Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Nutritional Biochemistry 16 (2005) 321–330

Journal of Nutritional Biochemistry

REVIEWS: CURRENT TOPICS

Molecular mechanisms of action of the soy isoflavones includes activation of promiscuous nuclear receptors. A review

Marie-Louise Ricketts[a], David D. Moore[a], William J. Banz[b,c,d], Orsolya Mezei[e], Neil F. Shay[e,*]

[a]*Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030, USA*
[b]*Department of Animal Science, Southern Illinois University, Carbondale, IL 62901, USA*
[c]*Department of Food and Nutrition, Southern Illinois University, Carbondale, IL 62901, USA*
[d]*Department of Physiology, Southern Illinois University, Carbondale, IL 62901, USA*
[e]*Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA*

Received 20 July 2004; received in revised form 25 October 2004; accepted 16 November 2004

Abstract

Consumption of soy has been demonstrated to reduce circulating cholesterol levels, most notably reducing low-density lipoprotein (LDL) cholesterol levels in hypercholesterolemic individuals. The component or components that might be responsible for this effect is still a matter of debate or controversy among many researchers. Candidate agents include an activity of soy protein itself, bioactive peptides produced during the digestive process, or the soy isoflavones. Although soy intake may provide other health benefits including preventative or remediative effects on cancer, osteoporosis and symptoms of menopause, this review will focus on isoflavones as agents affecting lipid metabolism. Isoflavones were first discovered as a bioactive agent disrupting estrogen action in female sheep, thereby earning the often-used term 'phytoestrogens'. Subsequent work confirmed the ability of isoflavones to bind to estrogen receptors. Along with the cholesterol-lowering effect of soy intake, research that is more recent has pointed to a beneficial antidiabetic effect of soy intake, perhaps mediated by soy isoflavones. The two common categories of antidiabetic drugs acting on nuclear receptors known as peroxisome proliferator activated receptors (PPARs) are the fibrates and glitazones. We and others have recently asked the research question 'do the soy isoflavones have activities as either "phytofibrates" or "phytoglitazones"?' Such an activity should be able to be confirmed both in vivo and in vitro. In both the in vivo and in vitro cases, this action has indeed been confirmed. Further work suggests a possible action of isoflavones similar to the nonestrogenic ligands that bind the estrogen-related receptors (ERRs). Recently, these receptors have been demonstrated to contribute to lipolytic processes. Finally, evaluation of receptor activation studies suggests that thyroid receptor activation may provide additional clues explaining the metabolic action of isoflavones. The recent advances in the discovery and evaluation of the promiscuous nuclear receptors that bind many different chemical ligands should prove to help explain some of the biological effects of soy isoflavones and other phytochemicals.

Keywords: Isoflavone; Soy; Cholesterol; Nuclear hormone receptors

1. Soy intake affects lipid metabolism

Soy intake produces improvements in blood lipid levels and atherosclerosis as shown in many animal and human nutrition studies. In 1999, an FDA health claim was established suggesting that intake of 25 g of soy per day in a diet low in saturated fat and cholesterol may help to reduce the risk of cardiovascular disease. Other results, mentioned below, suggest a beneficial effect of soy intake for individuals with type 2 diabetes. This review focuses on the cell and molecular actions of soy and the improvements in lipid metabolism resulting from soy intake. Other important aspects of soy research include the study of the relationship between soy intake and cancer, osteoporosis, and symptoms of menopause. These aspects of soy research are beyond the scope of this review and are not discussed herein.

A metaanalysis of 38 different controlled clinical trials concluded that in humans, consumption of soy protein, rather than animal protein, lead to significantly decreased serum concentrations of total cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides [1]. The search for the specific factor or factors within soy that are responsible for these improvements has led to a number of

* Corresponding author. Tel.: +1 574 631 9035; fax: +1 574 631 7413.
E-mail address: nshay1@nd.edu (N.F. Shay).

0955-2863/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2004.11.008

conflicting results, which may be a matter of controversy among nutrition researchers. Our guiding hypothesis is that one component of soy, the isoflavones, are one factor among others that exert a positive influence on lipid metabolism. Readers are referred to other reviews [1–4] for an overview of the lipid lowering effect of soy intake.

1.1. Soy isoflavones and lipid metabolism

Dietary soy includes protein, lipids, fiber and phytochemicals including the soy isoflavones. Isoflavones are particularly abundant in soy and clover. In soy, the predominant isoflavones are genistein, daidzein and glycitein. Metabolic conversion of daidzein into another isoflavone, equol, may be of particular importance as equol may have an unusually high bioactivity [5]. Early studies identifying isoflavones as bioactive agents determined that clover isoflavones were agonists of estrogenic activity [6]. Based on these early findings, a significant amount of research has been directed toward understanding soy isoflavones as phytoestrogens. Since females have a lower risk of heart disease until menopause, there is wisdom in looking at the effect of estrogen on the cardiovascular system. Investigating isoflavones as an agent separate from soy has produced a wide range of results including both positive and negative results. Some studies in animals clearly show effects of isoflavones on lipid parameters [7–9]. A clinical trial demonstrated differences in lipoprotein levels and monocyte LDL-receptor mRNA levels after feeding postmenopausal women different amounts of soy isoflavones [10]. In C57BL/6 mice, intake of isoflavone-containing soy protein significantly reduced VLDL+LDL cholesterol levels compared to feeding an isoflavone-free diet; further, aortic sinus lesions were significantly greater in the mice fed the isoflavone-free diet [11]. Low-density lipoprotein receptor null mice did not show any significant differences, although the authors noted that lesion areas were so great in all groups of LDLr-null mice that differences due to diet may be difficult to demonstrate. Adams et al. [12,13] demonstrated significantly lower amounts of cholesterol ester content in the aorta in the LDLr −/−; +apoB mice fed isoflavone-containing soy. They subsequently implicated estrogen receptor-α (ERα) in the atheroprotective effect of isoflavones. Interestingly, a recent report indicates decreased adipose deposition in C57BL/6 mice due to intake of the isoflavone genistein, apparently dependent on ERα [14]. It is well apparent that the estrogen receptor plays an important role in the development of atherosclerosis and adiposity. The exact degree of influence that the estrogen receptor contributes to atherosclerosis and/or blood lipoprotein levels remains to be resolved in contrast to other proteins singularly involved in lipid metabolism such as the LDL receptor, the apolipoproteins and the enzymes involved in lipid metabolism.

