http://www.expertreviews.org/

expert reviews in molecular medicine

Molecular mechanisms by which dietary isoflavones potentially prevent atherosclerosis

Aedin Cassidy, Sonia de Pascual Teresa and Gerald Rimbach

Dietary isoflavones are currently receiving much attention because of their potential role in preventing coronary artery disease and other chronic diseases. Accumulating evidence from cell culture and laboratory animal experiments indicates that isoflavones have the potential to prevent or delay atherogenesis. Suggested mechanisms of action include: a reduction in low-density lipoprotein (LDL) cholesterol and a potential reduction in the susceptibility of the LDL particle to oxidation; (2) an improvement in vascular reactivity; (3) an inhibition of pro-inflammatory cytokines, cell adhesion proteins and nitric oxide (NO) production; and (4) an inhibition of platelet aggregation. These mechanisms are consistent with the epidemiological evidence that a high consumption of isoflavone-rich soy products is associated with a reduced incidence of coronary artery disease. Biological effects of isoflavones are dependent on many factors, including dose consumed, duration of use, protein-binding affinity, and an individual's metabolism or intrinsic oestrogenic state. Further clinical studies are necessary to determine the potential health effects of isoflavones in specific population groups as we currently know little about age-related differences in exposure to these compounds and there are few guidelines on optimal dose for cardiovascular health benefits.

Aedin Cassidy (corresponding author)
Visiting Academic, Hugh Sinclair Human Nutrition Unit, School of Food Biosciences, University of Reading, Whiteknights, Reading, RG6 6AP, UK. Tel/Fax: +44 (0)1234 870498; E-mail: cassidyaedin@hotmail.com

Sonia de Pascual Teresa
Postdoctoral Scientist, Department of Plant Food Science and Technology, Instituto del Frio, Consejo Superior de Investigaciones Cientificas, Jose Antonio Novais 10, E-28040 Madrid, Spain. Tel: +34 91 549 23 00; Fax: +34 91 549 36 27; E-mail: soniapt@if.csic.es

Gerald Rimbach
Lecturer, Hugh Sinclair Human Nutrition Unit, School of Food Biosciences, University of Reading, Whiteknights, Reading, RG6 6AP, UK. Tel: +44 (0)118 931 6463; Fax: 0118 931 0080; E-mail: g.h.rimbach@reading.ac.uk

Accession information: DOI: 10.1017/S1462399403005264; Vol. 5; 30 September 2003

Interest in the physiological role of bioactive compounds present in plants has increased dramatically over the past decade. In relation to human health, particular attention has been given to the flavonoids, and especially one of their subclasses, the phytoestrogens. Phytoestrogens embody several groups of plant-derived, nonsteroidal compounds with oestrogen-like activity. The two major classes of dietary phytoestrogens are the isoflavones and lignans, both of which are widely distributed within the plant kingdom (Ref. 1). These compounds have a wide range of hormonal and nonhormonal activities in animals or in vitro, and these suggest plausible mechanisms for potential physiological effects of diets rich in isoflavones in humans (Refs 2, 3). In addition, experimental and epidemiological data are available to support the concept that isoflavone-rich diets exert physiological effects, and preliminary human studies suggest a role of isoflavones in the prevention of arteriosclerosis. This article reviews our current understanding of the potential molecular mechanisms of action by which dietary isoflavones potentially prevent atherosclerosis.

Introduction to phytoestrogens

The isoflavones are the most extensively studied of the phytoestrogen class; however, their occurrence in foods is limited largely to soyabeans and a few other legumes (Ref. 1). By contrast, lignans are widely distributed but have been relatively little studied due in part to difficulties in their isolation and analysis (Refs 4, 5).

Structure

The basic structural unit of the isoflavones comprises two benzene rings (A and B), linked via a heterocyclic pyrone ring (C) (Fig. 1) (Ref. 1). Although nonsteroidal in structure, it is the phenolic ring and, in particular, the 4'-OH (hydroxyl group) of the B-ring of isoflavones that are the essential structural component for interaction with oestrogen receptors (ERs); these structural elements are also found in raloxifene and tamoxifen, which are drug therapies used in breast cancer treatment because of their antagonistic effects on oestrogen (Ref. 6). By contrast, lignans as they occur in plants are not active oestrogens. Such activity is only achieved following degradation by the gut microflora to mammalian lignans. Lignans are defined as compounds that possess a 1,4-diarylbutane structure (Ref. 4).

Dietary sources

All soyabean-derived protein extracts and foods available for human consumption contain significant levels of isoflavones. However, there is a large variability in concentration and profile among these products that depends on species, geographical and environmental conditions,

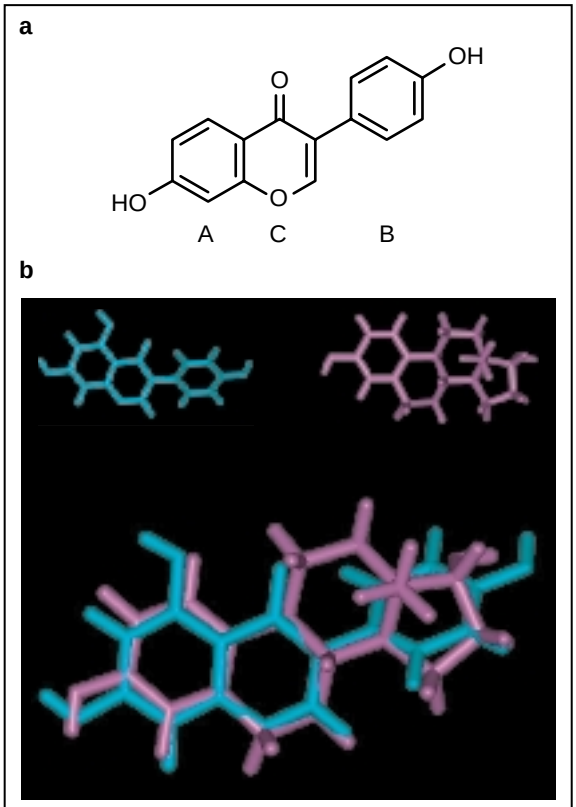


Figure 1. Structural similarity between isoflavones and oestrogens. (a) The basic structural unit of the isoflavones comprises two benzene rings (A and B), linked via a heterocyclic pyrone ring (C). (b) Isoflavones, such as genistein, are known as phytoestrogens because their chemical structure is similar to that of the female hormone oestrogen, as shown here from the superimposed structure of genistein (light blue) with oestradiol (purple), the most potent mammalian oestrogenic hormone **(fig001acr)**.

Accession information: DOI: 10.1017/S1462399403005264; Vol. 5; 30 September 2003

and the extent of industrial processing of the soyabeans (Ref. 1). Highest levels of isoflavones are found in soya flours and soya protein concentrates. Although tofu and soya milks contain significant amounts of isoflavones, concentrations vary considerably between type and brand. By contrast, soya oils contain only trace levels of isoflavones because the highly hydrophilic glycoside conjugates in soyabeans are unable to partition into the lipophilic oil (Ref. 1). In recent years, numerous extracts of soy or other sources of isoflavones have been produced commercially as supplements but, to date, there are limited data examining the relative clinical effectiveness of these preparations, and recent results suggest that quality assurance is a significant issue with commonly available isoflavone supplements (Ref. 7).

Although isoflavones have oestrogenic activity that is 100–1000-times weaker than oestradiol (the most potent mammalian oestrogenic hormone), some foods and dietary supplements contain comparatively high amounts of these compounds, so that plasma levels can exceed endogenous oestrogen levels by several orders of magnitude; therefore, these compounds have the potential to exert biological effects in vivo (Ref. 1). The daily dietary intake of isoflavones in Western populations is typically negligible (<1 mg/day). Soya has traditionally been a staple of Japanese and Chinese diets; however, the rapidly changing eating trends in Japan and China now make it difficult to determine accurately the intake of isoflavones in these countries. Recent estimates indicate intakes of 20–50 mg/day, but this might vary between urban and rural areas, and with other lifestyle factors (Ref. 1).

