone structures



6"-*O*-Acetylgenistin

IIsoflavone glycone structures



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

ADME of Phyto-oestrogens

- **Absorption**
- **Digestion**
- **Metabolism**
- **Excretion**

Isoflavones ingested mainly as glycosides in non-fermented soy products

Genistein derivatives >
daidzein derivatives >
glycitein derivatives

Most prevalent forms are :- **malonyl genistin**
malonyl daidzin
genistin derivatives
daidzin derivatives

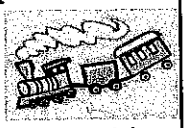
Isoflavones ingested mainly as aglycones in fermented soy products

For example

Food	Glucoside (mg/g dry wt)	Aglucone (mg/g dry wt)
soy milk	**3.02**	**0.24**
tofu	**1.81**	**0.23**
tempeh	**0.40**	**0.73**
miso	**0.11**	**1.27**
soy sauce	**nd**	**0.09**
soy cheese	**0.10**	**0.006**

Uptake
Isoflavones are absorbed across the enterocyte of healthy adults as aglucones

- Due to lower molecular weight
- increased hydrophobicity



- absorption of aglucones takes place mainly in the small and large intestine

Transfer

Acid hydrolysis of isoflavones may occur in the stomach

Liver and enterocytes of small intestine contain ß glucosidase

Glucosidase associated with gut microflora *Lactobacilli, Bifidobacteria and Bacteroides*

Enzymes in saliva may convert PE glucosides to aglucones

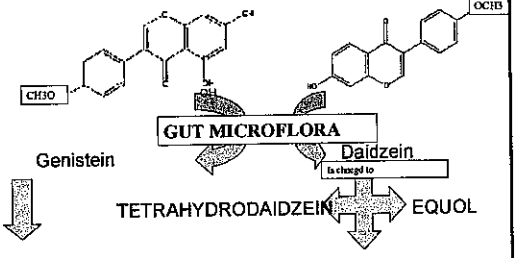
Gut microflora: genistein ⇒ p - ethyl phenol

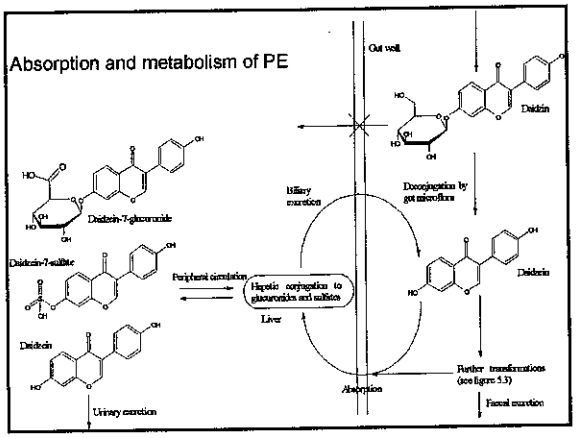
daidzein ⇒ equol or O - DMA

Absorption

Biochanin A
5,7-dihydroxy-4'-methoxyisoflavone

Formononetin
7-hydroxy-4'-methoxyisoflavone





Metabolism of phyto-oestrogens

- Ingestion as glycosides

Gut → faeces

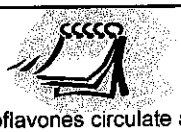
liver ← blood

kidneys

urine

30% of population excrete equol

Note



- Approx 5 - 50% of isoflavones circulate as free aglucones or monosulphates
- Genistein and daidzein (G & D) are conjugated on 4' and 7 positions
- peak plasma levels occur within 5 - 8 hours post ingestion of G & D
- plasma half lives for G and D ~ 5 - 8 hours
- some subjects exhibit more than one plasma peak indicating enterohepatic circulation
- form of ingestion may affect bioavailability

Factors which may affect metabolism of PE

- Age
- Gender
- Gut transit time
- Food matrix
- Habitual diet
- Genetic factors

*Babies and infants

Gut microflora

In an infant gut is sterile at birth

After 1 week microflora develop

Initial gut microflora capable of oxidative metabolism e.g.

Enterobacteria, *Streptococci*, and *Staphylocci (aerobic)*

As oxygen is metabolised *anaerobic* bacteria flourish e.g.