1.2. Soy isoflavones and type 2 diabetes

Although many researchers have focused mainly on plasma lipid markers as an indicator of effectiveness of soy and soy components, other parameters such as aortic lipid content, general adiposity and liver lipid content are important markers to help discern the effects of various soy components. Recently, intake of soy isoflavones or other botanicals have been associated with improvements in symptoms of type 2 diabetes [15–17]. Although an association was noted, no speculation regarding mechanism of action seemed to be discussed in these reports. Only a limited number of clinical studies [16–21] have investigated the antidiabetic effects of soy protein and/or soy isoflavones. Many of these studies have been relatively short and/or not optimally controlled when accounting for gender or isoflavone content of the diets [17–21]. Moreover, a few of the studies used a combination of plant-based foods in which soy protein and/or other soy foods were not the only variable; further, plasma isoflavone content was not measured to confirm compliance and efficacy of the dietary regimens [18,20,21]. In a recent well-controlled study utilizing a 12-week crossover design, 32 postmenopausal women with type 2 diabetes benefited from a high-isoflavone soy protein supplement. The high-isoflavone soy protein supplement significantly reduced fasting insulin, HbA(1c) and insulin-resistance values in this population [16]. The authors contributed many of the beneficial effects to the phytoestrogen (i.e., isoflavone) content of the supplement, although they did not have a soy protein-only supplement (i.e., low-isoflavone soy protein). Additionally, the study was limited by the control (30 g of cellulose) that was not well matched to the 30 g of soy protein dietary supplement. A limited amount of data currently exists in this important and potentially promising area of research.

1.3. Soy isoflavones as nuclear receptor ligands

Although it had been understood for many years that the soy isoflavones acted as phytoestrogens, little was done to evaluate the possibility that isoflavones activated other nuclear receptors such as the lipid-regulating peroxisome proliferator activated receptors (PPARs), the liver X receptor (LXR) and the farnesoid X receptor (FXR). In 2000, Repa et al. [22] demonstrated how ligands for receptors, such as the PPARs (fibrates or glitazones), LXR (oxysterols) or FXR (bile acids), could activate these ‘promiscuous’ receptors and produce effects on liver lipid levels, bile acid synthesis, net cholesterol absorption and lipoprotein metabolism. The known effects of soy intake on lipid metabolism seemed to resemble some of the actions caused by activation of these lipid-regulating nuclear receptors. At a qualitative level, it appears that the chemical structure of the isoflavones are similar to that of the fibrates, agonists of PPARα used to treat hyperlipidemia and type 2 diabetes [23]. Thus, reported improvements in liver lipid levels associated with soy intake could be caused by PPAR activation by isoflavones. After considering the report of Repa et al. among these other considerations, we formulated the hypothesis that one effect of soy intake on lipid metabolism may be due to isoflavones acting as activators of PPARs. Thus, we began a variety of

studies utilizing PPARα and PPARγ expression vectors in a murine macrophage model, in the Zucker diabetic rat and using a panel of chimeric nuclear receptors in HepG2 cells. In the following section, we describe several studies investigating the activity of soy isoflavones as activators of nuclear receptors, including the estrogen receptor and PPARs.

2. The estrogen receptors

2.1. Properties of the estrogen receptors

A cDNA for the estrogen receptor was first identified in 1985 [24], and later, a second receptor, ERβ, was identified [25]. Both ERα and ERβ are classical endocrine receptors, with estradiol and synthetic ligands being high-affinity ligands of the receptor. Activated estrogen receptor largely binds to target promoter sequences as a homodimer, but ERα and ERβ have also been shown to dimerize.

Predating the discovery of the genes for the estrogen receptors was the discovery that sheep fed mainly on red clover suffered reproductive dysfunction [6]. This failure was determined to be due to disruption of normal patterns of circulating steroid hormone concentrations in ewes grazing on clover. Both plasma estrogen and progesterone levels were disrupted. Later, these observations were understood to be the result of estrogenic activity of the clover isoflavones. This early study was later followed with many others evaluating the estrogenic activity of the soy isoflavones. As an agonist of the estrogen receptors, genistein has been measured to be more potent than daidzein, while both genistein and daidzein are more potent ligands for ERβ than ERα [26–28]. The differential agonist activity of genistein and daidzein has led some to describe the soy isoflavones as dietary components that may act as selective estrogen receptor modulators (SERMs). In almost all cases, glycitein has much lower potency as an estrogen receptor agonist. In contrast to the isoflavones genistein and daidzein, both bioavailable as a constituent of soy, is the metabolite of daidzein, equol. Equol, so named from its discovery in horse urine, is derived from daidzein in humans and other species [29]. Equol is of particular interest because although it is abundantly produced in most animal models used for cardiovascular research, it appears to be produced in significant amounts only in subsets of humans [5,30,31]. Equol has been found to be a much more potent ERα agonist compared to either genistein or daidzein, while it acts similarly to daidzein on ERβ. Thus, in individuals who are able to convert daidzein to equol, equol will be activating both ERα and ERβ, while those individuals who do not produce equol will be activating primarily ERβ. Many human nutrition studies evaluating the effect of dietary soy intake or soy isoflavones may have been confounded by the presence of different numbers of equol producers and equol nonproducers. It will be important to identify equol producers from nonproducers in future studies.

The activity of soy components as ER agonists can be demonstrated using a Gal4 fusion reporter system as shown in Fig. 1. The ligand-binding domain (LBD) of the human ERα receptor was joined to the DNA-binding domain (DBD) of the yeast Gal4 gene, and this chimera was evaluated in comparison to the intact Gal4 alone without the ERα domain. Human hepatoma HepG2 cells were transfected with an expression vector containing the Gal4 or ER-Gal4 chimera and with a Gal4/luciferase reporter plasmid containing a Gal4-driven promoter sequence. β-Galactosidase was used as an internal control to ensure equivalent transfection efficiencies between conditions. Following 24-h treatment periods during which cells were exposed to various soy compounds or mixtures, cells were lysed and luciferase levels measured as an indicator of Gal4- or ER/Gal4-induced transactivation. Cells were treated with a negative control; isoflavone-containing soy extracts containing mixtures of either conjugated (C-ISO) or unconjugated (U-ISO) isoflavones; individually with genistein, daidzein or glycitein; and also with soy saponins. Without the ERα LBD (Fig. 1, left panel), Gal4 is unresponsive to any of the treatments, that is, no transactivation is observed. In Fig. 1, the middle panel demonstrates the relative effect of the various soy components on ERα/Gal4-driven transactivation. Mixtures of both conjugated and unconjugated isoflavones induced ERα/Gal4-driven transactivation as did genistein and daidzein. Compared with other isoflavones, glycitein did not induce transactivation significantly. In contrast, treatment with soy saponins proved to have no ability to induce transactivation of the ERα/Gal4 chimera. This trial was then repeated with the exception that all previous treatments were tested in combination with estradiol (e.g., genistein+estradiol) along with a negative control, in addition to one group of cells that was incubated with estradiol alone (E2) (Fig. 1, right panel). In this case, the high affinity of estradiol for ERα in comparison to the isoflavones becomes immediately apparent. Estradiol stimulates ER/Gal4-driven transactivation approximately 50-fold, while simultaneous treatment of HepG2 cells with estradiol and any soy component did not significantly increase estradiol-induced ER/Gal4-driven transactivation further. Thus, the Gal4 receptor system serves as a sensitive and effective indicator of ligand-dependent transactivation in a cell culture system.