Absorption and metabolism

Over 20 years ago, it was established that the intestinal microflora plays a key role in the metabolism of phytoestrogens from the lignan and isoflavone classes (Refs 4, 8). Antibiotic administration blocks metabolism, and germ-free animals do not excrete the metabolites (Ref. 8). After ingestion, phytoestrogens are hydrolysed by intestinal glucosidases, and the resulting aglycones can be absorbed or further metabolised in the large gut to specific metabolites. Interest in gut metabolites has increased in recent years. In particular, the metabolism of the dietary isoflavone daidzein in the large intestine to equol has received attention because of the stronger binding affinity of equol compared with daidzein to ERs and preliminary evidence suggesting that it is a more potent modulator of hormonal status in healthy young women (Ref. 9).

Much of the available data on the absorption and metabolism of dietary phytoestrogens are of a qualitative nature in that it is known that dietary phytoestrogens are metabolised by intestinal bacteria, absorbed, conjugated in the liver, circulated in plasma and excreted in urine. Recent studies have addressed quantitatively what happens to isoflavones following ingestion, with data on pure compounds and stable isotopes complementing recent pharmacokinetic data for soy foods (Refs 7, 10). Knowledge of the pharmacokinetics of phytoestrogens is essential for making recommendations regarding long-term efficacy in clinical studies, as recent research suggests significant differences in bioavailability between foods rich in phytoestrogens and supplements (Ref. 7). In addition, the dose administered, the type of food and the chemical form of the compound appear to exert effects on the bioavailability (Ref. 10). Maintenance of a steady-state serum level should be optimal for clinical effectiveness of these compounds and, on the basis of recent pharmacokinetic data, this would be best achieved by divided doses of the soya food or supplement throughout the day, rather than by a single dose. Interestingly, the pharmacokinetic behaviour of phytoestrogens contrasts with that of synthetic environmental xenooestrogens: phytoestrogens have a half-life of 8–10 h, whereas xenooestrogens have long half-lifes and bioaccumulate in fat tissues (Ref. 10).

Mechanisms of action of phytoestrogens

ER-mediated mechanisms of action

The oestrogenic activity of isoflavones was first described in the 1940s when the infertility of sheep in Western Australia was proposed to be caused by ingestion of clover pastures rich in the isoflavone precursors formononetin and biochanin A (Ref. 11). Using an ER-dependent transcriptional response assay, Miksicek (Ref. 12) reported that of the isoflavone precursors, genistein had the highest oestrogenicity compared with daidzein, biochanin A and formononetin. It is this oestrogenic activity that is thought to be mainly responsible for several of the beneficial effects of isoflavones in hormone-dependent processes, such as reducing bone loss associated

Accession information: DOI: 10.1017/S1462399403005264; Vol. 5; 30 September 2003

with osteoporosis, improving menopausal symptoms, and lowering levels of plasma low-density lipoprotein (LDL) (which accumulates in blood vessel walls during arteriosclerosis) (Ref. 1).

There are two types of ER – ERα and ERβ – encoded by distinct genes: ERα and ERβ appear to serve distinct biological roles, as judged from the different phenotypes of mice devoid of each gene alone and the fact that they differ in the C-terminal ligand-binding domain and the N-terminal transactivation domain (Refs 13, 14, 15). ERβ is mainly expressed in nonreproductive tissues, such as the vascular system and bone, and seems to mediate in part the impact of oestrogens on the vasculature and the growth-promoting effects of oestrogens on nongonadal tissues (Ref. 14). By contrast, ERα is responsible for the classical hormonal effects, such as endometrial proliferation and mammary enlargement. The tissue distribution and binding affinities of ERs are summarised in Table 1. Following ligand binding, the ER dimerises before binding to target genes and modulating transcription. Activated ERs regulate transcription of target genes either directly by binding to regulatory DNA elements or indirectly by modulating the expression of other transcription factors, such as AP-1 or NF-κB. Thus, ERs can transactivate AP-1- or NF-κB-responsive genes (Refs 13, 14, 15).

The oestrogenic potency of isoflavones is low compared with 17-β-oestradiol, as soy isoflavones have ~1/3 and 1/1000 of the affinity of 17-β-oestradiol for ERβ and ERα, respectively (Refs 13, 15). Since genistein possesses a much higher binding affinity for ERβ than for ERα, isoflavones can be regarded as a type of natural 'selective ER modulator' (SERM). However, recent X-ray crystallographic studies examining the interaction of oestrogens, raloxifene and genistein with ERβ suggest that the orientation of raloxifene and genistein with ERβ is different from that of oestradiol, in particular in the interaction with helix 12 of the receptor (Ref. 1). Isoflavones lack specific lipophilic regions, which undoubtedly affect their ERβ-binding ability and the subsequent initiation of cellular events.

Use of the term SERMs for isoflavones suggests that they possess the beneficial physiological actions of natural oestrogens without the associated negative effects, in particular in tissues such as the breast (Ref. 16). Nevertheless, as mentioned above, although the reported oestrogenic potency of isoflavones is weak, their biological potential cannot be ignored, as typical circulating levels of isoflavones can exceed endogenous oestradiol concentrations by 10 000-fold following consumption of a diet containing soy foods (Ref. 17). However, once absorbed, only a small fraction of isoflavones circulates systemically in their unconjugated form. Most undergoes glucuronidation and sulphatation by the gut wall and liver, followed by enterohepatic circulation (Ref. 16). Recent data suggest that the formation of these glucuronide conjugates decreases the relative affinities of isoflavones for ERs (Ref. 18).

Table 1. Tissue distribution and binding affinities of oestrogen receptors (ERs) (tab001acr)

ER	Tissue distribution[a]	Relative binding affinity[b]		
		Oestrogen	Anti-oestrogen	Phytoestrogen
ERα	Adrenal gland, breast, kidney, ovary, testes, uterus	17-β-oestradiol (E_2): 100% 17-α-oestradiol: 58%	4-OH-tamoxifen: 178%	Genistein: 5%
ERβ	Bladder, bone, brain, breast, ovary, prostate, vascular system, uterus	17-β-oestradiol (E_2): 100% 17-α-oestradiol: 11%	4-OH-tamoxifen: 339%	Genistein: 36%

[a] Note that both ERα and ERβ are expressed in breast, ovary and uterus. For more information, see Refs 13 and 15.
[b] The relative binding affinity of each competitor was calculated as 'the ratio of concentrations of 17-β-oestradiol (E_2) and competitor required to reduce the specific radioligand binding by 50% (i.e. ratio of IC_{50} values)' according to Ref. 13. The relative binding affinity of E_2 was arbitrarily set at 100. The isoflavone genistein has a stronger binding affinity for ERβ (36%) than for ERα (5%).

Accession information: DOI: 10.1017/S1462399403005264; Vol. 5; 30 September 2003

Antioxidant activity

Several in vitro and in vivo studies have demonstrated that isoflavones might possess antioxidant properties, although it should be noted that most of these investigations have focused solely on the antioxidant effects of genistein (Ref. 19). Proposed molecular mechanisms responsible for its antioxidant potential include the ability to scavenge radicals, chelate metals, inhibit hydrogen peroxide (H_2O_2) production, and stimulate 'antioxidant' enzymes, including catalase, superoxide dismutase and glutathione peroxidase.

