Do dietary phytoestrogens influence susceptibility to hormone-dependent cancer by disrupting the metabolism of endogenous oestrogens?

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Abstract

Phytoestrogens are natural constituents of our diets that have been suggested to protect against hormone-dependent breast cancer. Some of the diverse effects of these compounds may be attributed to ligand-dependent differences in their interaction with oestrogen receptor sub-classes. However, phytoestrogens can also inhibit enzymes that are involved in the generation and removal of endogenous steroid hormones. Among the most potent effects of dietary phytoestrogens is their ability to inhibit the sulphotransferases that sulphate both oestrogenic steroids and a variety of environmental chemicals, including dietary procarcinogens. Circulating steroid sulphates are thought to be the major source of oestradiol in post-menopausal breast tumours and sulphation is a key step in the activation of some dietary procarcinogens.

Key words: breast cancer, flavonoids, sulphatase, sulphotransferase.

Abbreviations used: DHEA, dehydroepiandrosterone; SULT1E, oestrogen sulphotransferase; SULT1A1, the thermostable form of phenol sulphotransferase; SULT, sulphotransferase.

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carcinogens. Hence the inhibition of sulpho-transferases by dietary phytoestrogens may have complex effects upon human susceptibility to breast cancer.

Introduction
Breast cancer is responsible for approx. 20000 deaths per year in the U.K., and dietary factors are thought to have a role in the aetiology of the disease. High-fat diets may be a contributory factor [1]. However, phytoestrogens, including flavonoids, have been suggested to be protective, because Eastern populations with a high dietary intake of these compounds have a relatively low incidence of the disease [2,3]. Dietary fibre is also thought to protect against the disease, and this effect has been linked to the significant quantities of lignans found therein [4,5].

Some of the diverse effects of phytoestrogens may be attributed to ligand-dependent differences in their interaction with oestrogen receptor subclasses. However, where appropriate experiments have been performed, these compounds are often found to interact rather weakly at oestrogen receptors [6]. An alternative way in which phytoestrogens could influence cellular behaviour is by altering the metabolism, and hence the availability, of endogenous oestrogens. Such effects of phytoestrogens would be particularly significant for the development of hormone-dependent tumours, because the oestrogens which drive the growth of such tumours are thought to be generated locally [7].

In post-menopausal women, among whom breast cancer is most prevalent, there is no significant production of ovarian oestrogens. However, oestrogens continue to be synthesized from dehydroepiandrosterone (DHEA) and androstenedione, which are derived mainly from the adrenal cortex [7]. The major pathways involved in oestrogen biosynthesis from circulating steroids and steroid sulphates are shown in Scheme 1. All enzyme activities depicted in Scheme 1 are found in breast tissue [8], and several are reported to be elevated in mammary tumours [9–12].

Phytoestrogens in the diet
Isoflavones, flavones and coumestans are the most abundant phytoestrogens in the human diet, and soy is by far the richest source of these compounds.
centrations at or similar to the IC₅₀, and their 4'-methyl esters (biochanin A and formononetin respectively) are found in clover, but are less significant constituents of human diets [18]. Lignans, such as the enterolactone precursor secoisolariciresinol, are enriched in oilseeds, but are also found in legumes, cereals and vegetables [19].

The relative paucity of these compounds in Western diets is well illustrated by the comparison between circulating phytoestrogen levels in Japanese men consuming a traditional soy-based diet and in a group of Finnish vegetarians [20]. The former had plasma levels of daidzein and genistein (free plus conjugated) of ~1 μM, whereas the corresponding value for the Finnish vegetarians was ~18 nM. Western individuals consuming soy-based diets or dietary supplements for perceived therapeutic benefit are likely to exhibit plasma phytoestrogen levels similar to those reported for the Japanese men [21]. Dietary lignans and flavonoids are conjugated to sugar moieties, and absorption across the gut is preceded by deconjugation. After absorption, these compounds are mostly conjugated with glucuronic acid or sulphate in the liver [22], but the free compounds may be regenerated in target tissues.