3. Orphan and adopted orphan nuclear receptors

After discovery of the first nuclear receptors including the estrogen and glucocorticoid receptors (GRs), other members of the nuclear receptor family were identified by a variety of screening techniques. These candidate nuclear receptors had similar sequence features at the protein level including ligand-binding, DNA-binding and ligand-dependent and ligand-independent activation functions, but as newly discovered sequences, a corresponding endogenous ligand was not known. Thus, these receptors discovered without

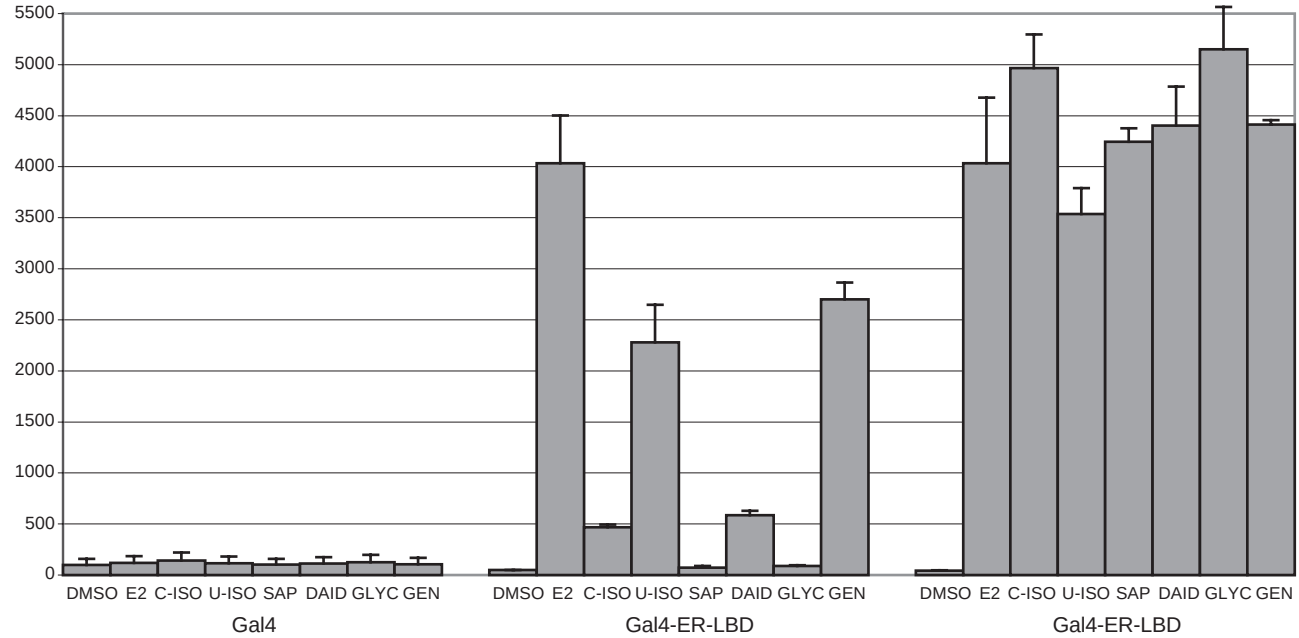

Fig. 1. Activation of ERα by soy compounds. HepG2 cells were cotransfected with the Gal4 luciferase reporter plasmid (Gal4) or a chimeric plasmid containing the Gal4 DBD fused to the LBD of ERα (Gal4-ER-LBD). After transfection, cells were treated for 24 h with either no ligand (DMSO), 10 ng/L of the indicated product or 1 μmol/L of estradiol (E2) (left and middle) or simultaneously with soy product and E2 (right). Each value represents the mean of three determinations with error bars showing S.E.M.s. DMSO, dimethyl sulfoxide vehicle (control); E2, estradiol; C-ISO, mixture of conjugated isoflavones; U-ISO, mixture of unconjugated isoflavones; SAP, soy saponins; DAID, daidzein; GLYC, glycitein; GEN, genistein.

known ligands were termed 'orphan receptors'. As ligands were determined for orphan receptors, they were then categorized as 'adopted orphan receptors'. This adopted orphan receptor group now includes PPAR, LXR, pregnane X receptor (PXR) and FXR.

3.1. Are the soy isoflavones ligands of promiscuous nuclear receptors?

As mentioned in the Soy isoflavones and lipid metabolism section (1.1), we considered the proposition that soy isoflavones might be ligands for nuclear receptors other than the estrogen receptors. Several studies substantiated that other natural products or bile acids can act as agonists of nuclear receptors [32–34]. To determine if isoflavones could activate PPARs, we utilized the Gal4 fusion system to determine if soy isoflavones could indeed serve as ligands for the promiscuous lipid-regulating nuclear receptors, and if so, for which specific receptors.