Guo et al. (Ref. 20) investigated the free-radical-scavenging and antioxidant activities of various structurally related isoflavones, including genistein, daidzein, biochanin A and genistin, in cell-free and endothelial cell model systems. The authors concluded that the free-radical-scavenging activities of some isoflavones might not substantially contribute to their antioxidant properties (Ref. 20). Thus, the ability of genistein and daidzein to increase cellular GSH (reduced glutathione) might make a more significant contribution to their biological action than their scavenging activities.

The antioxidant activities of genistein and daidzein might be affected by their extensive metabolism in the gut and liver. Indeed, Rimbach and coworkers (Ref. 21) have shown that isoflavone metabolites exhibit higher antioxidant activity than parent compounds in standard antioxidant assays, indicating that the metabolism of isoflavones affects their free-radical-scavenging and antioxidant properties. In a liposomal system, it has been demonstrated that genistein is a more effective antioxidant than daidzein, and this is likely to be attributable to its third OH group in the C-5 position (Ref. 22). Moreover, the precursors biochanin A and formononetin showed very weak antioxidant capacities in this in vitro system as they lack the C-4' OH group, which appears to be an important determinant of the antioxidant properties of isoflavones (Ref. 22). Equol showed superior antioxidant actions compared with both the precursor molecules and the parent isoflavones, suggesting that structural properties (the absence of the 2,3 double bond in conjunction with a loss of the 4-oxo group) enhances antioxidant properties (Refs 22, 23).

Isoflavones have also been shown to reduce LDL oxidation, as shown by significantly delayed LDL oxidation compared with baseline measurements in six healthy volunteers who consumed soy protein (60 mg/day isoflavones) for two weeks (Ref. 24). In vitro data are consistent with these findings, with Kapiotis et al. (Ref. 25) demonstrating that genistein inhibited the oxidation of LDL in the presence of copper ions or superoxide and nitric oxide (NO) radicals.

Cardiovascular effects of phytoestrogens: implications for arteriosclerosis

Cardiovascular disease (CVD) is the primary cause of death in Western societies (Ref. 26). Most myocardial infarctions, cerebrovascular events and peripheral vascular disease result from underlying atherosclerotic lesions, characterised by the focal accumulation of lipids, cells and connective tissue components within the arterial wall (Ref. 27). The mechanisms involved in the development of arteriosclerosis have not been fully established, but there is a consensus that the expression by endothelial cells of inflammatory cytokines, adhesion molecules and chemotactic proteins plays a key role (Ref. 28).

Epidemiological studies suggest that differences in diet might explain the lower incidence of CVD in Japan compared with other industrialised countries such as the USA or the UK. The high dietary intake of isoflavones in Japan is thought to be in part responsible (Ref. 29). Potential anti-atherogenic effects of isoflavones (summarised in Table 2) include: (1) a reduction in low-density lipoprotein (LDL) cholesterol and a potential reduction in the susceptibility of the LDL particle to oxidation; (2) an improvement in vascular reactivity; (3) an inhibition of pro-inflammatory cytokines, cell adhesion proteins and NO production; and (4) an inhibition of platelet aggregation.

Lipid metabolism

Animal studies

The hypocholesterolaemic effect of soy protein has been known for many decades (Ref. 30). In many animal species, substituting dietary animal protein with soy protein consistently reduces LDL cholesterol and total cholesterol levels (Ref. 31). Gerbils fed soy-based diets have significantly lower levels of total cholesterol, LDL plus VLDL (very-low-density lipoprotein) cholesterol, and apolipoprotein B concentrations (Ref. 32). Isoflavone consumption led to a 30% decrease in plasma cholesterol levels and a 50% reduction in

Accession information: DOI: 10.1017/S1462399403005264; Vol. 5; 30 September 2003

Table 2. Potential mechanisms by which isoflavones protect against arteriosclerosis (tab002acr)

Isoflavone property	Mechanisms
Antioxidant activity	Inhibition of low-density lipoprotein (LDL) oxidation Stimulation of antioxidant enzymes Induction of glutathione (GSH) synthesis
Hypocholesterolaemic effects	Increased bile acid secretion Increased LDL receptor activity Reduced cholesterol absorption from gut
Gene-regulatory activity	Inhibition of NF-κB-dependent signal transduction pathways Inhibition of protein tyrosine kinase activity Inhibition of inducible nitric oxide production in macrophages Downregulation of expression of cell adhesion molecules and pro-inflammatory cytokines
Platelet function and vascular effects	Inhibition of platelet aggregation Improvement of vascular reactivity

atherosclerotic lesion area in a strain of mice with low HDL (high-density lipoprotein) cholesterol (Ref. 33). Soy protein containing isoflavones decreased LDL cholesterol and increased HDL cholesterol in a group of female monkeys fed a moderately atherogenic diet (Refs 34, 35), and when a state of menopause was experimentally established in this animal model (by ovariectomy), soy protein consumption, as compared with casein consumption, significantly improved plasma lipids and lipoprotein concentrations.

The key issue of whether the response to soy protein is mediated through the presence of isoflavones has been the focus of much attention. It appears that when isolated soy protein is alcohol-washed and most, but not all, of the isoflavones are removed, there is only a marginal reduction in plasma cholesterol (Ref. 36). The hypocholesterolaemic effect, however, is restored when the isoflavones are added back to the alcohol-washed isolated soy protein. Nevertheless, isoflavones alone have little or no cholesterol-lowering effect in cynomologus monkeys (Refs 35, 36).

The mechanisms by which isoflavones lower plasma cholesterol levels have not been elucidated fully but evidence suggests that the LDL receptor is necessary for the isoflavones to affect lipoprotein metabolism (Refs 33, 36), which suggests that isoflavones increase LDL catabolism and thus bile acid excretion. However, further research is required to address these issues.

Clinical studies

Clinically, soy protein has been effectively used in the therapy of patients with hypercholesterolaemia for several decades (Ref. 31). The mechanism of action of the cholesterol-lowering effect is still poorly understood and is almost certainly multifactorial. A meta-analysis of 38 clinical studies concluded that the mean reduction in total serum cholesterol was 9.3%, whereas LDL decreased by 12.9% with soya protein extracts (Ref. 30). Individuals with the highest initial cholesterol levels experienced the greatest reduction. In one study of premenopausal women with normal cholesterol levels, a cholesterol-lowering effect from a diet of soy protein was observed: mean total cholesterol dropped 9.6% when 60 g of textured vegetable soy protein, delivering 45 mg of isoflavones, were consumed each day over a one-month period (Refs 9, 37).

An intake of 25 g/day of soya protein extract would be associated with a 0.23 mmol/l decrease in serum cholesterol (Ref. 30). On the basis of this evidence and further clinical studies, the US Food and Drug Administration (FDA) approved a health claim for cholesterol reduction based on an intake of 25 g soya protein per day. This intake is higher than the current daily intake in Japan and it is unknown whether life-time exposure to diets rich in these compounds accounts for the lower blood cholesterol and coronary heart disease rates in the Asian populations. A Japanese

Accession information: DOI: 10.1017/S1462399403005264; Vol. 5; 30 September 2003

health check-up study of 3596 women observed a strong inverse relationship between daily soy protein intake and serum cholesterol. The average soy protein intake for women was 6.88 g (Ref. 38), which is calculated as an isoflavone intake of 10–30 mg/day. The FDA drew no conclusion regarding the role of isoflavones in the cholesterol-lowering effect, but a recent study has shown that isoflavones present naturally within the soy food play a significant role in lowering plasma LDL and their absence from soya renders the food ineffective in reducing cholesterol levels. This work showed a linear dose–response relationship between dietary isoflavone content and cholesterol reduction, with no lowering effect observed when isoflavones were removed from the soy protein (Ref. 39). By contrast, isoflavones alone (fed as pure compounds or extract) have consistently been found to have no lipid-lowering effect (Refs 40, 41, 42, 43). This indicates that the mechanism of action of isoflavones on lipids is complex and probably involves an interaction with the soya food matrix.