Phytoestrogens and endogenous steroid metabolism in hormone-dependent tumours
Aromatase and the β-hydroxysteroid dehydrogenases
There has been much interest in the aromatase pathway as a source of oestrogens in hormone-dependent tumours, and in the use of aromatase inhibitors for breast cancer therapy [10]. Hence it is not surprising that about half of the papers published on the inhibition of steroid-metabolizing enzymes by phytoestrogens concern aromatase. Most authors have not attempted detailed kinetic analysis of the inhibitory effects of these compounds, but there is an abundance of data defining IC₅₀ values for aromatase inhibition by flavonoids, mostly determined at substrate concentrations at or similar to the Kᵥ value (Table 1). The IC₅₀ and Kᵥ values listed in Table 1 are as reported in the papers quoted, or are derived from the data therein. The listings of individual compounds are not exhaustive, but Table 1 does record the most potent reported effects of phytoestrogens and their sulphonyl conjugates upon steroid-metabolizing enzymes. Where detailed kinetic analysis was attempted, inhibition of aromatase activity by these compounds was found to be competitive [(23–27); Table 1]. The most potent aromatase inhibitors were flavones that were hydroxylated at the 7-position on the A ring. Further hydroxylation at the 5-position on the A ring or on the B ring had little effect upon the IC₅₀, but hydroxylation at the 3- and 8-positions on the C and A rings respectively yielded noticeably weaker aromatase inhibitors.

Much less is known about the possible role of phytoestrogens as inhibitors of the 17β- and 3β-hydroxysteroid dehydrogenases. Coumestrol and apigenin were the most potent inhibitors of the former, with IC₅₀ values of approx. 0.1 μM [29,30] (Table 1). Genistein inhibited 17β-hydroxysteroid dehydrogenase with an IC₅₀ of 1 μM [8] (Table 1). Only isoflavones, at relatively high concentrations, were found to be inhibitors of 3β-hydroxysteroid dehydrogenase [8,31] (Table 1).

The IC₅₀ and Kᵥ values reported in Table 1 should be interpreted in the light of the concentrations of these compounds that are found in individuals consuming diets with various phytoestrogen contents. As noted above, these cover a wide range, but it seems very unlikely that any of the aromatase inhibitors listed in Table 1, which are rather minor constituents of most diets, could reach sufficient concentrations in vivo to influence enzyme activity in human tissues. Of the inhibitors of the hydroxysteroid dehydrogenases, only genistein might be speculated to exert a physiologically relevant effect upon 17β-hydroxysteroid dehydrogenase, and then only at the upper concentration limit found in individuals consuming diets with a very high soy content.

Steroid sulphatase and sulphotransferases
Although some locally produced oestrogens in hormone-dependent tumours may be derived from the aromatase pathway, there is growing evidence that circulating steroid sulphates, and oestrone sulphate in particular, may be a more significant source of active hormones [11,12, 36–38]. Sulphotransferases in a variety of tissues contribute to a circulating pool of oestrone sulphate that is very much larger than that of free oestrone [7]. In post-menopausal women,
## Table I

