

The biological effects of genistein and its intracellular metabolite, 5,7,3',4'-tetrahydroxyisoflavone.

Giulia Corona^[1] and Jeremy P.E. Spencer

Molecular Nutrition Group, School of Chemistry, Food and Pharmacy, University of Reading,
Reading RG6 6AP, UK

[1] Corresponding author:

Giulia Corona

Food and Nutritional Sciences Department, School of Chemistry, Food and Pharmacy
The University of Reading, PO Box 226, Whiteknights Reading RG6 6AP United Kingdom
Tel: +44 (0) 118 378 7713
Fax: +44 (0) 118 931 0080
mail: g.corona@reading.ac.uk

Jeremy P E Spencer

Food and Nutritional Sciences Department, School of Chemistry, Food and Pharmacy
The University of Reading, PO Box 226, Whiteknights Reading RG6 6AP United Kingdom
Tel: +44 (0) 118 378 8724
Fax: +44 (0) 118 931 0080
mail: j.p.e.spencer@reading.ac.uk

Departmental contact

Maria Itta, Departmental secretary
Food and Nutritional Sciences Department, School of Chemistry, Food and Pharmacy
The University of Reading, PO Box 226, Whiteknights Reading RG6 6AP United Kingdom
Tel: +44 (0) 118 378 8703
Fax: +44 (0) 118 931 0080
mail: m.itta@reading.ac.uk

List of Abbreviations

3beta-HSD	hydroxysteroid dehydrogenase/isomerase
γ-GCS	Gamma-glutamyl cysteine synthetase
COX-2	cyclooxygenase-2
DR	diabetic retinopathy
GPx	glutathione peroxidase
GSH	reduced glutathione
IBD	inflammatory bowel disease
ICAM-1	intercellular adhesion molecule-1
IFN(gamma)	interferon-gamma
IL-1β	interleukin-1beta
IL-8	interleukin-8
LPS	lipopolysaccharide
MCP-1	monocyte chemoattractant protein-1
MPO	myeloperoxidase
SHBG	sex hormone-binding globuline
SOD	superoxide dismutase
THIF	5,7,3',4'-tetrahydroxyisoflavone.
TNF-α	tumour necrosis factor-alpha

Abstract

Genistein is abundant in soya and related products and its consumption is associated with numerous health benefits, including oestrogenic activity, anticancer effects, antioxidant actions and anti-inflammatory activity. However, whilst at high concentration in soy, the biological effects of genistein, in cells and tissues, will be dependent on its absorption, metabolism and distribution. In foods, isoflavones are predominantly present as glycosylated forms, i.e. genistin. However these are hydrolyzed prior to absorption, releasing the aglycone, i.e. genistein, which may be absorbed and may undergo further biotransformation, including intracellular metabolism. In the case of genistein this intracellular metabolism may lead to the formation of bioactive metabolites such as 5,7,3',4'-tetrahydroxyisoflavone (THIF), which itself will undergo conjugation with cellular thiols such as glutathione (GSH) to yield glutathionyl conjugates of THIF. Studies have suggested that THIF and its derivatives may be partly responsible for the biological effects of genistein in vivo. Indeed, it may act influence the proliferation of cancer cells through its effects of DNA oxidation and the activation of downstream signalling pathways which control the cell cycle.

1. Genistein and its biological activities

The isoflavone genistein (5,7,4'-trihydroxyisoflavone) (Figure 1) is present primarily in soybeans and a variety of legumes (Reinli and Block 1996), where its content can differ depending on the variety of soybean, the year harvested, geographic location and plant part (Delmonte and Rader 2006). Non-soy legumes such as lentils and other types of beans do not contain appreciable amounts of genistein (Liggins *et al* 2000). However, genistein can also occur in soy products in the form of its glycoside (Figure 1) genistin (Liggins *et al* 2000; Reinli and Block 1996). Genistein exhibits a wide range of biological effects that contribute to its potential health benefits (Head 1998), such as oestrogenic activity (Cassidy *et al* 1994), anticancer effects (Messina and Bennink 1998), antioxidant actions (Cai and Wei 1996), and anti-inflammatory activity (Fanti *et al* 2006).

1.1. Oestrogenic activity

Isoflavones are structurally similar to oestrogens (Miksicek 1995) and can function both as oestrogen agonists and antagonists depending on the hormonal milieu and the target tissue and species under investigation (Molteni *et al* 1995). Genistein's anti-oestrogenic effects are due to its ability to bind to the oestrogen receptor (Wang *et al* 1996; Yearley *et al* 2007), therefore suppressing the more harmful effects of oestrogens, such as their effects on oestrogen-sensitive cancers (Anthony *et al* 1998; Goodman *et al* 1997; Horn-Ross *et al* 2003). For example, in MCF-7 breast cancer cells, which are known to be estrogen receptor positive, genistein is able to stimulate estrogen-responsive pS2 mRNA expression and compete with [3H]-estradiol binding to the estrogen receptor with 50% inhibition (Wang *et al* 1996). Furthermore, isoflavones have been found to exert an estrogenic effect in both the absence or presence of endogenous estrogen (Adlercreutz and Mazur 1997). Alternatively, genistein may reduce oestrogenic activity via its ability to induce the production of sex hormone-binding globuline SHBG, thus leading to a faster clearance of sex hormones (including oestrogens)

(Mousavi and Adlercreutz 1993) and a reduced risk of hormone-sensitive breast and prostate cancer (Messina and Hilakivi-Clarke 2009; Smith *et al* 2008). Similarly, genistein is known to inhibit many of the enzymes involved in the biosynthesis and metabolism of steroid hormones (Head 1998). For example, genistein has direct effects on cellular progesterone synthesis which involve the inhibition of hydroxysteroid dehydrogenase/isomerase (3 β -HSD) enzyme activity across the post-cyclic AMP pathway. It directly affects cellular progesterone synthesis through its inhibition of 3 β -HSD gene expression and down-regulation of its transcription (Tiemann *et al* 2007).

There is convincing evidence that a diet rich in soy-protein, which contains genistein, has an impact on the hormonal status and the regulation of the menstrual cycle of premenopausal women (Cassidy *et al* 1994). A daily intake of 60g of soy protein (containing 45 mg isoflavones) for 1 month significantly increased follicular phase length and/or delayed menstruation, significantly suppressed the mid-cycle surges of luteinizing hormone and follicle-stimulating hormone and increased plasma estradiol concentrations in the follicular phase (Cassidy *et al* 1994). These effects were potentially associated with lower risk of breast cancer (Cassidy *et al* 1994; Henderson *et al* 1985).