In addition to the well-known effects of oestrogen on lipids, oestrogen exerts several endothelium-dependent effects related to both vasodilation and NO metabolism that might have a favourable effect on vascular health. However, this proposed benefit has not yet been confirmed in randomised clinical trials. Indeed recent large-scale clinical trials have failed to document a benefit of hormone replacement therapy in women with established coronary heart disease (Ref. 44), and these data are suggestive of oestrogen therapy having a prothrombic or pro-inflammatory effect that might offset any other potential benefits. The extent to which SERMs and phytoestrogens share in these beneficial and potentially harmful effects of oestrogen is not yet established.

Women appear to be protected from coronary heart disease in their premenopausal years but, after the menopause, rates increase dramatically (Ref. 29). The loss of endogenous oestrogen that occurs with menopause deleteriously alters lipoprotein metabolism, leading to an increased risk of coronary heart disease as a result of raised cholesterol levels, increased susceptibility of lipids to become oxidised, and adverse effects on the quality of blood vessels (Ref. 45). The lower incidence of coronary heart disease in Asian countries compared with Western countries, and the lower rates in vegetarians compared with omnivores (Refs 1, 8), suggest that this disease is largely preventable and dietary intake might be an important factor in its aetiology.

Vascular reactivity

Oestrogens exert potent effects on the arterial wall, and this was first recognised more than 40 years ago. The endothelial wall of the blood vessel has been found to have almost equal proportions of ERα and ERβ (Refs 13, 46), and these two receptors play an important role in the vasoreactivity of the blood vessels. ERα rapidly activates endothelial-derived NO synthase (eNOS), the key enzyme responsible for NO-induced dilation of the blood vessels (Ref. 47). This is a nongenomic and rapid event that is reduced in atherosclerotic arteries. Studies of the ERα knockout and ERβ knockout mice attest to the important role that these two receptors play in vascular events related to CVD; recent data show ERβ-deficient mice have numerous functional abnormalities in vascular smooth muscle cells and blood vessels, suggesting an essential role for ERβ in the regulation of blood pressure and vascular function (Ref. 48). Therefore, perhaps the greatest benefits of a diet rich in isoflavones might be their effects on improving the quality of blood vessels, rather than effects on blood cholesterol levels per se.

Vascular constriction is associated with an increased risk of arteriosclerosis and hypertension. The few animal and clinical studies conducted to date suggest that isoflavones can improve vascular compliance. Isoflavones increase blood vessel dilation and improve blood flow in rhesus monkeys (Ref. 49). Soy isoflavones were also shown to promote arterial dilation and inhibit constriction in a group of female rhesus monkeys fed an atherogenic diet containing soy isoflavones (Ref. 50). In vitro platelet aggregation in response to thrombin and serotonin was also less when compared with female monkeys consuming an atherogenic diet from which soy isoflavones had been removed. Most of these effects have also been confirmed in human studies (Refs 40, 51).

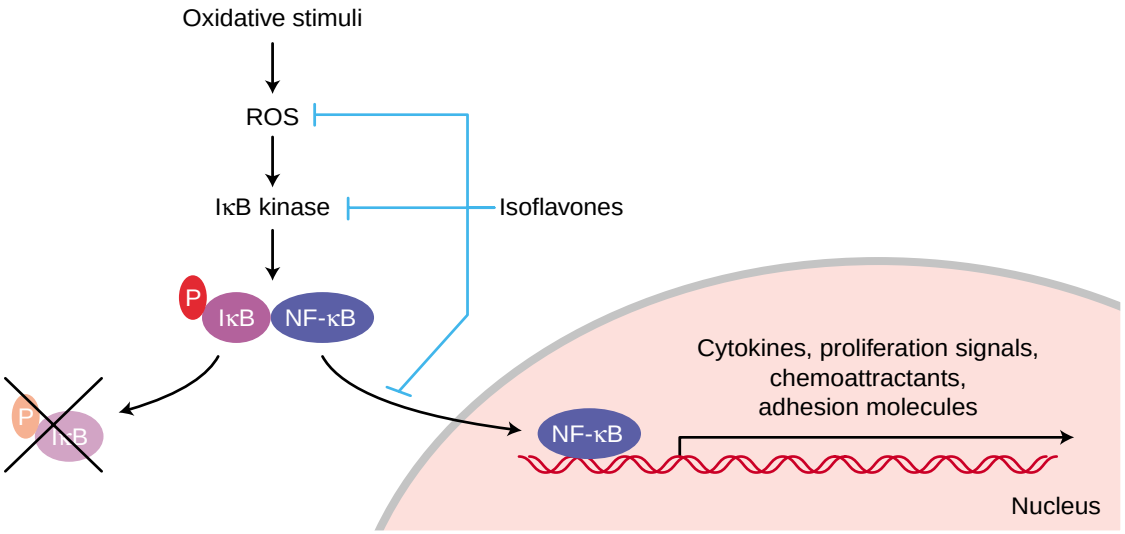
Inflammation and cell adhesion

Several in vitro model systems have been used to examine the potential anti-inflammatory and anti-cell-adhesion properties of compounds such as the phytoestrogens. However, most of the studies conducted have used concentrations of isoflavones that are unlikely to be achievable in vivo.

Accession information: DOI: 10.1017/S1462399403005264; Vol. 5; 30 September 2003

Activation of the endothelium results in the release of vascular cytokines such as interleukin 1β (IL-1β) and tumour necrosis factor α (TNF-α). These cytokines in turn induce the cell-surface expression of adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1), which are centrally involved in the endothelial recruitment of leukocytes (Ref. 52). Focal expression of ICAM-1 and VCAM-1 has been reported in arterial endothelium overlying early foam cell lesions in both dietary and genetic models of arteriosclerosis in rabbits (Ref. 53). This expression, together with the activation of monocyte chemoattractant protein 1 (MCP-1), leads to infiltration of mononuclear cells into the artery wall (Ref. 54). The uptake of oxidised LDL by these cells leads to the formation of lipid-laden foam cells, and the development or progression of atherosclerotic plaques (Ref. 55).

Transcription of ICAM-1, VCAM-1 and MCP-1 is dependent, at least in part, on the activation of NF-κB, a classical member of the Rel family of transcription factors. In unstimulated cells, NF-κB is inactivated by sequestration to the IκB family of inhibitor proteins. Agents that activate NF-κB induce degradation of IκB. Free NF-κB then enters the nucleus and binds to the regulatory regions of its target genes (Ref. 56). The activity of NF-κB is controlled by the redox status of the cell, and the generation of reactive oxygen species might be a common step in all of the



The NF-κB pathway as a potential molecular target of isoflavones

Figure 2. The NF-κB pathway as a potential molecular target of isoflavones. Simplified scheme of oxidant/antioxidant regulation of activation of the transcription factor NF-κB. In unstimulated cells, NF-κB is inactivated by sequestration to the IκB family of inhibitor proteins. Various stimuli, including H_2O_2, organic peroxides, phorbol esters, ultraviolet radiation, cytokines such as TNF-α, and lipopolysaccharide, that lead to an increase of intracellular reactive oxygen species (ROS) result in phosphorylation of the inhibitory subunit of IκB and its subsequent proteolysis. Once released from IκB, the dimeric NF-κB translocates into the nucleus and binds to corresponding promoter enhancer region of various target genes implicated in atherogenesis, such as those encoding cytokines (e.g. TNF-α, IL-1), proliferation signals (e.g. CSFs), chemoattractants (e.g. MCP-1) and adhesion molecules (e.g. ICAM-1 and VCAM-1). Isoflavones might counter activation of NF-κB by reducing the concentration of intracellular ROS, inhibiting IκB kinases, or reducing NF-κB translocation and DNA binding. Abbreviations: CSF, colony-stimulating factor; ICAM-1, intercellular adhesion molecule 1; IL-1, interleukin 1; MCP-1, monocyte chemoattractant protein 1; TNF-α, tumour necrosis factor α; VCAM-1, vascular cell adhesion molecule 1**(fig002acr)**.