As shown in Fig. 2, a set of Gal4/LBD fusion vectors were used to evaluate the effect of isoflavone-containing mixtures, individual isoflavones and soy saponins. The vectors used included a Gal4 DBD (amino acids 1–147) with no ligand domain fused, and Gal4 DBD fused to the LBDs of the retinoid X receptor (RXR), FXR, LXRα, PPARα, PPARγ, murine PXR (mPXR), thyroid hormone receptor β (TRβ), GR and vitamin D receptor (VDR). Treatment details are provided in Fig. 2. Luciferase expression was induced (+100% or greater) after treatment with isoflavone-containing mixtures or individual isoflavones for PPARα, PPARγ, PXR and TRβ. PPARγ and PXR appeared to be induced to the greatest degree, and both by mixtures of isoflavones and by individual isoflavones. For example, after 24 h of treatment with 10 μg/ml of a mixture of unconjugated soy isoflavones (U-ISO), transactivation driven by the Gal4-PPARγ chimera was increased 320% above control; by Gal4-PXR, +200%; by Gal4-PPARα, +140%; by Gal4-TRβ, +110%. Largely, soy saponins do not appear to be efficacious ligands for the receptors tested, perhaps limited by structural considerations or perhaps by limited cellular uptake. A mixture of soy sterols was also evaluated and no induction was observed with any receptor (data not shown). The bioactivity of natural products does not necessarily involve action as an agonist; for example, guggulsterone, the active ingredient in the ayurvedic extract gugulipid, was shown to be an antagonistic ligand for FXR [33]. Interactions between agonist and a bioactive compound might be synergistic or antagonistic in nature. To screen for possible interactions, we then utilized the Gal4 fusion system to evaluate the effects of isoflavones on agonist-stimulated expression for each reporter (Fig. 3). Transfected HepG2 cells were either untreated (DMSO), treated with known agonist (e.g., for RXR: 1 μmol/L 9-*cis*-retinoic acid) or with known agonist plus 10 ng/L of soy extract, individual isoflavone or saponins. Interestingly, when agonist-induced expression was set at 100%, the greatest further induction was observed with thyroid hormone. All combinatorial treatments stimulated Gal4-TRβ expression 100% or more above treatment with T_3 alone. A connection between soy intake and thyroid hormone metabolism or action has been discussed and

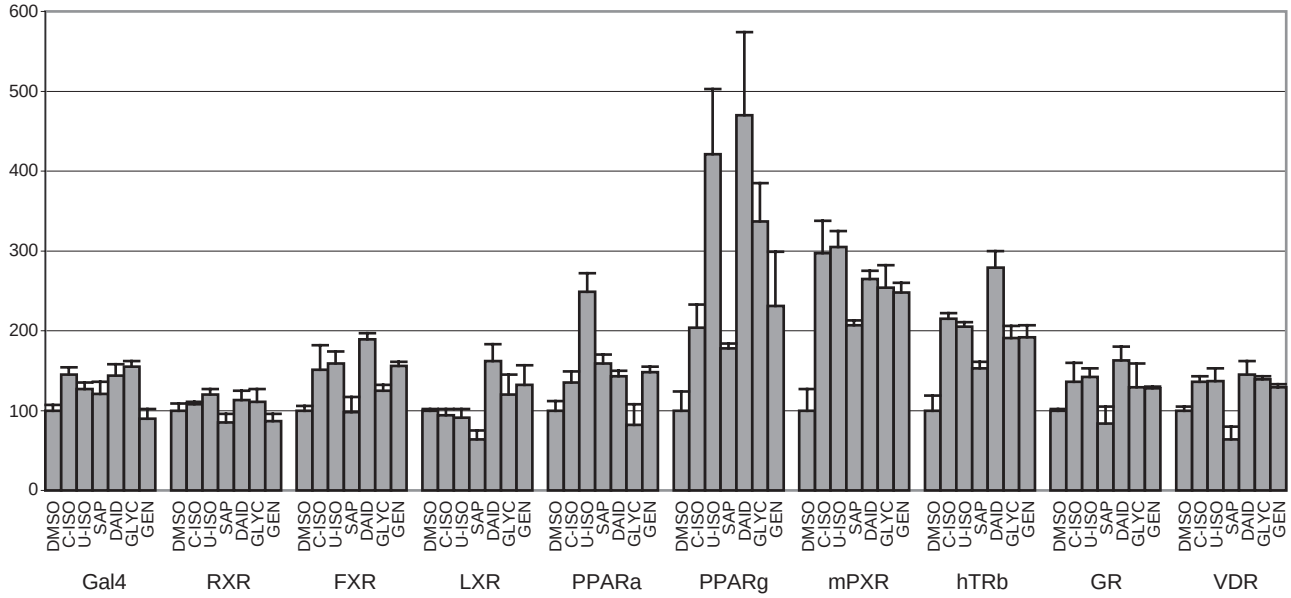

Fig. 2. Activation of nuclear receptors by soy compounds. HepG2 cells were cotransfected with the Gal4 luciferase reporter and a series of chimeric plasmids in which the Gal4 DBD is fused to the indicated nuclear hormone receptor LBD. After transfection, cells were treated for 24 h with 10 ng/L of the indicated soy compounds (see details in Fig. 1). Chimeric receptor plasmids were RXR, FXR, LXR, PPARα, PPARγ, mPXR, human thyroid hormone receptor β (hTRb), GR and VDR.

investigated to a limited degree. Several studies have evaluated the hypothesis that soy intake alters circulating thyroid hormone levels, but most have suggested that soy protein intake may have a small effect on thyroid hormone levels but most likely not clinically important [35]. However, the data from the screening experiment in Fig. 3 suggest that an interaction may indeed be occurring and further investigation may be warranted. It may be that thyroid hormone action is potentiated by isoflavones, although the levels of thyroid hormone itself are not changed significantly by soy intake. Other results from Fig. 3 that may be worthy of note are +40–50% increases above ligand-dependent activation for LXR and PXR, and +40–80% increases for PPARα and PPARγ. Further, resembling their effects when tested without the appropriate ligand, little of note was observed for RXR, FXR, GR and