1.2. Anticancer effects

Interest in genistein as a anticancer agent arose due to population-based data indicating a link between genistein consumption and a decreased risk of mortality from several types of cancer, in particular prostate and breast cancer (Pavese *et al* 2010). Epidemiological studies indicate a protective effect of isoflavone ingestion against breast cancer in premenopausal women and women who had high soy intakes during adolescence (Peeters *et al* 2003; Piller *et al* 2006), whereas in postmenopausal women the data vary depending on the study (Jones *et al* 2002; Ju *et al* 2006; Ju *et al* 2002). Genistein is a potent inhibitor of the growth of breast cancer cells, whereas its beta-glucoside genistin has little effect (Peterson and Barnes 1991, 1993).

The effects of genistein on breast cancer cell growth and proliferation were studied in estrogen-receptor negative (MDA-468) and positive (MCF-7 and MCF-7-D-40) cell lines and it was ascertained that the presence of the estrogen receptor is not required for genistein to inhibit the cancer cell growth (Peterson and Barnes 1991). The estrogen-independent effects of genistein can be due to their ability, or the ability of one of their metabolites, to block cell cycle progression through direct effects on intracellular signalling (Nguyen *et al* 2006). For example, genestein has been shown to be capable of causing a cell cycle block in the G₂/M phase in vitro (Cappelletti *et al* 2000; Santell *et al* 2000) and an overexpression of cyclin dependent kinase inhibitor p21WAF1 in breast cancer cells, leading to cell cycle arrest (Chinni *et al* 2003). Other mechanisms whereby genistein can exert its anticancer properties include induction of apoptosis (Sergeev 2004; Xu and Loo 2001), inhibition of tyrosine kinases (Mitropoulou *et al* 2002; Morton *et al* 1999), modulation of the MAPK kinase signalling (Li *et al* 2006) alterations in the phosphatidylinositol 3-kinase cascade (Lee *et al* 2001) and inhibition of DNA topoisomerases (McCabe and Orrenius 1993).

The relationship between isoflavone supplementation and prostate cancer has also been extensively investigated. Male rats fed with genistein (0, 25 and 250 mg /kg) over their lifetime led to an inhibition of the development of N-methylnitrosourea-induced prostate invasive adenocarcinomas, in a dose-dependent manner (Wang *et al* 2002). It has been proposed that the regulation of AR/Akt/PTEN pathway by genistein may be one of the molecular mechanisms by which it inhibits proliferation and induces apoptosis in prostate cancer cells (Wang *et al* 2009). The Akt-GSK-3 pathway and its downstream effectors have also been identified as targets for the chemopreventive action of genistein in transgenic adenocarcinoma mouse prostate model (TRAMP/FVB) mice, where incorporation of genistein in the diet significantly inhibited the activation of Akt, restored the activation of GSK-3beta, reduced cyclin D1 levels post-transcriptionally and maintained the expression of the cadherin-1 complex via down-regulation of snail-1, decreasing the proliferative potential, retarding cancer progression and maintaining the integrity of the prostatic epithelial cells in vivo (El Touny and Banerjee 2007).

Another mechanism of increased prostate cancer cell death by genistein is proposed to occur via its ability to inhibit NF-kappaB signalling, leading to altered expression of regulatory cell cycle proteins such as cyclin B and/or p21WAF1/Cip1, thus promoting G2/M arrest. These findings support the important and novel strategy of combining genistein with radiation for the treatment of prostate cancer (Raffoul *et al* 2006).

1.3. Antioxidant action

Genistein, similar to other isoflavones, possesses a relatively strong antioxidant potential *in vitro* (Han *et al* 2009), and exerts its antioxidant actions by scavenging free radicals (Kim *et al*), chelating metals (Dowling *et al*), inhibiting the production of oxidizing species such as H₂O₂ (Sethy-Coraci *et al* 2005), and by enhancing the activity of endogenous antioxidant enzymatic systems, such as catalase (Cai and Wei 1996). Whilst direct antioxidant actions *in vivo* are highly unlikely (Williams *et al* 2004), at physiologically achievable concentrations of 5 nM genistein increased intracellular-reduced glutathione (GSH) levels by approximately 10 %, whereas cellular alpha-tocopherol and uric acid remained unchanged (Guo *et al* 2002). The mechanisms behind increases in GSH may include the effect of isoflavones on enzymes involved in the synthesis of GSH such as γ -GCS (Guo *et al* 2002). Dietary administration of genistein for 30 days in mice increases the activity of antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase and glutathione in various organs, including skin, small intestine, liver, kidney, and lung (Cai and Wei 1996). Furthermore, treatment of macrophages with genistein reduces LPS induced GSH depletion and induces superoxide dismutase (SOD) and catalase (Choi *et al* 2003). Similar effects have been reported in rats, where feeding the animals with an isoflavone-rich diet increased glutathione peroxidase and glutathione reductase activities, blood glutathione levels and glutathione S-transferase levels in kidney (Appelt and Reicks 1999).

1.4. Anti-inflammatory activity

Genistein has been shown to have an effect on the production of inflammatory mediators in human peripheral blood mononuclear and polymorphonuclear leukocytes stimulated with lipopolysaccharide (LPS) and interferon-gamma (IFN(γ)) (Richard *et al* 2005). Genistein's anti-inflammatory activity has also been noted in human brain microvascular endothelial cells, where it dose-dependently inhibited cytokine-induced up-regulation of pro-inflammatory mediators such as tumour necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8), and intercellular adhesion molecule-1 (ICAM-1) and cytokine-induced transmigration of blood leukocytes (Lee and Lee 2008). Exposure to high amounts of genistein, as occurs in traditional East Asian diets, where the mean total intake of isoflavones is estimated between 17 and 47 mg/day (Vergne *et al* 2009), may be associated with a lower incidence of inflammatory bowel disease (IBD) (Loftus 2004). Indeed, the risk of IBD appears to increase in Asian immigrants adopting western lifestyles, suggesting a protective role for isoflavones (Loftus 2004). To confirm this hypothesis, the effect of orally administered genistein on the inflammatory response to 2,4,6-trinitrobenzenesulfonic acid-induced chronic colitis in rats has been investigated and indicates that genistein exerts beneficial anti-inflammatory effects by inhibiting molecular and biochemical inflammatory markers in the colon, specifically cyclooxygenase-2 (COX-2) and myeloperoxidase (MPO) (Seibel *et al* 2009). Diabetic retinopathy (DR) is also associated with microglial activation and increased levels of inflammatory cytokines and the efficacy of genistein for alleviation of diabetes-induced retinal inflammation has been studied in an animal model of diabetes. Genistein was found to be effective in dampening diabetes-induced retinal inflammation by reducing TNF- α release and inhibiting ERK and P38 phosphorylation in activated microglial cells (Ibrahim *et al* 2010).