Accession information: DOI: 10.1017/S1462399403005264; Vol. 5; 30 September 2003

signalling pathways that lead to IκB degradation (Fig. 2). NF-κB activation is inhibited by several chemically distinct antioxidants, including *N*-acetylcysteine (NAC), dithiocarbamates, vitamin E derivatives, glutathione peroxidase (GPx) activators and various metal chelators (Ref. 57), as well as flavonoids (Ref. 58).

Several observations suggest a key role for NF-κB in atherogenesis. Importantly, activated NF-κB has been identified in situ in human atherosclerotic plaques (Ref. 59) as well as in an arterial injury model (Ref. 60), but not in cells of normal vessels devoid of arteriosclerosis. Furthermore, NF-κB is activated by an atherogenic diet and by oxidised LDL (Ref. 61), as well as by advanced glycosated end products (Ref. 62).

In an in vitro model system using human monocytes, lipopolysaccharide (LPS) increased both NF-κB binding to DNA consensus sites and TNF-α release (Ref. 63). At pharmacological concentrations, genistein (40 μM) attenuated both NF-κB DNA binding and TNF-α release. Genistein is known to inhibit protein tyrosine kinase (PTK) (Ref. 64), so these results suggest that LPS-induced NF-κB activation and TNF-α release in human monocytes is PTK dependent (Ref. 63). At these high concentrations, genistein also inhibited NF-κB DNA-protein binding in LPS-stimulated monocytes by approximately 50% (Ref. 47), and genistein (50 μM), but not daidzein, inhibited TNF-α-induced NF-κB activation in cultured human lymphocytes. Furthermore, consumption of a soy-based dietary supplement containing 50 mg of an isoflavone mixture (genistein, daidzein and glycitin) twice daily for three weeks was shown to reduce ex vivo NF-κB activation induced by TNF-α in peripheral lymphocytes in healthy male volunteers (Ref. 65). These in vitro and in vivo data suggest anti-inflammatory properties of isoflavones.

Following stimulation with the pro-inflammatory cytokines IL-1 and TNF-α, endothelial cells express the leukocyte adhesion molecules E-selectin, VCAM-1 and ICAM-1. Several studies have investigated cytokine expression following exposure to genistein and the intracellular signalling mechanisms that might be involved. Genistein dose-dependently inhibited the maximal E-selectin expression induced by incubation of human umbilical vein endothelial cells (HUVEC) for 4 h with TNF-α and IL-1 (Ref. 66). Furthermore, VCAM-1 secretion was inhibited by genistein after stimulation with TNF-α or IL-1. By contrast, genistein did not alter ICAM-1 secretion after a 24 h incubation with either of the two cytokines (Ref. 66). Weber et al. (Ref. 67) investigated the role of tyrosine phosphorylation in the induction of endothelial leukocyte adhesion molecule 1 (ELAM-1), VCAM-1 and ICAM-1. Pre-treatment of the cells with genistein resulted in a dose-dependent inhibition of TNF-α-induced ELAM-1, VCAM-1 and ICAM-1 surface expression. In a further study examining the effects of genistein as a PTK inhibitor of leukocyte (neutrophil, lymphocyte and monocyte) adhesion to endothelial cells, IL-1 and TNF-α-stimulated neutrophil and monocyte adhesion was significantly inhibited by genistein compared with stimulated control cells (Ref. 68).

Monocyte-derived macrophages are the principal inflammatory cell in the atheromata. In early stages of artherosclerotic lesion formation, macrophages and endothelial cells interact to trigger a cycle of events that exacerbates endothelial dysfunction, resulting in a loss of homeostatic control (Ref. 69). Activated macrophages generate large amounts of NO from L-arginine by the action of inducible NOS (iNOS), and its overproduction has been associated with oxidative stress and chronic inflammation (Ref. 70). NO is an important intracellular and intercellular regulator of many biological functions, including macrophage-mediated cytotoxicity (Refs 71, 72). Cytokines such as interferon γ (IFN-γ) and other inflammatory stimuli such as bacterial LPS regulate the activity of iNOS in macrophages (Refs 73, 74). Genistein was found to inhibit nitrite production in a dose-dependent manner when rat mesangial cells were activated with IL-1β. This finding suggests a central role for PTK in the signalling pathway of IL-1β, resulting in the activation of iNOS in rat mesangial cells (Ref. 75). Li et al. (Ref. 76) showed that genistein had an inhibitory effect on NO production in an immortalised astrocyte cell line that was activated using a three-cytokine mixture (TNF-α, IL-1 and IFN-γ) designed to induce iNOS maximally.

In a further study, Gottstein et al. (Ref. 77) found that in a macrophage cell line activated with IFN-γ plus LPS, genistein and daidzein significantly inhibited NO production and TNF-α secretion. iNOS mRNA levels remained unchanged by the isoflavone treatment, which suggests that the inhibitory effect is post-transcriptional. This suppression of LPS activity

Accession information: DOI: 10.1017/S1462399403005264; Vol. 5; 30 September 2003

has also been shown for other flavonoids (Ref. 78). Sheu et al. (Ref. 79) reported an inhibitory effect of genistein and daidzein on LPS-induced expression of the iNOS gene in macrophages, but the coincubation of LPS and isoflavones might have given rise to this effect: inhibition of iNOS expression might have been caused by a direct interaction of isoflavones with the LPS molecule, rather than a direct effect on the cell. In the Gottstein et al. study (Ref. 77), macrophages were preincubated with genistein and daidzein for 24 h before the addition of IFN-γ and LPS in order to avoid any direct chemical interaction. The inhibition of NO production observed in this study might reflect inhibition of TNF-α secretion by genistein and daidzein, as it has previously been demonstrated that TNF-α is crucial for the induction of NO synthesis in IFN-γ- and/or LPS-stimulated macrophages (Refs 80, 81).

MCP-1 is a CC chemokine that might play a key role in atherogenesis since it is involved in the recruitment of monocytes and T cells into the arterial wall. MCP-1 mRNA has been detected in atherosclerotic lesions by in situ hybridisation (Refs 82, 83). Furthermore, a decrease in atherosclerotic lesion size is seen in mice deficient for the MCP-1 receptor CCR-2, and fewer macrophages and monocytes are present in their aortas (Ref. 84). Therapeutic drugs and dietary factors targeting MCP-1 and/or its receptor might prove useful in the prevention of atherosclerotic lesion development. Recently, it has been shown that genistein and daidzein dose-dependently downregulated MCP-1 secretion (Ref. 77), indicating that both of these isoflavones might have the potential to inhibit monocyte infiltration into the arterial wall. It is known that the expression of MCP-1 is regulated at the transcriptional level (Ref. 54). Therefore, it is hypothesised that genistein and daidzein might regulate TNF-α-induced MCP-1 expression through transcription factors such as NF-κB and AP-1, which have binding sites in the promoter region of the MCP-1 gene.

Platelet aggregation and endothelium reactivity

In vitro data clearly suggest that both genistein and daidzein have anti-aggregatory activity in human platelets (Ref. 77). This finding is consistent with earlier reports showing that the consumption of soy protein and its isoflavone-enriched fraction lowers platelet aggregation in rats (Ref. 85). The exact molecular mechanisms by which isoflavones affect platelet aggregation are unclear and currently under investigation. Apart from PTK inhibition (Ref. 86) within the cyclooxygenase pathway, several other reported molecular effects of flavonoids might influence platelet function. The modification of platelet cyclic-3',5'-adenosine monophosphate (cAMP) via the inhibition of phosphodiesterase activity is the most supported pathway for anti-aggregatory effects of flavonoids (Ref. 87). Inhibition of lipoxygenase activity, as demonstrated principally for the flavonoids myricetin and quercetin (Ref. 88), is another possible mechanism. Stimulation of adenylate cyclase, leading to increased cAMP levels, has been proposed as a further anti-aggregatory signal transduction pathway (Ref. 89).