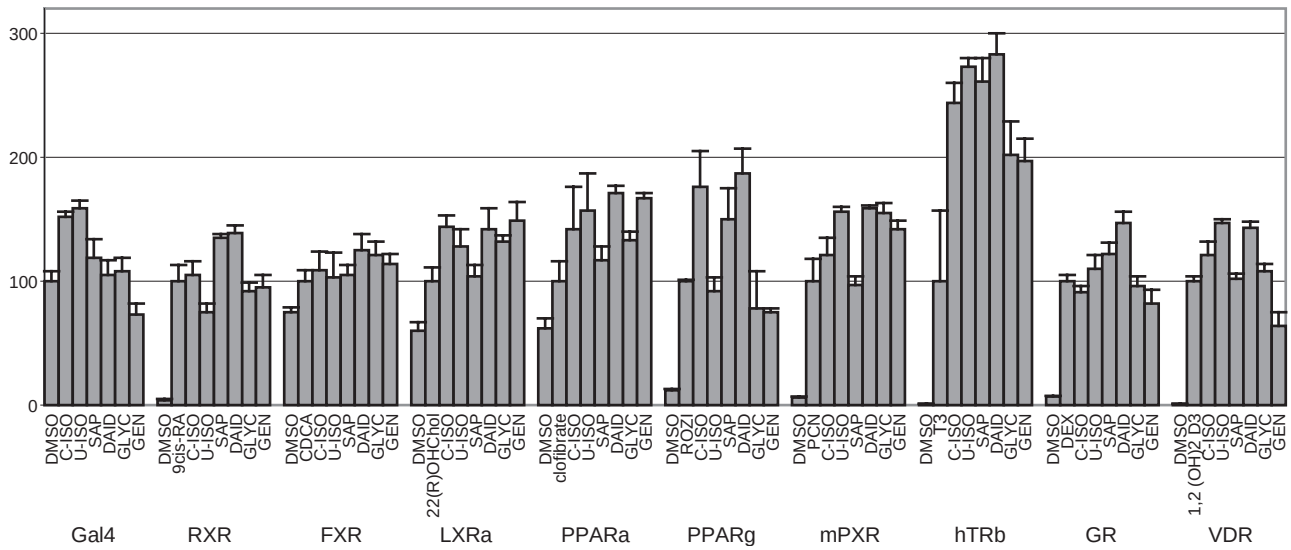

Fig. 3. Activation of nuclear receptors after simultaneous treatment with soy compounds and known receptor agonist. HepG2 cells were cotransfected with the Gal4 luciferase reporter and a series of chimeric plasmids in which the Gal4 DBD is fused to the indicated nuclear hormone receptor LBD. Details of the soy compounds and the concentrations used are given in Fig. 1. Chimeric receptors are detailed in Fig. 2. After transfection, cells were treated for 24 h with a concentration of agonist known to activate the chimeric receptor or was simultaneously treated with agonist and a given soy product (10 ng/L). The ligands used and their concentrations were RXR, 1 μmol/L 9-*cis*-retinoic acid; FXR, 100 μmol/L chenodeoxycholic acid (CDCA); LXR, 10 μmol/L 22(*R*)-hydroxycholesterol [22(*R*)OHChol]; PPARα, 300 nmol/L clofibrate; PPARγ, 1 μmol/L rosiglitazone (Rozi); PXR, 10 μmol/L pregnenolone 16α-carbonitrile (PCN); TRβ, 10 μmol/L T_3; GR, 100 nmol/L dexamethasone (DEX); VDR, 1 μmol/L vitamin D3 [1,25$(OH)_2$-D3].

Table 1

Isoform	Tissue distribution	Metabolic/physiological roles	Ligands
PPARα	Liver	Regulation of β-oxidation genes	Natural eicosanoids (8-*S*-HETE* and leukotriene B4),
	Kidney	Stimulation of fatty acid catabolism	oxidized phospholipids, fibrate medications
	Heart	Inhibition of NF-κB transcription	
	Muscle	Inhibition of inflammatory response	
	Sites of fatty acid	at the vascular wall	
	Catabolism	Increased glucose tolerance	
PPARγ	Brown adipose tissue	Increased adipocyte differentiation	Arachidonic acids (PG-J*2 and 15-HETE),
	White adipose tissue	Increased glucose tolerance	fatty acid-derived compounds from oxLDLs,
	Intestine	Increased insulin resistance	glitazone medications
	Sites of cellular	Inhibition of VSMC proliferation	
	Differentiation and	Inhibition of macrophage activation	
	Lipid storage	Inhibition of NF-κB transcription	
PPARβ/δ	Heart	Increased fatty acid oxidation	Polyunsaturated FAs
	Adipose tissue	Lower TG and free fatty acid levels	Prostaglandins
	Brain	Protects against fatty liver	Retinoic acid
	Muscle	Controls inflammatory status of	Synthetic ligands (carbaprostacyclin, GW501516)
	Spleen	macrophages	
	Lung	Activates fatty acid oxidation in adipose tissue	
	Adrenal glands		
	Ubiquitously expressed	May have role in adipocyte precursor cell proliferation	

VDR. In a separate set of trials, equol was tested using this Gal4 system as a potential agonist. At 1 μmol/L, equol was able to activate the ER/Gal4 chimera to the same degree as did 1 μmol/L estradiol. Further, equol significantly induced PXR-mediated luciferase expression, and to a lesser degree, PPARα-mediated expression. Equol did not appear to up-regulate PPARγ transactivation (data not shown).

3.2. The peroxisome proliferator activated receptors

The PPAR receptors (α, γ and δ) have distinctly different tissue distributions and have been shown to impact lipid metabolism in the liver, muscle, adipocyte and macrophage among other tissues, and general background may be reviewed in Refs. [36–39] and more recent information for PPARγ in Refs. [40] and [41]; for PPARα in Refs. [42] and [43]; and PPARδ in Refs. [44–46]. Summaries listing specific roles and agonists of the PPARs are in Table 1 and Fig. 4.

Both cardiovascular disease and type 2 diabetes affect millions, are significant causes of mortality and are huge contributors to health care costs. Both diseases involve dysregulation of lipid metabolism. Drugs used to improve lipid levels include PPARα agonists (fenofibrate, 'Tricor') and PPARγ agonists (rosiglitazone, 'Avandia'). Fibrates stimulate the oxidation of fatty acids via PPAR activation, acting in liver and muscle, among other tissues. Glitazones acting on the PPARγ receptor are insulin-sensitizing agents and typically produce dramatic improvements in regulation of blood glucose levels. Activation of PPARγ also produces an increase in adiposity; this may represent a redistribution of lipids to adipocytes, perhaps due to the significant role that PPARγ plays in adipocyte differentiation. The long-term consequences of this gain in body fat accompanying glitazone treatment are not understood at this time; however, the dramatic improvement of blood glucose concentrations make this accompanying side effect a tolerable part of therapy. Dysregulation of PPARs may contribute to foam cell development and atherosclerosis. Three papers published in 2001 [47–49] described actions of PPARs on the macrophage. Consideration of these papers led us to the hypothesis that if soy isoflavones are indeed PPAR ligands, then they may be acting on macrophages in a similar manner to PPAR agonists to retard foam cell and fatty streak development. This effect may relate to experimental measurements of atherosclerosis as indicated by blood vessel lipid content.