2. Intracellular formation of the genistein metabolite 5,7,3',4'-tetrahydroxyisoflavone (THIF)

The biological effects of genistein are undoubtedly affected by its metabolism by phase II metabolic conjugation to glucuronic acid or sulphuric acid, catalyzed by UDP-glucuronyl transferase or sulfotransferase enzymes in the intestinal epithelium and in the liver. In addition, intracellular metabolism of genistein may also act to affect its cellular actions. Genistein undergoes further transformation intracellularly to yield novel bioactive metabolites. For example, in tumorigenic breast epithelial cells, genistein is selectively taken up into the cell and is subjected to significant intracellular metabolism by CYP450 enzymes leading to the formation of both 5,7,3',4'-tetrahydroxyisoflavone (THIF; orobol) and two glutathionyl conjugates of THIF (Nguyen *et al* 2006). A scheme of the described metabolic pathways is presented in figure 2. It is also shown that the co-treatment with cimetidine prevents the conversion of genistein to THIF, and because cimetidine is known to inhibit the CYP450 isoforms 1A2, 2C9, 2C19, 2D6, and 3A4, it is likely that one of these isoenzymes is responsible for the conversion of genistein to THIF in T47D cells (Nguyen *et al* 2006). Previously, it has been suggested that isoflavone metabolism in transformed but not non-transformed breast epithelial cells may modulate the growth inhibitory effects of genistein (Peterson *et al* 1996). Glutathionyl conjugates of THIF in cells may be formed either enzymatically via the action of glutathione S-transferase or non-enzymatically via oxidative metabolism of THIF and subsequent reaction of THIF o-quinone with the cellular thiol GSH. However, the inhibition of glutathione S-transferase does not block glutathione conjugate formation, indicating that the latter is more likely to occur (Nguyen *et al* 2006). Conjugations with thiols, such as glutathione, represent a major target for quinones, and the detoxification of quinones by GSH conjugation is generally considered to be cytoprotective (Monks and Lau 1997). Glutathionyl conjugates from a variety of polyphenol quinones have been observed in cellular systems and display a wide array of biological activities (Corona *et al* 2006; Monks and Lau 1997; Spencer *et al* 2003). Indeed, the redox activity of polyphenols is frequently enhanced following conjugation

with GSH (Monks and Lau 1997) and as GSH conjugation is often coupled to the subsequent export of the adduct from cells, the conjugation of THIF appears to represent a detoxification pathway (Monks and Lau 1997; Spencer *et al* 2003).

3. 5,7,3',4'-tetrahydroxyisoflavone (THIF) and cancer

The exposure of breast epithelial cells to physiological concentrations of genistein selectively induces growth arrest and G2-M phase cell cycle block in tumorigenic (T47D) but not non-tumorigenic (MCF10A) breast epithelial cells (Nguyen *et al* 2006). These effects of cancer cell proliferation were paralleled by significant differences in the association of genistein with cells and in particular its intracellular metabolism (Nguyen *et al* 2006). Previously, it had been suggested that isoflavone metabolism in transformed but not non-transformed breast epithelial cells may modulate the growth inhibitory effects of genistein (Peterson *et al* 1996). In agreement with this hypothesis, genistein appeared to be selectively taken up into tumorigenic breast epithelial cells and subjected to significant intracellular metabolism leading to the formation of THIF and two glutathionyl conjugates of THIF (Nguyen *et al* 2006). In contrast, there was minimal cell-association of genistein with MCF10A cells and no subsequent formation of free THIF (Nguyen *et al* 2006). In breast cancer cells THIF formation triggers the activation of the MAP kinase p38 (Figure 3). Active p38 prevents the phosphorylation of cyclin B1 and hence its transport to the nucleus, an event essential for correct functioning of the cdc2-cyclin B1 complex. In addition, active p38 may undergo translocation to the nucleus where it directly inhibits the phosphorylation/activation of cdc2, thereby blocking entry of cells into mitosis (G2-M block) (Nguyen *et al* 2006). These data suggest that the formation of THIF is crucial in driving the anti-cancer effects of genistein *in vivo*, in particular by inhibiting the proliferation of cancer cells but not affecting normal cell function. Further evidence relating to the cellular actions of the genistein metabolite, THIF, were elucidated in human breast carcinoma cells and summarized in figure 3 (Vauzour *et al* 2007). Here THIF induced a G2-

M cell cycle arrest in T47D tumorigenic breast epithelial cells which was mediated by the activation of ataxia telangiectasia and Rad3-related kinase (ATR) via its phosphorylation at Ser⁴²⁸ (Vauzour *et al* 2007). This activation of ATR appeared to result from THIF-induced increases in intracellular oxidative stress, a depletion of cellular GSH and an increase in DNA strand breakage (Vauzour *et al* 2007). These events led to the downstream inhibition of cdc2, which was accompanied by the phosphorylation of both p53 (Ser¹⁵) and Chk1 (Ser²⁹⁶) and the de-activation of cdc25C phosphatase. It was suggested by the authors that the anti-proliferative actions of THIF may be mediated by initial oxidative DNA damage, activation of ATR and downstream regulation of the p53 and Chk1 pathways leading to cell cycle arrest in G2-M (Vauzour *et al* 2007). These data sets suggest that the formation of THIF may mediate the effects of genistein on cancer cells. This hypothesis is in agreement with other investigations, which reported the effects of THIF (orobol) and other isoflavones (genistein, daidzein and 7,8,4'-trihydroxyisoflavone) on angiogenesis and endothelial cell proliferation (Kiriakidis *et al* 2005). In a chicken chorioallantoic membrane assay; all compounds had the capacity to inhibit angiogenesis, albeit with different potencies (genistein > THIF > daidzein (48.98 %) and 7,8,4'-TriOH (24.42 %), and also inhibited endothelial cell proliferation, with THIF causing the greatest inhibition at lower concentrations (Kiriakidis *et al* 2005).