**Flavonoids and other phytoestrogens are inhibitors of steroid-metabolizing enzymes**

Data are as quoted in, or derived from, the sources given. The listings are not exhaustive, but do include the most potent reported effects of those phytoestrogens tested. MeOH, methoxy.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Inhibitor [ref.]</th>
<th>(IC_{50}) or (K^*) ((\mu)M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aromatase</td>
<td>7-OH-flavone [8,24]</td>
<td>0.2, 0.25*</td>
</tr>
<tr>
<td></td>
<td>5,7-OH-flavone (chrysin) [8,23,26,28]</td>
<td>0.5–11, 0.24*, 0.26*</td>
</tr>
<tr>
<td></td>
<td>4',5,7-OH-flavone (apigenin) [8,23]</td>
<td>1.2, 2.9</td>
</tr>
<tr>
<td></td>
<td>3',4',5,7-OH-flavone (luteolin) [27]</td>
<td>4.8*</td>
</tr>
<tr>
<td></td>
<td>4',7-OH-flavone [24]</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Flavone [23,24]</td>
<td>8, 10</td>
</tr>
<tr>
<td></td>
<td>Flavanone [23,24]</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Enterolactone [25,27]</td>
<td>6*, 14*</td>
</tr>
<tr>
<td></td>
<td>3',4',5,7-OH-flavone (quercetin) [23]</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>3',4',5,7-OH-flavone (kaempferol) [27]</td>
<td>27*</td>
</tr>
<tr>
<td></td>
<td>7,12-OH-coumestan (coumestrol) [8,27]</td>
<td>25, 1.3*</td>
</tr>
<tr>
<td></td>
<td>7,8-OH-flavone [28]</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>4',7-OH-isoflavane (equol) [25]</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>4',7-OH-isoflavone (daidzein) [25]</td>
<td>&gt; 1000</td>
</tr>
<tr>
<td>17(\beta)-Hydroxysteroid dehydrogenase</td>
<td>7,12-OH-coumestan (coumestrol) [29,30]</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>4',5,7-OH-flavone (apigenin) [30]</td>
<td>~ 0.12</td>
</tr>
<tr>
<td></td>
<td>7-OH-flavone [8]</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>4',5,7-OH-isoflavone (genistein) [8]</td>
<td>1.0</td>
</tr>
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<td></td>
<td>3',4',7-OH-isoflavone (fisetin) [30]</td>
<td>~ 1.2</td>
</tr>
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<td></td>
<td>4'-MeOH-5,7-OH-isoflavone (biochanin A) [8]</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>4',7-OH-isoflavone (daidzein) [8]</td>
<td>10</td>
</tr>
<tr>
<td>3(\beta)-Hydroxysteroid dehydrogenase</td>
<td>4'-MeOH-7-OH-isoflavone (formononetin) [8,31]</td>
<td>3.5, &gt; 50</td>
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<tr>
<td></td>
<td>4',5,7-OH-isoflavone (genistein) [8,31]</td>
<td>2.9, 10</td>
</tr>
<tr>
<td></td>
<td>4'-MeOH-5,7-OH-isoflavone (biochanin A) [31]</td>
<td>7.5</td>
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<tr>
<td></td>
<td>4',7-OH-isoflavone (daidzein) [8,31]</td>
<td>10, 11</td>
</tr>
<tr>
<td>Oestron sulphatase</td>
<td>3',4',5,7-OH-flavone (quercetin) [32]</td>
<td>&lt; 10</td>
</tr>
<tr>
<td></td>
<td>3',4',5,7-OH-flavone (kaempferol) [32]</td>
<td>~ 10</td>
</tr>
<tr>
<td></td>
<td>4',5,7-OH-flavanone (naringenin) [32]</td>
<td>~ 10</td>
</tr>
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<td>DHEA sulphatase</td>
<td>Daidzein-4'-O-sulphate [33]</td>
<td>5.9*</td>
</tr>
<tr>
<td></td>
<td>Daidzein-4',7-dio-sulphate [33]</td>
<td>1.0*</td>
</tr>
<tr>
<td>DHEA sulphotransferase</td>
<td>Daidzein-4'-O-sulphate [33]</td>
<td>&gt; 100</td>
</tr>
<tr>
<td></td>
<td>Daidzein-4',7-dio-sulphate [33]</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Phenol sulphotransferase (SULT1A1)</td>
<td>3',4',5,7-OH-flavone (quercetin) [34,35]</td>
<td>0.1, 0.1*</td>
</tr>
<tr>
<td></td>
<td>3',4',7-OH-flavone (fisetin) [34]</td>
<td>0.1</td>
</tr>
<tr>
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<td>3,5,7-OH-flavone (galangin) [34]</td>
<td>0.2</td>
</tr>
<tr>
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<td>4',5,7-OH-isoflavone (genistein) [35]</td>
<td>0.21*</td>
</tr>
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<td>3',4',5,5',7-OH-flavone (myricetin) [34]</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>4',7-OH-isoflavone (daidzein) [35]</td>
<td>0.34*</td>
</tr>
<tr>
<td></td>
<td>3',4',5,7-OH-flavone (kaempferol) [34]</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>5,7-OH-flavone (chrysin) [34]</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>4',5,7-OH-flavone (apigenin) [34]</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>4',7-OH-isoflavane (equol) [35]</td>
<td>0.49*</td>
</tr>
<tr>
<td></td>
<td>Daidzein-4'-O-sulphate [33]</td>
<td>&gt; 100</td>
</tr>
<tr>
<td></td>
<td>Daidzein-4',7-dio-sulphate [33]</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>
much oestrone derived from DHEA and andro-
stenedione synthesized in the adrenal gland will
enter this pool.