In 2000, Smith et al. [50] demonstrated that administration of the PPAR agonist rosiglitazone retarded the

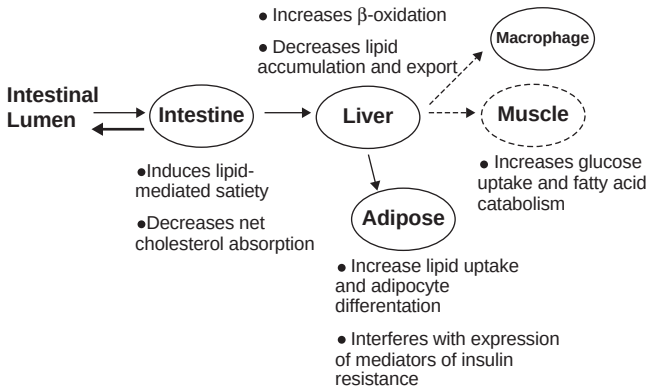

Fig. 4. Tissue-specific actions of the PPARs. PPARγ serves as a direct regulator of adipogenesis, and along with the LXRα receptor, regulates cholesterol efflux from enterocytes through ATP-binding cassette cholesterol transporters. In contrast, PPARα serves a primary function to stimulate fatty acid oxidation, primarily in liver, and to a lesser extent, in heart and muscle. Less well studied is PPARδ. PPARδ appears to be counter-regulatory to PPARγ, at least with respect to adipose tissue. While PPARγ serves to promote adipogenesis and fat accumulation, PPARδ appears to promote lipolysis and thermogenesis. In skeletal muscle, the stimulatory effect of PPARδ on fatty acid oxidation appears to greatly exceed the contribution provided by the less-expressed PPARα. Solid lines represent tissues where PPARs may direct lipid accumulation; dotted lines represent tissues where catabolism may be most regulated.

development of diabetes in the obese Zucker rat (OZR). At that same time, it was also shown that in male OZR, graded improvements in liver triglycerides, cholesterol and cholesterol ester levels, and improved plasma cholesterol, but not plasma triglyceride levels when diets contained (1) casein, (2) low-isoflavone containing soy and (3) high-isoflavone containing soy [8]. Another study [51] detailed additional findings in male and female OZR, whereby both male and female OZR consuming a high isoflavone soy protein diet displayed improvements in lipid and metabolic parameters associated with the diabetic phenotype. Moreover, female OZRs consuming a high isoflavone soy protein diet displayed improvements in glucose tolerance consistent with results observed in humans treated with antidiabetic PPARα agonists such as the glitazones. Liver triglyceride, liver cholesterol and plasma cholesterol levels decreased significantly in all OZR fed high-isoflavone soy protein diets compared to the casein-fed rats ($P<.05$) [51]. These findings are consistent with the hypothesis that soy isoflavones improve lipid metabolism and produce an antidiabetic effect by activating PPAR receptors.

In 2003, Dang et al. [52] confirmed that genistein was indeed a ligand for PPARγ with a $K_i=5.7$ μM, comparable to that of some known PPARγ ligands [53]. The dissociation constant of rosiglitazone determined in saturation experiments in the presence of high concentrations of genistein was reduced compared to tests completed in the absence of genistein, while the number of binding sites was not altered. Further, using a PPRE-driven luciferase vector and an expression vector coding for human PPARγ, a dose-dependent relationship was observed between genistein and luciferase expression in a concentration range of 1–50 μmol/L of genistein. Taken together, Dang et al. provided convincing evidence that genistein is a ligand for PPARγ. Further, functional assays were performed to evaluate dose effects of genistein on osteogenesis in KS483 cells. At doses focused around 1 μmol/L, genistein positively impacted markers of osteogenesis while higher concentrations of genistein decreased these effects. Adipogenic markers were also evaluated over the same range of concentrations for genistein. Interestingly, at ~1 μmol/L, genistein has a negligible effect on adipogenesis, but at higher concentrations, up to 50 μmol/L, genistein promotes adipogenesis. Thus, genistein appears to have two separate actions: first, at low concentrations, acting via the estrogen receptor, genistein promotes osteogenesis. At higher concentrations, genistein, acting as a ligand for PPARγ, is stimulating adipogenesis. Cotransfection studies implicated PPARγ as a factor down-regulating ER-mediated transcriptional activity at the higher doses of genistein tested. Their results suggest an antiestrogenic action of genistein at higher concentrations acting via an ER-independent pathway. These important data demonstrate that in vivo levels of genistein or isoflavones are a very important consideration. Serum levels of isoflavones may vary greatly depending on soy intake, perhaps ranging more than 100-fold in humans, from perhaps 40 nmol/L to 4 μmol/L [54–56]. Although serum concentrations have been determined after consumption of a single bolus of individual isoflavones, to our knowledge, no study has measured serum levels of isoflavones in individuals consuming isoflavone-containing soy supplements. In these individuals, there is the potential for serum levels to approach or exceed 10 μmol/L. In addition to these concerns, one must also take into consideration differential effects of genistein and daidzein as ER and PPAR agonists, the metabolic conversion of daidzein into equol, the heterogeneity of equol producers in the population and differential effects on males versus females.

Other experiments evaluated PPAR-activated gene expression in the macrophage [51]. The murine macrophage cell line RAW 264.7 was used as a model. Cells were transfected with a PPRE-driven luciferase plasmid, a PPAR-containing expression vector (α or γ) and a control plasmid to monitor transfection efficiency. The soy products evaluated in these tests included mixtures of conjugated and unconjugated isoflavones, and individually, genistein, daidzein and glycitein. When challenged with a mixture of unconjugated soy isoflavones present at 2.5 μg/ml (total isoflavone concentration ~6 μmol/L), cells demonstrated induced levels of both PPARα- and PPARγ-dependent luciferase expression of approximately two-fold or greater over a 24-h time course. When challenged for a 24-h period, RAW cells expressing either the PPARα or PPARγ receptor demonstrated significantly increased levels of PPRE-driven luciferase activity when treated with a mixture of unconjugated soy isoflavones, genistein alone (~10 μmol/L), daidzein alone (~10 μmol/L), but not by a mixture of conjugated isoflavones or by glycitein alone. We hypothesize that both conjugated isoflavones and glycitein are either not well transported into RAW cells or the structural differences make them less potent ligands of PPARα or PPARγ.

Taken together, in vivo data showing improvements in liver lipids with increasing dietary isoflavones [51], in vitro data from adipocytes and osteoblasts [52], in vitro data from a macrophage cell line [51] and Figs. 1–3 from this article all point consistently to an activity of isoflavones as PPAR agonists. Further, carnitine palmitoyltransferase I (CPT-I), a PPAR-regulated gene that is coordinately regulated with hepatic fatty acid oxidation, was identified in a genetic screen searching for soy- and isoflavone-regulated mRNAs [57]. A recent evaluation of the PPAR-induced acyl CoA oxidase (ACO) gene demonstrated in a set of 55 OZRs that ACO mRNA was significantly increased in rats fed diets containing either casein with rosiglitazone (+49%, $P<.1$) or isoflavone-containing diets (+35%, $P<.1$) compared to levels in rats fed casein alone [58]. Thus, at this time, it appears that many independent lines of evidence point to a new action of soy isoflavones as PPAR agonists.