In addition, the antioxidant character of isoflavones might play a role in inhibiting platelet aggregation. Pignatelli et al. (Ref. 90) showed that collagen-induced platelet aggregation was associated with the production of H_2O_2, which acts as an important second messenger in platelets, stimulating both the phospholipase C pathway and arachidonic acid metabolism. Consistent with this finding, platelets primed with nonactivating concentrations of arachidonic acid or collagen were activated by nanomolar concentrations of H_2O_2 (Ref. 91). Since isoflavones possess antioxidant properties (Refs 22, 92) and can scavenge radicals, the evidence that reactive oxygen species are involved in platelet stimulation suggests another anti-aggregatory mechanism. In comparison with daidzein, the genistein molecule contains an additional hydroxyl group in the C-5 position, possibly resulting in a higher antioxidant activity (Ref. 93). This might explain why genistein has been demonstrated to be a more potent inhibitor of platelet aggregation than daidzein.

Conclusion

Dietary isoflavones are currently receiving much attention because of their potential role in preventing coronary artery disease and other chronic diseases. In the overall scheme of cardiovascular protection, isoflavones appear potentially to have a more important role in conditioning the vascular tree than in influencing cholesterol levels. The preferential binding of isoflavones to ERβ and the increasing recognition of the role of this receptor in the endothelial wall

Accession information: DOI: 10.1017/S1462399403005264; Vol. 5; 30 September 2003

provide justification for increasing the awareness of the 'heart-health effects' of diets rich in these phytoestrogens. Furthermore, such effects are not restricted to soy isoflavones but also apply to lignans and other flavonoids, which are abundant in plant foods. Potential anti-atherogenic effects of isoflavones include: a reduction in LDL cholesterol; modulation of pro-inflammatory cytokines, cell adhesion proteins and NO formation; protection of LDL against oxidation; inhibition of platelet aggregation; and an improvement in vascular reactivity. To date, most of the published in vitro data have reported beneficial effects only following exposure to pharmacological doses of isoflavones; thus further research is required to understand the importance of this mechanism following exposure to physiologically relevant levels of isoflavones and their corresponding liver and gut metabolites. Although epidemiological data and laboratory studies allude to the possible protective effects of soy isoflavones at specific target tissues, randomised placebo-controlled clinical trials are necessary to address further the relative importance of these compounds for cardiovascular health.

Acknowledgements and funding

The authors thank the peer reviewers for refereeing this manuscript. This work was supported by the EU grant Isoheart QLK1-CT-2001-00221.

References

1 Setchell, K.D. and Cassidy, A. (1999) Dietary isoflavones: biological effects and relevance to human health. J Nutr 129, 758S-767S, PubMed: 10082786

2 Cassidy, A. and Faughnan, M. (2000) Phyto-oestrogens through the life cycle. Proc Nutr Soc 59, 489-496, PubMed: 10997682

3 Bingham, M. et al. (2003) Gut metabolism and cardioprotective effects of dietary isoflavones. Curr Topics Nutraceutical Res 1, 31-48

4 Setchell, K.D. et al. (1981) Lignan formation in man—microbial involvement and possible roles in relation to cancer. Lancet 2, 4-8, PubMed: 6113409

5 Thompson, L.U. et al. (1991) Mammalian lignan production from various foods. Nutr Cancer 16, 43-52, PubMed: 1656395

6 Brzozowski, A.M. et al. (1997) Molecular basis of agonism and antagonism in the oestrogen receptor. Nature 389, 753-758, PubMed: 9338790

7 Setchell, K.D. et al. (2001) Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. J Nutr 131, 1362S-1375S, PubMed: 11285356

8 Setchell, K.D. et al. (1984) Nonsteroidal estrogens of dietary origin: possible roles in hormone-dependent disease. Am J Clin Nutr 40, 569-578, PubMed: 6383008

9 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, 333-340, PubMed: 8074062

10 Setchell, K.D. et al. (2003) Comparing the pharmacokinetics of daidzein and genistein with the use of 13C-labeled tracers in premenopausal women. Am J Clin Nutr 77, 411-419, PubMed: 12540402

11 Bennets, H.W., Underwood, E.J. and Shier, F.L. (1946) A specific breeding problem of sheep in subterranean clover pastures in western Australia. Australian Veterinary Journal 22, 2-12

12 Miksicek, R.J. (1994) Interaction of naturally occurring nonsteroidal estrogens with expressed recombinant human estrogen receptor. J Steroid Biochem Mol Biol 49, 153-160, PubMed: 8031711

13 Kuiper, G.G. et al. (1997) Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138, 863-870, PubMed: 9048584

14 Kuiper, G.G. et al. (1996) Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci U S A 93, 5925-5930, PubMed: 8650195

15 Kuiper, G.G. et al. (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 139, 4252-4263, PubMed: 9751507

16 Setchell, K.D. (2001) Soy isoflavones—benefits and risks from nature's selective estrogen receptor modulators (SERMs). J Am Coll Nutr 20, 354S-362S; discussion 381S-383S, PubMed: 11603644

17 Adlercreutz, H., Markkanen, H. and Watanabe, S. (1993) Plasma concentrations of phyto-oestrogens in Japanese men. Lancet 342, 1209-1210, PubMed: 7901532

18 Zhang, Y. et al. (1999) Daidzein and genistein glucuronides in vitro are weakly estrogenic and activate human natural killer cells at nutritionally relevant concentrations. J Nutr 129, 399-405, PubMed: 10024618