Bifidobacteria, Clostridia and Bacteroides

Equol

- More oestrogenic than genistein or daidzein
- present in human and cows milk
- not present in soy milk
- low levels found in urine of children > 6 months

Uptake of phyto-oestrogens in tissues

- In rat studies, phyto-oestrogens are detected in brain, muscle, spleen, heart, testis
- Higher levels found in plasma, liver, lung, and kidney

Uptake of phyto-oestrogens in tissues

- In human studies, phyto-oestrogens are detected in plasma, urine, faeces, prostatic fluid, semen, bile, saliva, breast milk, breast aspirate and cyst fluid

Effect of ER beta on tissue uptake in humans

Currently unknown

Additional effects mediated by PE

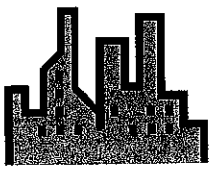
Effects on sex hormone concentrations

May be by

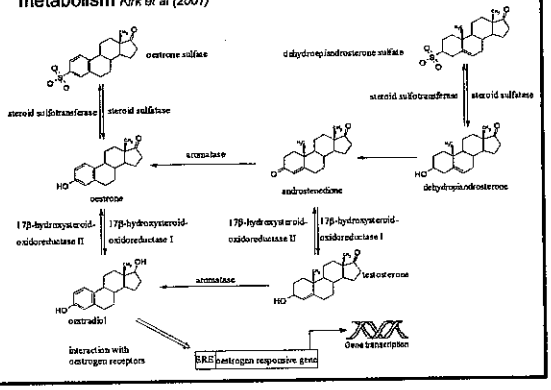
binding to SHBG to inhibit oestrogens and androgens binding causing ⇧ circulating oestrogens levels

stimulating synthesis of SHBG thus ⇩ circulating oestrogen levels

Effect on oestrogen biosynthesis and metabolism



Enzymes involved in oestrogen biosynthesis and metabolism *Kirk et al (2001)*



Action of steroid sulfatase and sulfotransferase

- Sulfatase catalyses the removal of sulfate from steroid like compounds
- Sulfotransferase catalyses the addition of sulfate to steroid like molecules
- equilibrium lies towards the sulfated compounds I.e sulfated compounds are 10 - 30 x higher than unconjugated forms
- steroid sulfatase is important in postmenopausal wome

Steroid sulfatase and sulfotransferase inhibition by PE

Phyto-oestrogen	Enzyme inhibited	IC_{50}(μM)
D - 4'-O -sulfate	steroid sulfatase	6
D -7,4'-di-O-sulfate	"	1.5
D - 4'-O -sulfate	steroid sulfotranferase	>100
D -7,4'-di-O-sulfate	"	>100

Effects of PE *in vivo* and *in vitro*

Aromatase Inhibition

Compound	Ki (μM)
naringenin	5.1± 0.2
7,8-dihydroxyflavone	10 ± 1
biochanin A	12 ± 5
genistein	123 ± 8

Other biochemical effects of PE

Thyroid peroxidase

(TPO) is an enzyme involved in the production of thyroid hormones.

TPO catalyses the oxidation of I_2 to $I^{\bullet}$ during synthesis of T_3 and T_4. *In vitro* studies indicate PE inhibit TPO. *In vivo* reducing levels of thyroid hormones may ⇧ TSH levels

Other biochemical effects

- Inhibition of topoisomerase II
- Inhibition of tyrosine specific protein kinases
- In vitro inhibition of cancer cells at high concentrations
- antioxidant properties (prevent oxidation of LDL cholesterol)

Phyto-oestrogens and osteoporosis

Animal studies

- Data from rodent studies consistently demonstrate a protective effect of phyto-oestrogens on BMD in ovriectomised animals
- * note, Rats are equol generating machines

Effects of PE on bone mineral density BMD

- Limited studies but several of the latest have shown **bone preserving action** of **PE**
- *Potter et al, 1998*
- **post menopausal** women taking 90mg/d isoflavones resulted in
- ⇧bone mineral content &⇧ density of the lumbar spine

Bone protective effects of PE

Scheiber & Rebar, 1999

Post-menopausal women taking 60-70 mg/ day isoflavones resulted in ⇩ urinary excretion of D-pyridinoline and N-telopeptide and markers of bone metabolism

Alekel et al, 2000

Peri-menopausal women taking 80mg/day isoflavones resulted in **attenuation** of bone loss from the lumbar spine

More recently

- The greatest effects on BMD seen in women who are equol excretors

Overall conclusion re BMD and phyto-oestrogens

- Women consuming moderate to high amounts of phyto-oestrogens both pre-menopausally and peri-menopausally will maintain BMD as they enter menopause

Phyto-oestrogens and cognitive function in younger populations

- *File SE et al, 2001* reported increased cognitive function in students taking 100mg phyto-oestrogens per day for 10 weeks

Phyto-oestrogens and cognitive function in younger populations

Students on high soy diet showed improvement in short term recall,

long term memory

mental flexibility

Other tests

For fluency tests and test of planning only the women performed better

Older women and phyto-oestrogens

- *Duffy et al, 2003*
- 33 post-menopausal women age 50 -65 years not on HRT
- 60 mg isoflavones/day or placebo for 12 weeks