4. 5,7,3',4'-tetrahydroxyisoflavone (THIF) and endothelial function

Genistein has been shown to be able to protect endothelial cells against damage induced by oxidative stress and decreases intracellular glutathione levels (Hernandez-Montes *et al* 2006). These effects appear to also be mediated by genistein intracellular metabolism. When genistein enters cells it is subject to the CYP450-induced intracellular metabolism yielding THIF. THIF may also react with intracellular GSH to form the adduct THIF-GSH (figure 3), inducing a reduction of intracellular GSH and an increased DNA damage (Hernandez-Montes *et al* 2006). Genistein and THIF are also able to induce the transcription of antioxidant enzymes:

more specifically genistein directly activate the translocation of Nrf2, which will increase the transcription of γ -GCS, leading to increases in GSH synthesis, whereas THIF stimulates the release of Nrf1 with the subsequent translocation into the nucleus where it may interact with the electrophile response element and induce the transcription of GPx (Hernandez-Montes *et al* 2006). The intracellular formation of glutathionyl conjugates of THIF may result either from the action of glutathione-S-transferase or from the autoxidation of THIF and subsequent reaction of THIF o-quinone with GSH (Hernandez-Montes *et al* 2006). These findings are in agreement with other studies that indicate that glutathionyl conjugates from a variety of polyphenol quinones have been observed in cellular systems and display a wide array of biological activities (Corona *et al* 2006; Monks and Lau 1997; Spencer *et al* 2003). It appeared that the protective effects observed in endothelial cells in response to genistein exposure were dependent on the induction of the enzyme glutathione peroxidase (GPx), due to increases in both GPx mRNA and enzyme activity. It was suggested that genistein and its intracellular metabolites protective effects on endothelial cells were dependent primarily on the activation of glutathione peroxidase mediated by Nrf1 activation, and not on Nrf2 activation or increases in glutathione synthesis (Hernandez-Montes *et al* 2006).

5. Summary

The cellular mechanism of action of genistein will be dependent on the cellular metabolism and uptake of the compound. Genistein enters the cells where it is hydroxylated to form 5,7,3',4'-tetrahydroxyisoflavone (THIF). It is suggested that THIF formation is crucial for its cellular activity and can be mediated by interactions of the newly formed cellular metabolite with signalling pathways. THIF is generated by the action of CYP450 enzymes in cells (Nguyen *et al* 2006) and the catechol group-containing metabolite is observed to persist within the cells for up to 24 h without undergoing O-methylation (Vauzour *et al* 2007) as has been observed for other catechol containing polyphenols (Corona *et al* 2006; Spencer *et al* 2003).

Indeed cellular actions of isoflavones are cell-type specific and also depend on its P450-related metabolism, oxidative metabolism, and GSH-conjugation (Hernandez-Montes *et al* 2006). Experiments conducted by testing both genistein and daizein in endothelial cells showed that only genistein significantly protected against the oxidative injury induced by hydrogen peroxide whereas daizein had no protective effect. In the same cell line, both genistein and daidzein significantly increased γ -glutamylcysteine-synthetase levels and were able to induce increases in cytosolic accumulation and nuclear translocation of Nrf2. In contrast GSH levels increased only in the cells treated with daidzein; whereas with genistein, they were significantly lower, due to its sequestration by metabolism. In addition, cytosolic levels of Nrf1, and the degree to which Nrf1 underwent nuclear translocation, were significantly higher in cells exposed to genistein than in those exposed to daidzein. Therefore it appeared that protection by genistein depended on its induction of GPx and changes in Nrf1 activation, suggesting that they may underlie its protective effects in endothelial cells, and be mediated by intracellular metabolism to THIF that is only relevant to genistein but not daidzein were hydroxylation cannot occur. Future work would be needed to fully investigate these metabolic difference in view of a more complete understanding on their molecular mechanism of action, and ascertain the presence of intracellular metabolites in plasma and urines following genistein intake.

List of Figures

Figure 1.

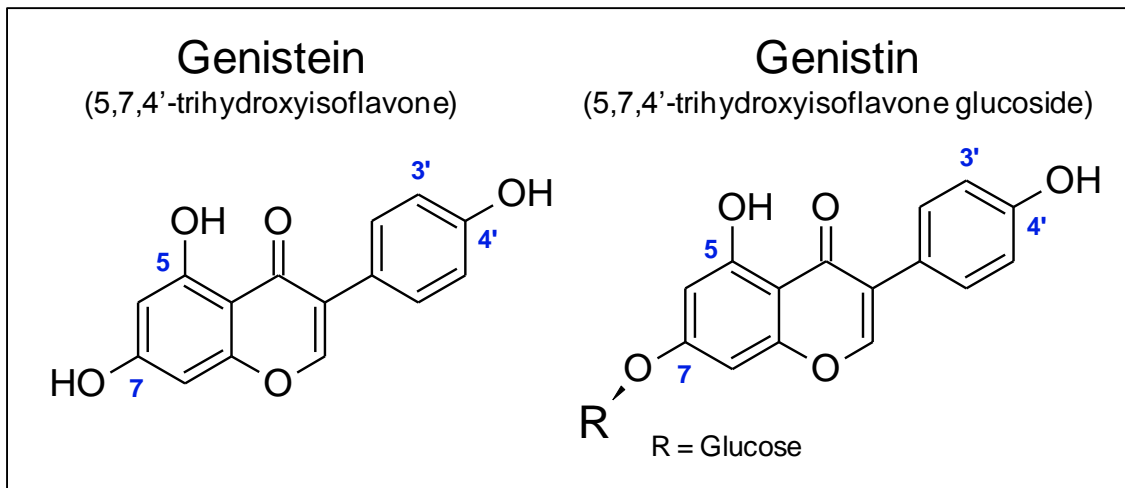


Figure 2.