19 Wei, H. et al. (1993) Inhibition of tumor

Accession information: DOI: 10.1017/S1462399403005264; Vol. 5; 30 September 2003

promoter-induced hydrogen peroxide formation in vitro and in vivo by genistein. Nutr Cancer 20, 1-12, PubMed: 8415125
20 Guo, Q. et al. (2002) ESR and cell culture studies on free radical-scavenging and antioxidant activities of isoflavonoids. Toxicology 179, 171-180, PubMed: 12204553
21 Rimbach, G. et al. Antioxidant and free radical scavenging activity of isoflavone metabolites. Xenobiotica (in press)
22 Arora, A., Nair, M.G. and Strasburg, G.M. (1998) Antioxidant activities of isoflavones and their biological metabolites in a liposomal system. Arch Biochem Biophys 356, 133-141, PubMed: 9705203
23 Mitchell, J.H. et al. (1998) Antioxidant efficacy of phytoestrogens in chemical and biological model systems. Arch Biochem Biophys 360, 142-148, PubMed: 9826439
24 Tikkanen, M.J. et al. (1998) Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. Proc Natl Acad Sci U S A 95, 3106-3110, PubMed: 9501223
25 Kapiotis, S. et al. (1997) Genistein, the dietary-derived angiogenesis inhibitor, prevents LDL oxidation and protects endothelial cells from damage by atherogenic LDL. Arterioscler Thromb Vasc Biol 17, 2868-2874, PubMed: 9409268
26 Poulter, N. (1999) Coronary heart disease is a multifactorial disease. Am J Hypertens 12, 92S-95S, PubMed: 10555607
27 Badimon, L. (2001) New challenges in the etiopathogenesis of atherothrombosis. Cerebrovasc Dis 11 Suppl 1, 80-84, PubMed: 11244204
28 Collins, T. and Cybulsky, M.I. (2001) NF-kappaB: pivotal mediator or innocent bystander in atherogenesis? J Clin Invest 107, 255-264, PubMed: 11160146
29 Cassidy, A. and Griffin, B. (1999) Phyto-oestrogens: a potential role in the prevention of CHD? Proc Nutr Soc 58, 193-199, PubMed: 10343357
30 Anderson, J.W., Johnstone, B.M. and Cook-Newell, M.E. (1995) Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med 333, 276-282, PubMed: 7596371
31 Sirtori, C.R., Even, R. and Lovati, M.R. (1993) Soybean protein diet and plasma cholesterol: from therapy to molecular mechanisms. Ann N Y Acad Sci 676, 188-201, PubMed: 8489131
32 Tovar-Palacio, C. et al. (1998) Intake of soy protein and soy protein extracts influences lipid metabolism and hepatic gene expression in gerbils. J Nutr 128, 839-842, PubMed: 9566990
33 Kirk, E.A. et al. (1998) Dietary isoflavones reduce plasma cholesterol and atherosclerosis in C57BL/6 mice but not LDL receptor-deficient mice. J Nutr 128, 954-959, PubMed: 9614153
34 Anthony, M.S. et al. (1996) Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. J Nutr 126, 43-50, PubMed: 8558324
35 Anthony, M.S. (1998) Comparison of soy phytoestrogens and conjugated equine estrogens on atherosclerosis progression in post-menopausal monkeys. Circulation 97, 829 (abstract)
36 Anthony, M.S., Clarkson, T.B. and Williams, J.K. (1998) Effects of soy isoflavones on atherosclerosis: potential mechanisms. Am J Clin Nutr 68, 1390S-1393S, PubMed: 9848505
37 Cassidy, A., Bingham, S. and Setchell, K. (1995) Biological effects of isoflavones in young women: importance of the chemical composition of soyabean products. Br J Nutr 74, 587-601, PubMed: 7577895
38 Nagata, C. et al. (1998) Decreased serum total cholesterol concentration is associated with high intake of soy products in Japanese men and women. J Nutr 128, 209-213, PubMed: 9446845
39 Crouse, J.R., 3rd et al. (1999) A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. Arch Intern Med 159, 2070-2076, PubMed: 10510993
40 Nestel, P.J. et al. (1997) Soy isoflavones improve systemic arterial compliance but not plasma lipids in menopausal and perimenopausal women. Arterioscler Thromb Vasc Biol 17, 3392-3398, PubMed: 9437184
41 Hodgson, J.M. et al. (1998) Supplementation with isoflavonoid phytoestrogens does not alter serum lipid concentrations: a randomized controlled trial in humans. J Nutr 128, 728-732, PubMed: 9521635
42 Nestel, P.J. et al. (1999) Isoflavones from red clover improve systemic arterial compliance but not plasma lipids in menopausal women. J Clin Endocrinol Metab 84, 895-898, PubMed: 10084567
43 Baum, J.A. et al. (1998) Long-term intake of soy protein improves blood lipid profiles and increases mononuclear cell low-density-

Accession information: DOI: 10.1017/S1462399403005264; Vol. 5; 30 September 2003

lipoprotein receptor messenger RNA in hypercholesterolemic, postmenopausal women. Am J Clin Nutr 68, 545-551, PubMed: 9734729
44 Cherry, N. et al. (2002) Oestrogen therapy for prevention of reinfarction in postmenopausal women: a randomised placebo controlled trial. Lancet 360, 2001-2008, PubMed: 12504395
45 Potter, S.M. (1998) Soy protein and cardiovascular disease: the impact of bioactive components in soy. Nutr Rev 56, 231-235, PubMed: 9735676
46 Register, T.C. and Adams, M.R. (1998) Coronary artery and cultured aortic smooth muscle cells express mRNA for both the classical estrogen receptor and the newly described estrogen receptor beta. J Steroid Biochem Mol Biol 64, 187-191, PubMed: 9605413
47 Chen, C.C., Wang, J.K. and Lin, S.B. (1998) Antisense oligonucleotides targeting protein kinase C-alpha, -beta I, or -delta but not -eta inhibit lipopolysaccharide-induced nitric oxide synthase expression in RAW 264.7 macrophages: involvement of a nuclear factor kappa B-dependent mechanism. J Immunol 161, 6206-6214, PubMed: 9834107
48 Zhu, Y. et al. (2002) Abnormal vascular function and hypertension in mice deficient in estrogen receptor beta. Science 295, 505-508, PubMed: 11799247
49 Honore, E.K. et al. (1997) Soy isoflavones enhance coronary vascular reactivity in atherosclerotic female macaques. Fertil Steril 67, 148-154, PubMed: 8986700
50 Williams, J.K. and Clarkson, T.B. (1998) Dietary soy isoflavones inhibit in-vivo constrictor responses of coronary arteries to collagen-induced platelet activation. Coron Artery Dis 9, 759-764, PubMed: 9919424
51 Washburn, S. et al. (1999) Effect of soy protein supplementation on serum lipoproteins, blood pressure, and menopausal symptoms in perimenopausal women. Menopause 6, 7-13, PubMed: 10100174
52 Cybulsky, M.I. and Gimbrone, M.A., Jr. (1991) Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science 251, 788-791, PubMed: 1990440
53 Thiery, J. et al. (1996) Study of causes underlying the low atherosclerotic response to dietary hypercholesterolemia in a selected strain of rabbits. Atherosclerosis 121, 63-73, PubMed: 8678925
54 Rimbach, G. et al. (2000) Macrophages stimulated with IFN-gamma activate NF-kappa B and induce MCP-1 gene expression in primary human endothelial cells. Mol Cell Biol Res Commun 3, 238-242, PubMed: 10891398
55 Rubanyi, G.M. (1993) The role of endothelium in cardiovascular homeostasis and diseases. J Cardiovasc Pharmacol 22 Suppl 4, S1-14, PubMed: 7523767
56 Baeuerle, P.A. and Henkel, T. (1994) Function and activation of NF-kappa B in the immune system. Annu Rev Immunol 12, 141-179, PubMed: 8011280
57 Kunsch, C. and Medford, R.M. (1999) Oxidative stress as a regulator of gene expression in the vasculature. Circ Res 85, 753-766, PubMed: 10521248
58 Saliou, C., Valacchi, G. and Rimbach, G. (2001) Assessing bioflavonoids as regulators of NF-kappa B activity and inflammatory gene expression in mammalian cells. Methods Enzymol 335, 380-387, PubMed: 11400387
59 Brand, K. et al. (1996) Activated transcription factor nuclear factor-kappa B is present in the atherosclerotic lesion. J Clin Invest 97, 1715-1722, PubMed: 8601637
60 Lindner, V. and Collins, T. (1996) Expression of NF-kappa B and I kappa B-alpha by aortic endothelium in an arterial injury model. Am J Pathol 148, 427-438, PubMed: 8579106
61 Brand, K. et al. (1997) Role of nuclear factor-kappa B in atherogenesis. Exp Physiol 82, 297-304, PubMed: 9129944
62 Yan, S.D. et al. (1994) Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. J Biol Chem 269, 9889-9897, PubMed: 8144582
63 Shames, B.D. et al. (1999) LPS-Induced NF-kappaB activation and TNF-alpha release in human monocytes are protein tyrosine kinase dependent and protein kinase C independent. J Surg Res 83, 69-74, PubMed: 10210645
64 Barnes, S. et al. (2000) Isoflavonoids and chronic disease: mechanisms of action. Biofactors 12, 209-215, PubMed: 11216488
65 Davis, J.N. et al. (2001) Soy isoflavone supplementation in healthy men prevents NF-kappa B activation by TNF-alpha in blood lymphocytes. Free Radic Biol Med 30, 1293-1302, PubMed: 11368927
66 May, M.J., Wheeler-Jones, C.P. and Pearson, J.D. (1996) Effects of protein tyrosine kinase inhibitors