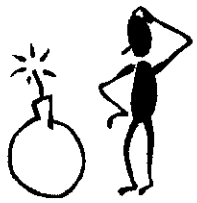
Results

- Those on high isoflavones showed
- greater recall
- greater improvement in learning rule reversals
- improvement in planning task

Phyto-oestrogens and prostate health

- Phyto-oestrogens inhibit 5 alpha reductase
- phyto-oestrogens are anti-oxidants
- 5 alpha reductase is involved in the formation of 5 alpha dihydrotestosterone (DHT)
- phyto-oestrogens may help reduce the production of DHT
- DHT affects growth factors and indirectly may affect BPH

Phyto-oestrogen research at St. Andrews University/Dundee



Phyto-oestrogen research at St Andrews University in collaboration with Ninewells Hospital, Dundee

- Phyto-oestrogen database constructed containing values assigned to >600 foods commonly eaten in Britain
- Database validated
- Biomarkers of phyto-oestrogen exposure validated

What is a biomarker of exposure and why is it useful?

- A biomarker of phyto-oestrogen is a biochemical snapshot of human exposure to a compound of interest
- Our research has validated three biomarkers of phyto-oestrogen exposure namely:-
- 24h urine collection
- Timed spot plasma sample
- Timed spot urine sample

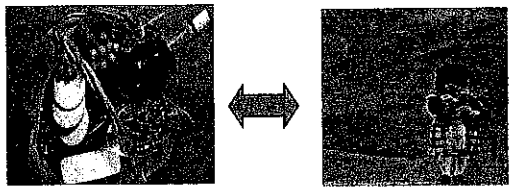
How can a biomarker of phyto-oestrogen exposure help?

- ✓ A single timed urine sample, single timed plasma sample or 24h urine collection can accurately predict phyto-oestrogen intake over 24h
- ✓ Analysis of several samples can provide information about variation in dietary intake
- ✓ By assessing dietary exposure women can decide if supplementation is necessary and by how much

Future research involving biomarkers of phyto-oestrogen exposure

- Assessment of dietary phyto-oestrogen exposure in women diagnosed with breast cancer or men diagnosed with prostate cancer
- Investigation of the effect of phyto-oestrogen intake on breast cancer or prostate cancer treatment, prognosis or progression

Development of a Biomarker of PE intake



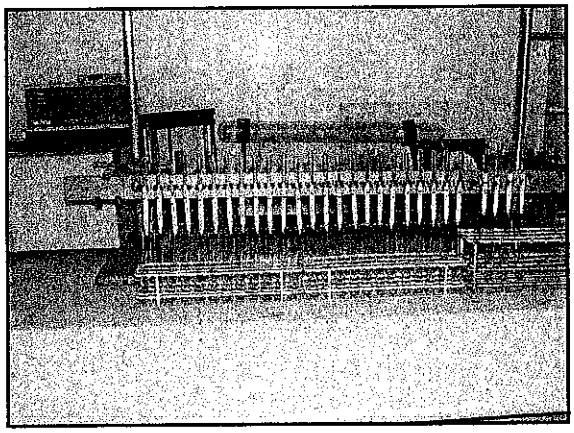
Duplicate diet analysis for validation of database and biomarkers of intake

Method for urine sample preparation

- 250µl urine, add 2ml of 0.1M Na Ac buffer (pH 5)
- 50µl internal standard containing 250ng analyte
- add1000 units activity glucucuronidase/ sulphatase (helix pomatia juice)
- incubate overnight at 37°C
- extract with 5 & 3 ml ethyl acetate and remove organic layer using ice/salt
- blow dry under N_2

Extraction of phyto-oestrogens

- Prepare columns using silanised pasteur pipettes and silanised glass wool
- pack with sephadex LH20
- dissolve sample in 200 µl $CHCl_3$:C_7H_{16}:CH_3OH
- 10 : 10 : 1
- add to column, repeat, then add 3.6ml solvent mixture
- PE eluted with 4ml MeOH
- blow dry under N_2
- re dissolve in 80µl, MeOH:H_2O, 1:1
- analyse using LC-MS (SCRI)



Where do we go from here?

- Biomarker used in prospective studies investigating the effect of phyto-oestrogens on cancer treatment
- breast cancer risk in high risk groups
- retrospective studies
- investigating the effect of phyto-oestrogens on tumour characteristics
- prostate cancer risk

Phyto-oestrogens and *in vitro* studies

Many studies have been carried out using cell lines exposed to isoflavones such as genistein

studies need to look at synergism of the components

Acknowledgements

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- Mr. Johnston Pickles et al
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