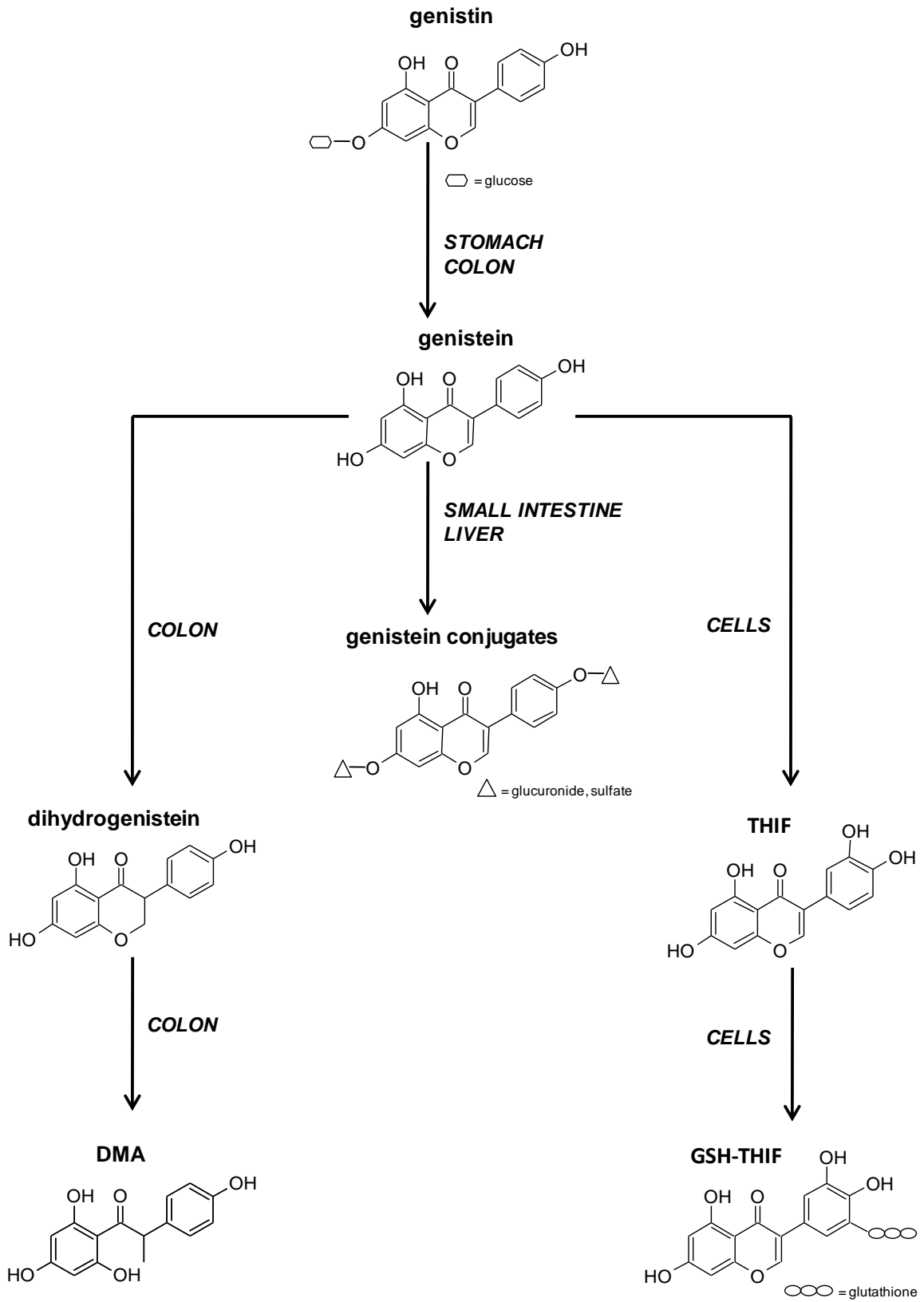


Figure 3.

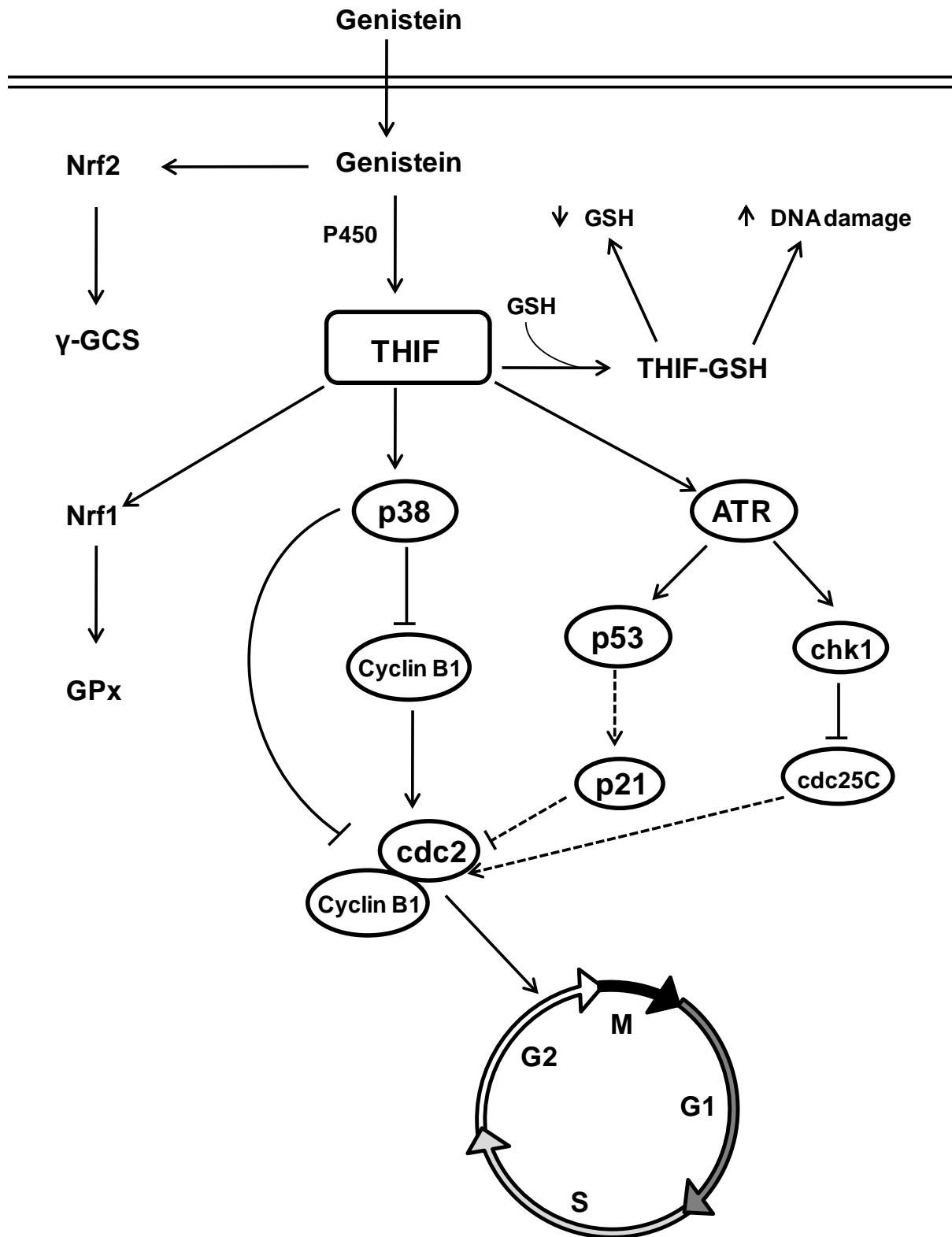


Figure legends

Figure 1. Chemical structure of genistein and genistin.

Figure 2. Genistein metabolic transformations. The glycoside genistin is not absorbed intact but hydrolyzed in the stomach and in the colon. Genistein can be absorbed by passive diffusion in the small intestine or undergo further biotransformation to a range of metabolites, such as dihydrogenistein and 6'-hydroxy-O demethylangolensin (DMA) formed in the colon. Genistein can also undergo metabolic conjugation to glucuronic acid or sulphuric acid in the intestinal epithelium and in the liver. Intracellularly, genistein is hydroxylated to 5,7,3',4'-tetrahydroxyisoflavone (THIF; orobol) and two glutathionyl conjugates of THIF can also be formed.