3.3. Estrogen-related receptors

Another report [59] provides additional information regarding flavones and isoflavones as nuclear receptor

ligands, in this case, the estrogen-related receptors (ERRs). The ERRs were identified by a variety of methods [60–62], and although they share amino acid homologies to the estrogen receptors, estrogens are not ligands of ERRs. Estrogen-related receptor appears to be constitutively active, but antagonists have also been identified. The search for agonists of these receptors has perhaps been more difficult due to the constitutive activity of the ERRs. Computer modeling of the ERRα LBD was used to perform structural-based virtual binding studies using commercially available natural products [59]. After completing a virtual screening project, several flavone and isoflavone compounds were identified as agonists of ERRs using mammalian transfection and two-hybrid experiments. Genistein, daidzein and biochanin A were isoflavones identified in this report. The flavone 6,3′,4′-trihydroxyflavone was similarly identified. When challenged with 10 μmol/L of each compound, HeLa cells transfected with ERRα or ERRβ demonstrated increased luciferase expression. Cells transfected with ERRα had increased luciferase expression when treated with biochanin A and 6,3′,4′-trihydroxyflavone, but not with genistein or daidzein. The isoflavones were subsequently shown to enhance interactions between ERRs and coactivator PNRC. Suetsugi et al. suggest that in vivo induction of ERRα may influence the development of breast cancer. Others have determined that ERRα is one of several important factors regulating fatty acid β-oxidation [63,64]. It should be noted that in the study reported by Suetsugi et al., transfection studies were performed without cotransfection of an additional reporter to monitor transfection efficiency (e.g., β-galactosidase). As isoflavones are well known to possess kinase inhibitor activities [65], phosphorylation changes, especially within nuclear receptors or their coactivators, may potently change transcription rates mediated by the nuclear receptors.

The PPARγ coactivator PGC-1β was recently shown to function as a protein ligand for ERRα and may act to enhance fatty oxidation and prevent obesity. Involvement of ERRs in the lipid-lowering effects of soy isoflavones or other natural compounds may prove to be a productive direction for future research.

4. Conclusions

Long known as phytoestrogens, it appears that the soy isoflavones need to be considered as ‘phytoglitazones’ and/or ‘phytofibrates’ if indeed the isoflavones are ligands for PPARγ and PPARα, respectively. In direct binding studies, genistein has been demonstrated to bind to PPARγ. In receptor-based activation studies, genistein and daidzein are more potent activators of the PPARs while glycitein does not appear to be a potent ligand. Also tested as potential activators were soy sterols and saponins. In both cases, these components did not appear to have substantial activity. Further testing will be required to determine if this lower activity is due to reduced uptake into cells or reduced activity as a ligand.

The relative potency of isoflavones and that of the metabolite equol is an important issue for future study. Equol is thought to be produced by the metabolic action of the intestinal flora only in a subset of humans. If, in the future, various isoflavone mixtures may be produced as dietary supplements, the specific daidzein content of those mixtures may be of considerable significance to the ‘equol-producer’ population.

Of further consideration and not extensively discussed in this review is the PXR. PXR mediates the response to xenobiotics by activating a set of phase I and phase II enzymes in the liver. PXR may play a significant role in lipid metabolism in at least two ways. First, PXR mediates the modification and potential disposal of sterols and bile acids in the body. Second, PXR can mediate the metabolism and turnover of prescription drugs, including those used to treat hypercholesterolemia and type 2 diabetes. As an example, PXR regulates the metabolism of atorvastatin, the world’s most commonly prescribed drug, by activating the transcription of cytochrome P450-3A4 [66]. It appears that a critical issue for the study of natural products and their impact on lipid metabolism is to ensure that there is no significant negative interaction between the consumption of a supplement (e.g., isoflavones) and the metabolism of a prescribed drug. The possibility exists for the isoflavones that if PXR is activated by isoflavones, as Gal4 reporter studies suggest, this activation may enhance the turnover of atorvastatin and reduce the potency of this statin. Further study is clearly warranted.

Finally, other works in progress include readdressing the interaction of the isoflavones with thyroid hormone action. Many researchers have attempted to ascribe the lipid-lowering effects of soy intake on the modulation of thyroid hormone levels. Although it has been difficult to demonstrate clear-cut effects of soy intake on thyroid hormone levels, again, reporter studies predict a possible potentiation of thyroid hormone activation by isoflavones. As with TR action, a potential impact on ERR action suggests a potential activation pathway worthy of additional study.

The isoflavones and other related natural compounds may have several features that allow them to serve as ligands for several different receptors: their size, relatively hydrophobic nature and similar structure to the sterols. As sterols and sterol derivatives are known to activate the LXR and FXR receptors and perhaps also serve as sterol cleavage-activating protein (SCAP) ligands that regulate sterol response element binding protein activation and sterol homeostasis, it is not unlikely that specific molecules may activate several different nuclear receptors. Clarifying the effects of the various components of soy and the specific cellular action of the isoflavones will prove to be a significant challenge for molecular nutrition studies of the next decade.

References

[1] Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med 1995;333: 276–82.

[2] Lichtenstein AH. Got soy? Am J Clin Nutr 2001;73:667–8.

[3] Lichtenstein AH. Soy protein, isoflavones and cardiovascular disease risk. J Nutr 1998;128:1589–92.

[4] Anthony MS. Soy and cardiovascular disease: cholesterol lowering and beyond. J Nutr 2000;130:662S–3S.

[5] Setchell KD, Brown NM, Lydeking-Olsen E. The clinical importance of the metabolite equol — a clue to the effectiveness of soy and its isoflavones. J Nutr 2002;132(12):3577–84.

[6] Obst JM, Seamark RF. Hormone studies on ewes grazing an oestrogenic (Yarloop clover) pasture during the reproductive cycle. Aust J Biol Sci 1975;28(3):279–90.

[7] Yamakoshi J, Piskula MK, Izumi T, Tobe K, Saito M, Kataoka S, et al. Isoflavone aglycone-rich extract without soy protein attenuates atherosclerosis development in cholesterol-fed rabbits. J Nutr 2000; 130:1887–93.