Oestrone sulphatase activity is elevated in
many hormone-dependent tumours [11,12] and in
hormone-dependent breast cancer cell lines [39].
The activity of oestrogen sulphotransferase
(SULT1E) is reported to be extremely low or
absent in human breast carcinoma cells [40-42],
where it is thought that oestrone sulphation is
catalysed mainly by the thermostable phenol
sulphotransferase (SULT1A1) [40,41]. Further-
more, transfection of hormone-dependent MCF-
7 breast cancer cells with cDNA for SULT1E
reduced hormone-stimulated proliferation by
46 %, suggesting that a diminished capacity to
sulphate oestrogens may contribute to the trans-
formed phenotype [43].

It is clear that the balance between steroid
sulphatase and steroid sulphotransferase activities
determine the availability of active steroids in
hormone-dependent tumours. Some progesterin
and other therapeutic agents have been shown to
reduce sulphatase and enhance sulphotransferase
activity in T47-D hormone-dependent breast can-
cer cells [44], but there have been rather few
reports detailing the effects of phytoestrogens
upon these activities (Table 1).

We have undertaken an extensive screen of 32
potential phytoestrogens and found no effects of
the unconjugated compounds upon oestrone sul-
phatase activity at physiologically relevant con-
centrations; the most potent effects are listed in
Table 2. Our results are in broad agreement with
those in the one other published paper on the
subject [32] (Table 1). In contrast, daidzein
sulphates, in the low micromolar concentration
range, inhibited sulphatase activity against either
oestrone sulphate (Table 2) or DHEA sulphate
[33] (Table 1). Inhibition of DHEA sulphatase
was competitive [33]. Since much of the cir-
culating daidzein in individuals on high soy diets
is sulphated [22], the possible physiological signi-
ficance of these effects should not be ignored.

However, by far the most potent effects of
naturally occurring flavonoids upon enzymes in-
volved in steroid metabolism are seen with the
sulphotransferase family. We, and others, have
examined the effects of a variety of flavonoids
upon SULT1A1 in platelets and liver [34,35]
(Tables 1 and 2). SULT1A1 is among the most
active sulphotransferases in many tissues. It is
generally thought to have a role in xenobiotic
detoxification, but it can also sulphate oestrogens
at relatively high concentrations [45]. This char-
acteristic may be important in cancer cells that do
not express SULT1E, but which may be exposed
to high concentrations of the hormone [40,41].
The most potent inhibitor of SULT1A1 activity
was the synthetic compound 3',4'-dihydroxy-
flavone, with IC50 values against the 'model'
substrate 4-nitrophenol (3 µM) and oestradiol
(20 µM) of 2 nM and 20 nM respectively (Table
2). However, luteolin (a natural constituent of
celery) was almost as potent, with IC50 values of
8 nM and 50 nM respectively against these sub-
strates. Quercetin inhibited SULT1A1 activity
against 4-nitrophenol and oestradiol with IC50
values of 60 nM and 100 nM respectively, well
within the concentration range found in the circu-
lation of individuals eating diets rich in onions
[46]. Of the abundant soy phytoestrogens, geni-
stein was the most potent, with IC50 values of
400 nM and 300 nM for activity against 4-nitro-
phenol and oestradiol respectively. This is within
the range of circulating concentrations for geni-
stein and its conjugates that are found in indi-
viduals on high soy diets [20,21]. The most potent
inhibitors of SULT1A1 activity against both
substrates were all hydroxylated in the 3'- and 4'-
positions (Table 2). The inhibition of SULT1A1
activity against 4-nitrophenol by those flavonoids
for which kinetic data exist appears to be non-
competitive [35].