Figure 3. Genistein and THIF: intracellular mechanism of action. Genistein enters cells where it is subject to the CYP450-induced intracellular metabolism yielding THIF. THIF may also react with intracellular GSH to form the adduct THIF-GSH, inducing a reduction of intracellular GSH and an increased DNA damage. Genistein and THIF might induce the transcription of antioxidant enzymes: more specifically genistein directly activates the translocation of Nrf2, which will increase the transcription of γ -GCS, leading to increases in GSH synthesis, whereas THIF stimulates the release of Nrf1 with the subsequent translocation into the nucleus where it may interact with the electrophile response element and induce the transcription of GPx. THIF triggers the activation of the MAP kinase p38. Active p38 prevents the phosphorylation of cyclin B1 and hence its transport to the nucleus, an event essential for correct functioning of the cdc2-cyclin B1 complex. In addition, active p38 may undergo translocation to the nucleus where it directly inhibits the phosphorylation/activation of cdc2, thereby blocking entry of cells into mitosis (G2-M block).

THIF formation also induces the activation of ATR, accompanied by the phosphorylation of both p53 and Chk1, downstream signals playing an important role in DNA damage checkpoint control. One of p53 downstream targets is the tumour suppressor protein p21 Waf1/Cip1,

which can act as an inhibitor of cell cycle progression via its ability to inhibit cdc2. THIF-induced activation of Chk1 is paralleled by an inactivation of cdc25C phosphatase, causing it to be sequestered in the cytoplasm and preventing it from de-phosphorylating cdc2.

Summary points

- The isoflavone genistein is present in soy products, mainly in the form of glycosides such as genistin.
- Genistein consumption is associated to numerous potential health benefits, such as estrogenic activity, anticancer effects, antioxidant actions, anti-inflammatory activity and cardiovascular effects.
- The biological effects of genistein in cells and tissues will be dependent on its absorption, metabolism and distribution.
- The most prevalent forms in the diet do not necessarily give rise to the highest concentrations in vivo and metabolites may be present in relevant amounts.
- Glycosilated forms of genistein are not absorbed intact in humans and their bioavailability requires hydrolysis to occur in the stomach and/or large intestine.
- Genistein can be absorbed by passive diffusion in the small intestine, or can be converted to dihydrogenistein and 6'-hydroxy-O demethylangolensin (DMA) in the colon.
- Genistein can undergo phase II metabolic conjugation to glucuronic acid or sulphuric acid, catalyzed by UDP-glucuronyl transferase or sulfotransferase enzymes in the intestinal epithelium and in the liver.
- Intracellularly, genistein is subjected to metabolic transformation by CYP450 enzymes leading to the formation of both 5,7,3',4'-tetrahydroxyisoflavone (THIF; orobol) and two glutathionyl conjugates of THIF.
- THIF formation is crucial for cellular activity, acts via a mechanism involving oxidative DNA oxidation and the activation of downstream signaling pathways, and may mediate the effects of genistein intracellularly.

Key facts of flavonoids metabolism

- The biological properties of flavonoids in the diet and their activity in vivo are dependent on the extent of their biotransformation and conjugation during absorption.
- The most prevalent flavonoids in the diet do not necessarily correspond to the most bioactive forms in vivo.
- Glycosylation has a great influence on flavonoid absorption and glycosylated forms may not be absorbed intact in humans.
- Dietary flavonoids are subjected to extensive phase I (oxidation, reduction, hydrolysis) and phase II (conjugation to glucuronic acid, sulfate, glutathione) metabolic reactions catalized by enzymes such as cytochrome P450 found both in the small intestine and the liver.
- Further transformations of flavonoids may occur in the colon, where bacterial enzymes may catalyse many reactions including hydrolysis, dehydroxylation, demethylation, ring cleavage and decarboxylation as well as rapid de-conjugation, and are also able to catalyse the breakdown of the flavonoid backbone itself to simpler molecules such as phenolic acids.

References

- Adlercreutz, H. and Mazur, W. (1997). Phyto-oestrogens and Western diseases. *Ann Med.* 29(2): 95-120.
- Anthony, M. S., Clarkson, T. B. and Williams, J. K. (1998). Effects of soy isoflavones on atherosclerosis: potential mechanisms. *Am J Clin Nutr.* 68(6 Suppl): 1390S-1393S.
- Appelt, L. C. and Reicks, M. M. (1999). Soy induces phase II enzymes but does not inhibit dimethylbenz[a]anthracene-induced carcinogenesis in female rats. *J Nutr.* 129(10): 1820-1826.
- Cai, Q. and Wei, H. (1996). Effect of dietary genistein on antioxidant enzyme activities in SENCAR mice. *Nutr Cancer.* 25(1): 1-7.
- Cappelletti, V., Fioravanti, L., Miodini, P. and Di Fronzo, G. (2000). Genistein blocks breast cancer cells in the G(2)M phase of the cell cycle. *J Cell Biochem.* 79(4): 594-600.
- Cassidy, A., Bingham, S. and Setchell, K. D. (1994). Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. *Am J Clin Nutr.* 60(3): 333-340.
- Chinni, S. R., Alhasan, S. A., Multani, A. S., Pathak, S. and Sarkar, F. H. (2003). Pleiotropic effects of genistein on MCF-7 breast cancer cells. *Int J Mol Med.* 12(1): 29-34.
- Choi, C., Cho, H., Park, J., Cho, C. and Song, Y. (2003). Suppressive effects of genistein on oxidative stress and NFkappaB activation in RAW 264.7 macrophages. *Biosci Biotechnol Biochem.* 67(9): 1916-1922.
- Corona, G., Tzounis, X., Assunta Dessi, M., Deiana, M., Debnam, E. S., Visioli, F. and Spencer, J. P. (2006). The fate of olive oil polyphenols in the gastrointestinal tract: implications of gastric and colonic microflora-dependent biotransformation. *Free Radic Res.* 40(6): 647-658.
- Delmonte, P. and Rader, J. I. (2006). Analysis of isoflavones in foods and dietary supplements. *J AOAC Int.* 89(4): 1138-1146.