Accession information: DOI: 10.1017/S1462399403005264; Vol. 5; 30 September 2003

on cytokine-induced adhesion molecule expression by human umbilical vein endothelial cells. Br J Pharmacol 118, 1761-1771, PubMed: 8842442
67 Weber, C. et al. (1995) Inhibitors of protein tyrosine kinase suppress TNF-stimulated induction of endothelial cell adhesion molecules. J Immunol 155, 445-451, PubMed: 7541425
68 McGregor, P.E., Agrawal, D.K. and Edwards, J.D. (1994) Attenuation of human leukocyte adherence to endothelial cell monolayers by tyrosine kinase inhibitors. Biochem Biophys Res Commun 198, 359-365, PubMed: 8292041
69 Tedesco, F. et al. (1999) Complement-endothelial cell interactions: pathophysiological implications. Mol Immunol 36, 261-268, PubMed: 10403479
70 Moncada, S., Palmer, R.M. and Higgs, E.A. (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 43, 109-142, PubMed: 1852778
71 Ignarro, L.J. (1994) Regulation of cytosolic guanylyl cyclase by porphyrins and metalloporphyrins. Adv Pharmacol 26, 35-65, PubMed: 7913618
72 Beckman, J.S. and Koppenol, W.H. (1996) Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol 271, C1424-1437, PubMed: 8944624
73 Stuehr, D.J. and Marletta, M.A. (1987) Induction of nitrite/nitrate synthesis in murine macrophages by BCG infection, lymphokines, or interferon-gamma. J Immunol 139, 518-525, PubMed: 3110273
74 Narumi, S., Finke, J.H. and Hamilton, T.A. (1990) Interferon gamma and interleukin 2 synergize to induce selective monokine expression in murine peritoneal macrophages. J Biol Chem 265, 7036-7041, PubMed: 2108965
75 Tetsuka, T. and Morrison, A.R. (1995) Tyrosine kinase activation is necessary for inducible nitric oxide synthase expression by interleukin-1 beta. Am J Physiol 269, C55-59, PubMed: 7543244
76 Li, W., Xia, J. and Sun, G.Y. (1999) Cytokine induction of iNOS and sPLA2 in immortalized astrocytes (DITNC): response to genistein and pyrrolidine dithiocarbamate. J Interferon Cytokine Res 19, 121-127, PubMed: 10090397
77 Gottstein, N. et al. (2003) Effect of genistein and daidzein on platelet aggregation and monocyte and endothelial function. Br J Nutr 89, 607-616, PubMed: 12720581
78 Azumi, S., Tanimura, A. and Tanamoto, K. (1997) A novel inhibitor of bacterial endotoxin derived from cinnamon bark. Biochem Biophys Res Commun 234, 506-510, PubMed: 9177302
79 Sheu, F., Lai, H.H. and Yen, G.C. (2001) Suppression effect of soy isoflavones on nitric oxide production in RAW 264.7 macrophages. J Agric Food Chem 49, 1767-1772, PubMed: 11308324
80 Green, S.J. et al. (1990) Leishmania major amastigotes initiate the L-arginine-dependent killing mechanism in IFN-gamma-stimulated macrophages by induction of tumor necrosis factor-alpha. J Immunol 145, 4290-4297, PubMed: 2124240
81 Jun, C.D. et al. (1995) Involvement of protein kinase C during taxol-induced activation of murine peritoneal macrophages. J Immunol 154, 6541-6547, PubMed: 7759887
82 Nelken, N.A. et al. (1991) Monocyte chemoattractant protein-1 in human atheromatous plaques. J Clin Invest 88, 1121-1127, PubMed: 1843454
83 Yla-Herttuala, S. et al. (1991) Expression of monocyte chemoattractant protein 1 in macrophage-rich areas of human and rabbit atherosclerotic lesions. Proc Natl Acad Sci U S A 88, 5252-5256, PubMed: 2052604
84 Boring, L. et al. (1998) Decreased lesion formation in CCR2-/- mice reveals a role for chemokines in the initiation of atherosclerosis. Nature 394, 894-897, PubMed: 9732872
85 Peluso, M.R. et al. (2000) A cooperative interaction between soy protein and its isoflavone-enriched fraction lowers hepatic lipids in male obese Zucker rats and reduces blood platelet sensitivity in male Sprague-Dawley rats. J Nutr 130, 2333-2342, PubMed: 10958832
86 Nakashima, S., Koike, T. and Nozawa, Y. (1991) Genistein, a protein tyrosine kinase inhibitor, inhibits thromboxane A2-mediated human platelet responses. Mol Pharmacol 39, 475-480, PubMed: 2017148
87 Beretz, A. et al. (1982) Role of cyclic AMP in the inhibition of human platelet aggregation by quercetin, a flavonoid that potentiates the effect of prostacyclin. Biochem Pharmacol 31, 3597-3600, PubMed: 6295405
88 Landolfi, R., Mower, R.L. and Steiner, M. (1984) Modification of platelet function and arachidonic acid metabolism by bioflavonoids. Structure-activity relations. Biochem Pharmacol 33, 1525-1530, PubMed: 6329230

Accession information: DOI: 10.1017/S1462399403005264; Vol. 5; 30 September 2003

89 Packham, M.A. and Mustard, J.F. (1977) Clinical pharmacology of platelets. Blood 50, 555-573, PubMed: 332253
90 Pignatelli, P. et al. (1998) Hydrogen peroxide is involved in collagen-induced platelet activation. Blood 91, 484-490, PubMed: 9427701
91 Iuliano, L. et al. (1994) Role of hydroxyl radicals in the activation of human platelets. Eur J Biochem 221, 695-704, PubMed: 8174549
92 Ruiz-Larrea, M.B. et al. (1997) Antioxidant activity of phytoestrogenic isoflavones. Free Radic Res 26, 63-70, PubMed: 9018473
93 Wei, H. et al. (1995) Antioxidant and antipromotional effects of the soybean isoflavone genistein. Proc Soc Exp Biol Med 208, 124-130, PubMed: 7892286

Further reading, resources and contacts

Setchell, K.D. (2001) Soy isoflavones—benefits and risks from nature's selective estrogen receptor modulators (SERMs). J Am Coll Nutr 20, 354S-362S; discussion 381S-383S, PubMed: 11603644

Setchell, K.D. and Cassidy, A. (1999) Dietary isoflavones: biological effects and relevance to human health. J Nutr 129, 758S-767S, PubMed: 10082786

Cassidy, A. and Griffin, B. (1999) Phyto-oestrogens: a potential role in the prevention of CHD? Proc Nutr Soc 58, 193-199, PubMed: 10343357

Bingham, M. et al. (2003) Gut metabolism and cardioprotective effects of dietary isoflavones. Curr Topics Nutraceutical Res 1, 31-48

Features associated with this article

Figures

Figure 1. Structural similarity between isoflavones and oestrogens (fig001acr).
Figure 2. The NF-κB pathway as a potential molecular target of isoflavones (fig002acr).

Tables

Table 1. Tissue distribution and binding affinities of oestrogen receptors (ERs) (tab001acr).
Table 2. Potential mechanisms by which isoflavones protect against arteriosclerosis (tab002acr).

Citation details for this article

Aedin Cassidy, Sonia de Pascual Teresa and Gerald Rimbach (2003) Molecular mechanisms by which dietary isoflavones potentially prevent atherosclerosis. Exp. Rev. Mol. Med. Vol. 5, 30 September, DOI: 10.1017/S1462399403006732

Accession information: DOI: 10.1017/S1462399403005264; Vol. 5; 30 September 2003