We have also examined the effects of a more
limited group of naturally occurring flavonoids,
chosen because of their effects upon SULT1A1,
upon the sulphation of a physiological concen-
tration (10 nM) of oestradiol. We recently reported
a sulphotransferase (SULT) activity in human
platelets, with a Km for oestradiol of this order, that
may be SULT1E [45]. Although this activity is
present only at low levels, platelets are a useful
source of assay material in the human population
because of the relative ease of tissue collection.
Quercetin, daidzein, genistein and luteolin all
inhibited SULT activity against oestradiol in
human platelets with IC50 values of <1 µM.
Again, these are flavonoid concentrations that may
be achieved in the circulation of individuals on
high soy diets, among those taking certain dietary
supplements [20,21] or, in the case of quercetin, in
subjects on diets rich in onions [46]. Kinetic
analysis, using plots of 1/v against [I] (Dixon) and
of [S]/v against [I], showed the inhibition of this
activity by genistein and daidzein to be com-
petitive, with KI values of 0.3 µM and 0.4 µM
respectively. As this paper was in the final stages of
Inhibition by flavonoids of sulphatase and sulphotransferase enzymes active against oestrogens in human platelets

Steroid sulphatase activity was determined against 1 nM [3H]oestrone 3-sulphate in particulate fractions from human platelets incubated for 60 min in 20 mM Tris/HCl (pH 6.5) [12]. Reactions were quenched with NaHCO₃ and unconjugated steroids were extracted into toluene prior to the determination of radioactivity. SULT activity against 3 nM 4-nitrophenol and 20 nM oestradiol was determined as described previously [45]. SULT I E I activity in platelet cytosol fractions [45], prepared in a buffer containing 0.25 M sucrose, 2 mM 2-mercaptoethanol and 10 mM sodium phosphate, pH 7.0, was assayed against 10 nM [3H]oestradiol [43] in a modified assay buffer containing 0.1 mM EDTA, 10 mM magnesium acetate and 100 mM Tris/acetate, pH 7.9. nd, not determined.

<table>
<thead>
<tr>
<th>Flavonoid</th>
<th>Platelet sulphotransferases versus:</th>
<th>3 μM 4-nitrophenol</th>
<th>20 μM oestradiol</th>
<th>10 nM oestradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Platelet steroid sulphatase versus 1 μM oestrone sulphate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3',4'-OH-flavone</td>
<td>nd</td>
<td>0.002</td>
<td>0.02</td>
<td>nd</td>
</tr>
<tr>
<td>3',4',5,7-OH-flavone (luteolin)</td>
<td>&gt; 25</td>
<td>0.008</td>
<td>0.05</td>
<td>0.4</td>
</tr>
<tr>
<td>3',4,7-OH-isoflavone</td>
<td>&gt; 25</td>
<td>0.02</td>
<td>0.08</td>
<td>nd</td>
</tr>
<tr>
<td>3',3',4',5,7-OH-flavone (quercetin)</td>
<td>nd</td>
<td>0.06</td>
<td>0.1</td>
<td>0.5</td>
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<tr>
<td>3,3',4',7-OH-flavone (fisetin)</td>
<td>nd</td>
<td>0.08</td>
<td>nd</td>
<td>nd</td>
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<tr>
<td>5,6,7-OH-flavone (baicalein)</td>
<td>&gt; 25</td>
<td>0.08</td>
<td>nd</td>
<td>nd</td>
</tr>
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<td>4',5,7-OH-flavone (apigenin)</td>
<td>&gt; 50</td>
<td>0.1</td>
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<td>nd</td>
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<tr>
<td>4',5,7-OH-isoflavone (genistein)</td>
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<td>0.3</td>
<td>0.8</td>
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<td>4',7-OH-isoflavone (daidzein)</td>
<td>&gt; 50</td>
<td>0.6</td>
<td>0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Daidzein sulphate</td>
<td>8.6</td>
<td>nd</td>
<td>nd</td>
<td>&gt; 10</td>
</tr>
</tbody>
</table>

preparation, Otake et al. [47] reported that SULT1E, from both human recombinant sources and from human mammary epithelial cells, was inhibited competitively by quercetin, with IC₅₀ values of 1.4 μM and 0.13 μM respectively. These data are in broad agreement with our own results, and emphasize further the potential significance of these effects of dietary flavonoids upon SULT1E activity in relation to breast cancer.