- Dowling, S., Regan, F. and Hughes, H. The characterisation of structural and antioxidant properties of isoflavone metal chelates. *J Inorg Biochem.* 104(10): 1091-1098.
- El Touny, L. H. and Banerjee, P. P. (2007). Akt GSK-3 pathway as a target in genistein-induced inhibition of TRAMP prostate cancer progression toward a poorly differentiated phenotype. *Carcinogenesis.* 28(8): 1710-1717.
- Fanti, P., Asmis, R., Stephenson, T. J., Sawaya, B. P. and Franke, A. A. (2006). Positive effect of dietary soy in ESRD patients with systemic inflammation--correlation between blood levels of the soy isoflavones and the acute-phase reactants. *Nephrol Dial Transplant.* 21(8): 2239-2246.
- Goodman, M. T., Wilkens, L. R., Hankin, J. H., Lyu, L. C., Wu, A. H. and Kolonel, L. N. (1997). Association of soy and fiber consumption with the risk of endometrial cancer. *Am J Epidemiol.* 146(4): 294-306.
- Guo, Q., Rimbach, G., Moini, H., Weber, S. and Packer, L. (2002). ESR and cell culture studies on free radical-scavenging and antioxidant activities of isoflavonoids. *Toxicology.* 179(1-2): 171-180.
- Han, R. M., Tian, Y. X., Liu, Y., Chen, C. H., Ai, X. C., Zhang, J. P. and Skibsted, L. H. (2009). Comparison of flavonoids and isoflavonoids as antioxidants. *J Agric Food Chem.* 57(9): 3780-3785.
- Head, K. A. (1998). Isoflavones and other soy constituents in human health and disease. *Alternative medicine review.* 3(1): 433-450.
- Henderson, B. E., Ross, R. K., Judd, H. L., Krailo, M. D. and Pike, M. C. (1985). Do regular ovulatory cycles increase breast cancer risk? *Cancer.* 56(5): 1206-1208.
- Hernandez-Montes, E., Pollard, S. E., Vauzour, D., Jofre-Montseny, L., Rota, C., Rimbach, G., Weinberg, P. D. and Spencer, J. P. (2006). Activation of glutathione peroxidase via Nrf1 mediates genistein's protection against oxidative endothelial cell injury. *Biochem Biophys Res Commun.* 346(3): 851-859.

- Horn-Ross, P. L., John, E. M., Canchola, A. J., Stewart, S. L. and Lee, M. M. (2003). Phytoestrogen intake and endometrial cancer risk. *J Natl Cancer Inst.* 95(15): 1158-1164.
- Ibrahim, A. S., El-Shishtawy, M. M., Pena, A., Jr. and Liou, G. I. (2010). Genistein attenuates retinal inflammation associated with diabetes by targeting of microglial activation. *Mol Vis.* 16: 2033-2042.
- Jones, J. L., Daley, B. J., Enderson, B. L., Zhou, J. R. and Karlstad, M. D. (2002). Genistein inhibits tamoxifen effects on cell proliferation and cell cycle arrest in T47D breast cancer cells. *Am Surg.* 68(6): 575-577; discussion 577-578.
- Ju, Y. H., Allred, K. F., Allred, C. D. and Helferich, W. G. (2006). Genistein stimulates growth of human breast cancer cells in a novel, postmenopausal animal model, with low plasma estradiol concentrations. *Carcinogenesis.* 27(6): 1292-1299.
- Ju, Y. H., Doerge, D. R., Allred, K. F., Allred, C. D. and Helferich, W. G. (2002). Dietary genistein negates the inhibitory effect of tamoxifen on growth of estrogen-dependent human breast cancer (MCF-7) cells implanted in athymic mice. *Cancer Res.* 62(9): 2474-2477.
- Kim, S. J., Kwon do, Y., Kim, Y. S. and Kim, Y. C. Peroxyl radical scavenging capacity of extracts and isolated components from selected medicinal plants. *Arch Pharm Res.* 33(6): 867-873.
- Kiriakidis, S., Hogemeier, O., Starcke, S., Dombrowski, F., Hahne, J. C., Pepper, M., Jha, H. C. and Wernert, N. (2005). Novel tempeh (fermented soyabean) isoflavones inhibit in vivo angiogenesis in the chicken chorioallantoic membrane assay. *Br J Nutr.* 93(3): 317-323.
- Lee, J. M., Hanson, J. M., Chu, W. A. and Johnson, J. A. (2001). Phosphatidylinositol 3-kinase, not extracellular signal-regulated kinase, regulates activation of the antioxidant-responsive element in IMR-32 human neuroblastoma cells. *J Biol Chem.* 276(23): 20011-20016.

- Lee, Y. W. and Lee, W. H. (2008). Protective effects of genistein on proinflammatory pathways in human brain microvascular endothelial cells. *J Nutr Biochem.* 19(12): 819-825.
- Li, J., Li, Z. and Mo, B. Q. (2006). [Effects of ERK5 MAPK signaling transduction pathway on the inhibition of genistein to breast cancer cells]. *Wei Sheng Yan Jiu.* 35(2): 184-186.
- Liggins, J., Bluck, L. J., Runswick, S., Atkinson, C., Coward, W. A. and Bingham, S. A. (2000). Daidzein and genistein contents of vegetables. *Br J Nutr.* 84(5): 717-725.
- Loftus, E. V., Jr. (2004). Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology.* 126(6): 1504-1517.
- McCabe, M. J., Jr. and Orrenius, S. (1993). Genistein induces apoptosis in immature human thymocytes by inhibiting topoisomerase-II. *Biochem Biophys Res Commun.* 194(2): 944-950.
- Messina, M. and Bennink, M. (1998). Soyfoods, isoflavones and risk of colonic cancer: a review of the in vitro and in vivo data. *Baillieres Clin Endocrinol Metab.* 12(4): 707-728.
- Messina, M. and Hilakivi-Clarke, L. (2009). Early intake appears to be the key to the proposed protective effects of soy intake against breast cancer. *Nutr Cancer.* 61(6): 792-798.
- Miksicek, R. J. (1995). Estrogenic flavonoids: structural requirements for biological activity. *Proc Soc Exp Biol Med.* 208(1): 44-50.
- Mitropoulou, T. N., Tzanakakis, G. N., Nikitovic, D., Tsatsakis, A. and Karamanos, N. K. (2002). In vitro effects of genistein on the synthesis and distribution of glycosaminoglycans/proteoglycans by estrogen receptor-positive and -negative human breast cancer epithelial cells. *Anticancer Res.* 22(5): 2841-2846.
- Molteni, A., Brizio-Molteni, L. and Persky, V. (1995). In vitro hormonal effects of soybean isoflavones. *J Nutr.* 125(3 Suppl): 751S-756S.
- Monks, T. J. and Lau, S. S. (1997). Biological reactivity of polyphenolic-glutathione conjugates. *Chem Res Toxicol.* 10(12): 1296-1313.
- Morton, M. S., Turkes, A., Denis, L. and Griffiths, K. (1999). Can dietary factors influence prostatic disease? *BJU Int.* 84(5): 549-554.

- Mousavi, Y. and Adlercreutz, H. (1993). Genistein is an effective stimulator of sex hormone-binding globulin production in hepatocarcinoma human liver cancer cells and suppresses proliferation of these cells in culture. *Steroids*. 58(7): 301-304.
- Nguyen, D. T., Hernandez-Montes, E., Vauzour, D., Schonthal, A. H., Rice-Evans, C., Cadenas, E. and Spencer, J. P. (2006). The intracellular genistein metabolite 5,7,3',4'-tetrahydroxyisoflavone mediates G2-M cell cycle arrest in cancer cells via modulation of the p38 signaling pathway. *Free Radic Biol Med*. 41(8): 1225-1239.
- Pavese, J. M., Farmer, R. L. and Bergan, R. C. (2010). Inhibition of cancer cell invasion and metastasis by genistein. *Cancer Metastasis Rev*. 29(3): 465-482.
- Peeters, P. H., Keinan-Boker, L., van der Schouw, Y. T. and Grobbee, D. E. (2003). Phytoestrogens and breast cancer risk. Review of the epidemiological evidence. *Breast Cancer Res Treat*. 77(2): 171-183.
- Peterson, G. and Barnes, S. (1991). Genistein inhibition of the growth of human breast cancer cells: independence from estrogen receptors and the multi-drug resistance gene. *Biochem Biophys Res Commun*. 179(1): 661-667.
- Peterson, G. and Barnes, S. (1993). Genistein and biochanin A inhibit the growth of human prostate cancer cells but not epidermal growth factor receptor tyrosine autophosphorylation. *Prostate*. 22(4): 335-345.
- Peterson, T. G., Coward, L., Kirk, M., Falany, C. N. and Barnes, S. (1996). The role of metabolism in mammary epithelial cell growth inhibition by the isoflavones genistein and biochanin A. *Carcinogenesis*. 17(9): 1861-1869.
- Piller, R., Chang-Claude, J. and Linseisen, J. (2006). Plasma enterolactone and genistein and the risk of premenopausal breast cancer. *Eur J Cancer Prev*. 15(3): 225-232.
- Raffoul, J. J., Wang, Y., Kucuk, O., Forman, J. D., Sarkar, F. H. and Hillman, G. G. (2006). Genistein inhibits radiation-induced activation of NF-kappaB in prostate cancer cells promoting apoptosis and G2/M cell cycle arrest. *BMC Cancer*. 6: 107.
- Reinli, K. and Block, G. (1996). Phytoestrogen content of foods--a compendium of literature values. *Nutr Cancer*. 26(2): 123-148.

- Richard, N., Porath, D., Radspieler, A. and Schwager, J. (2005). Effects of resveratrol, piceatannol, tri-acetoxystilbene, and genistein on the inflammatory response of human peripheral blood leukocytes. *Mol Nutr Food Res.* 49(5): 431-442.
- Santell, R. C., Kieu, N. and Helferich, W. G. (2000). Genistein inhibits growth of estrogen-independent human breast cancer cells in culture but not in athymic mice. *J Nutr.* 130(7): 1665-1669.
- Seibel, J., Molzberger, A. F., Hertrampf, T., Laudenschlager, U. and Diel, P. (2009). Oral treatment with genistein reduces the expression of molecular and biochemical markers of inflammation in a rat model of chronic TNBS-induced colitis. *Eur J Nutr.* 48(4): 213-220.
- Sergeev, I. N. (2004). Genistein induces Ca²⁺ -mediated, calpain/caspase-12-dependent apoptosis in breast cancer cells. *Biochem Biophys Res Commun.* 321(2): 462-467.
- Sethy-Coraci, I., Crock, L. W. and Silverstein, S. C. (2005). PAF-receptor antagonists, lovastatin, and the PTK inhibitor genistein inhibit H₂O₂ secretion by macrophages cultured on oxidized-LDL matrices. *J Leukoc Biol.* 78(5): 1166-1174.
- Smith, S., Sepkovic, D., Bradlow, H. L. and Auburn, K. J. (2008). 3,3'-Diindolylmethane and genistein decrease the adverse effects of estrogen in LNCaP and PC-3 prostate cancer cells. *J Nutr.* 138(12): 2379-2385.
- Spencer, J. P., Kuhnle, G. G., Williams, R. J. and Rice-Evans, C. (2003). Intracellular metabolism and bioactivity of quercetin and its in vivo metabolites. *Biochem J.* 372(Pt 1): 173-181.
- Suzuki, K., Koike, H., Matsui, H., Ono, Y., Hasumi, M., Nakazato, H., Okugi, H., Sekine, Y., Oki, K., Ito, K., Yamamoto, T., Fukabori, Y., Kurokawa, K. and Yamanaka, H. (2002). Genistein, a soy isoflavone, induces glutathione peroxidase in the human prostate cancer cell lines LNCaP and PC-3. *Int J Cancer.* 99(6): 846-852.
- Tiemann, U., Schneider, F., Vanselow, J. and Tomek, W. (2007). In vitro exposure of porcine granulosa cells to the phytoestrogens genistein and daidzein: effects on the biosynthesis of reproductive steroid hormones. *Reprod Toxicol.* 24(3-4): 317-325.

- Vauzour, D., Vafeiadou, K., Rice-Evans, C., Cadenas, E. and Spencer, J. P. (2007). Inhibition of cellular proliferation by the genistein metabolite 5,7,3',4'-tetrahydroxyisoflavone is mediated by DNA damage and activation of the ATR signalling pathway. *Arch Biochem Biophys.* 468(2): 159-166.
- Vergne, S., Sauvant, P., Lamothe, V., Chantre, P., Asselineau, J., Perez, P., Durand, M., Moore, N. and Bennetau-Pelissero, C. (2009). Influence of ethnic origin (Asian v. Caucasian) and background diet on the bioavailability of dietary isoflavones. *Br J Nutr.* 102(11): 1642-1653.
- Wang, J., Eltoum, I. E., Carpenter, M. and Lamartiniere, C. A. (2009). Genistein mechanisms and timing of prostate cancer chemoprevention in lobund-wistar rats. *Asian Pac J Cancer Prev.* 10(1): 143-150.
- Wang, J., Eltoum, I. E. and Lamartiniere, C. A. (2002). Dietary genistein suppresses chemically induced prostate cancer in Lobund-Wistar rats. *Cancer Lett.* 186(1): 11-18.
- Wang, T. T., Sathyamoorthy, N. and Phang, J. M. (1996). Molecular effects of genistein on estrogen receptor mediated pathways. *Carcinogenesis.* 17(2): 271-275.
- Williams, R. J., Spencer, J. P. and Rice-Evans, C. (2004). Flavonoids: antioxidants or signalling molecules? *Free Radic Biol Med.* 36(7): 838-849.
- Xu, J. and Loo, G. (2001). Different effects of genistein on molecular markers related to apoptosis in two phenotypically dissimilar breast cancer cell lines. *J Cell Biochem.* 82(1): 78-88.
- Yearley, E. J., Zhurova, E. A., Zhurov, V. V. and Pinkerton, A. A. (2007). Binding of genistein to the estrogen receptor based on an experimental electron density study. *J Am Chem Soc.* 129(48): 15013-15021.