Conclusions

Although some phytoestrogens may provoke endocrine disruption by acting directly at oestrogen receptors [6], the most potent effects of these compounds are exerted upon enzymes involved in steroid metabolism, especially those that interconvert free steroids and steroid sulphates. Steroid sulphotransferases and sulphatases are inhibited significantly by flavonoids and flavonoid sulphates respectively, at concentrations that may be attained in the blood of subjects on phytoestrogen-enriched diets. Since active oestrogens in mammary tumours are thought to be provided mainly by the sulphatase route [11,12,36–38], dietary flavonoids could exert profound effects upon their bio-availability in tumour cells.

The net effect of a diet rich in phytoestrogens upon the interconversion of active oestrogens and their sulphates probably depends upon the chemical nature of the circulating flavonoids and their distribution between free and sulphated forms. Although most circulating flavonoids are thought to be conjugated, free flavonoids may be generated by the action of sulphatases in target tissues. The exquisite sensitivity of sulphotransferase enzymes to inhibition by free flavonoids may ensure that this effect predominates over the inhibition of the sulphatase by flavonoid sulphates in individuals with a high dietary flavonoid intake, thus increasing the local availability of oestrogens to drive tumour growth. This could be especially significant in some breast cancer cells, where the 'front line' defence of SULT1E is reported to be compromised, since flavonoids also inhibit SULT1A1, which acts as a secondary enzyme system for oestrogen sulphation in these cells [40,41].

While the effects of flavonoids upon sulphotransferase activities imply that their presence in the diet might increase susceptibility to breast cancer, epidemiological evidence has been taken to suggest the reverse [2,3]. This paradox
may possibly be explained by some other roles that sulphotransferases fulfil in these cells. For example, sulphotransferases can catalyse the bio-
activation of heterocyclic amine pro-carcinogens [48]. These are found in overcooked meats and have also been implicated in the aetiology of breast cancer [49,50]. Clearly, further investigation of the biochemical effects of phytoestrogen-rich diets in vivo is essential before any clear advice can be formulated concerning dietary phytoestrogens and human health.

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References
Actions of the soy phytoestrogen genistein in models of human chronic disease: potential involvement of transforming growth factor β

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Abstract
The structural similarity, but non-identity, between 17β-oestradiol and the soy phytoestrogen genistein suggests that the two compounds will have actions that may be identical in some target biological systems, but different in others. Epidermal growth factor (EGF)-stimulated proliferation of human mammary epithelial cells (that do not express the oestrogen receptor) was significantly suppressed at genistein concentrations (5–10 µM) that are attainable physiologically. Others have shown previously that transforming growth factor β (TGFβ) has similar growth-inhibitory effects on human cells. Analysis of the conditioned medium of human mammary epithelial cells exposed to genistein plus EGF showed increased levels of TGFβ relative to those in the medium of cells exposed to EGF or genistein alone. Related experiments in a primate model of menopause demonstrated that ingestion of soy containing isoflavones was correlated with the suppression of neurodegeneration-relevant phosphorylation of the microtubule-associated protein tau, while intake of Premarin (a hormone replacement therapy that is commonly prescribed for women) was not correlated. The results discussed here indicate that genistein, and probably other related phytoestrogens, have pleiotropic actions, some of which may involve TGFβ activity.

Introduction
Women living in South-East Asian countries have a lowered incidence of breast cancer compared with those in Western countries [1–3]. Since one of the striking differences between these two populations is diet, much experimentation has addressed the question of whether dietary components that are either enriched in the Asian diet, or missing from the Western diet, could lower the risk of breast cancer. Soy-based foods are an obvious difference between the South-East Asian and the Western diets; people in South-East Asian countries consume probably 1–5-fold more soy protein than those in Western countries [4,5]. Since the initial finding that ingested plant isoflavones might have anti-oestrogenic activity in animals [6], experiments have shown that the isoflavones, the expression of which is essentially restricted to the soybean [7], bind to both oestrogen receptors (ERs), ERα and ERβ [8–10], suggesting that these isoflavones could affect receptor-mediated actions of oestrogen. More recently, isoformones were shown to inhibit the proliferation of mammalian cells, including human mammary epithelial (HME) cells, suggesting inhibition of cell proliferation as the basis of a

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