[8] Peluso MR, Winters TA, Shanahan MF, Banz WJ. 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 2000;130: 2333–42.

[9] Mezei O, Chou CN, Kennedy KJ, Tovar-Palacio C, Shay NF. Hepatic cytochrome P450-2A and phosphoribosylpyrophosphate synthetase-associated protein mRNAs are induced in the gerbil after consumption of isoflavone containing protein. J Nutr 2002;132:2538–44.

[10] Baum J, Teng H, Erdman JW, Klein BP, Persky VW, Freels S, et al. Long-term intake of soy protein lowers the risk of cardiovascular disease in postmenopausal women with high cholesterol. Am J Clin Nutr 1998;68:545–61.

[11] Kirk EA, Sutherland P, Wang SA, Chait A, LeBoeuf RC. Dietary isoflavones reduce plasma cholesterol and atherosclerosis in C57BL/6 mice but not LDL receptor-deficient mice. J Nutr 1998;128(6):954–9.

[12] Adams MR, Golden DL, Register TC, Anthony MS, Hodgin JB, Maeda N, et al. The atheroprotective effect of dietary soy isoflavones in apolipoprotein E−/− mice requires the presence of estrogen receptor-alpha. Arterioscler Thromb Vasc Biol 2002;22(11): 1859–64.

[13] Adams MR, Golden DL, Anthony MS, Register TC, Williams JK. The inhibitory effect of soy protein isolate on atherosclerosis in mice does not require the presence of LDL receptors or alteration of plasma lipoproteins. J Nutr 2002;132(1):43–9.

[14] Naaz A, Yellayi S, Zakroczymski MA, Bunick D, Doerge DR, Lubahn DB, et al. The soy isoflavone genistein decreases adipose deposition in mice. Endocrinology 2003;144(8):3315–20.

[15] Bhathena SJ, Velasquez MT. Beneficial role of dietary phytoestrogens in obesity and diabetes. Am J Clin Nutr 2002;76(6):1191–201.

[16] Jayagopal V, Albertazzi P, Kilpatrick ES, Howarth EM, Jennings PE, Hepburn DA, et al. Beneficial effects of soy phytoestrogen intake in postmenopausal women with type 2 diabetes. Diabetes Care 2002;25: 1709–14.

[17] Anderson JW, Blake JE, Turner J, Smith BM. Effects of soy protein on renal function and proteinuria in patients with type 2 diabetes. Am J Clin Nutr 1998;68:1347S–53S.

[18] Meshcheriakova VA, Plotnikova OA, Sharafetdinov K, Iatsyshina TA. The use of the combined food products with soy protein in diet therapy for patients with diabetes mellitus type 2. Vopr Pitan 2002;71: 19–24.

[19] Jenkins DJ, Kendall CW, Jackson CJ, Connelly PW, Parker T, Faulkner D, et al. Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. Am J Clin Nutr 2002;76:365–72.

[20] Wheeler ML, Fineberg SE, Fineberg NS, Gibson RG, Hackward LL. Animal versus plant protein meals in individuals with type 2 diabetes and microalbuminuria: effects on renal, glycemic, and lipid parameters. Diabetes Care 2002;25:1277–82.

[21] Hermansen K, Sondergaard M, Hoie L, Carstensen M, Brock B. Beneficial effects of a soy-based dietary supplement on lipid levels and cardiovascular risk markers in type 2 diabetic subjects. Diabetes Care 2001;24:228–33.

[22] Repa JJ, Turley SD, Lobaccaro JA, Medina J, Li L, Lustig K, et al. Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. Science 2000;289(5484):1524–9.

[23] Elisaf M. Effects of fibrates on serum metabolic parameters. Curr Med Res Opin 2002;18(5):269–76.

[24] Walter P, Green S, Greene G, Krust A, Bornert JM, Jeltsch JM, et al. Cloning of the human estrogen receptor cDNA. Proc Natl Acad Sci U S A 1985;82(23):7889–93.

[25] Pace P, Taylor J, Suntharalingam S, Coombes RC, Ali S. Human estrogen receptor beta binds DNA in a manner similar to and dimerizes with estrogen receptor alpha. J Biol Chem 1997;272(41): 25832–8.

[26] Kostelac D, Rechkemmer G, Briviba K. Phytoestrogens modulate binding response of estrogen receptors alpha and beta to the estrogen response element. J Agric Food Chem 2003;51(26):7632–5.

[27] Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 1998;139(10): 4252–63.

[28] Casanova M, You L, Gaido KW, Archibeque-Engle S, Janszen DB, Heck HA. Developmental effects of dietary phytoestrogens in Sprague–Dawley rats and interactions of genistein and daidzein with rat estrogen receptors alpha and beta in vitro. Toxicol Sci 1999;51(2): 236–44.

[29] Axelson M, Sjovall J, Gustafsson BE, Setchell KD. Soya — a dietary source of the non-steroidal oestrogen equol in man and animals. J Endocrinol 1984;102(1):49–56.

[30] Atkinson C, Berman S, Humbert O, Lampe JW. In vitro incubation of human feces with daidzein and antibiotics suggests interindividual differences in the bacteria responsible for equol production. J Nutr 2004;134(3):596–9.

[31] Rafii F, Davis C, Park M, Heinze TM, Beger RD. Variations in metabolism of the soy isoflavonoid daidzein by human intestinal microfloras from different individuals. Arch Microbiol 2003;180(1): 11–6.

[32] Moore LB, Goodwin B, Jones SA, Wisely GB, Serabjit-Singh CJ, Willson TM, et al. St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. Proc Natl Acad Sci U S A 2000;97(13):7500–2.

[33] Urizar NL, Liverman AB, Dodds DT, Silva FV, Ordentlich P, Yan Y, et al. A natural product that lowers cholesterol as an antagonist ligand for FXR. Science 2002;296(5573):1703–6.

[34] Makishima M, Lu TT, Xie W, Whitfield GK, Domoto H, Evans RM, et al. Vitamin D receptor as an intestinal bile acid sensor. Science 2002;296(5571):1313–6.

[35] Persky VW, Turyk ME, Wang L, Freels S, Chatterton Jr R, Barnes S, et al. Effect of soy protein on endogenous hormones in postmenopausal women. Am J Clin Nutr 2002;75(1):145–53 [erratum in: Am J Clin Nutr 2002 Sep;76(3):695].

[36] Nagy L, Tontonoz P, Alvarez JGA, Chen H, Evans RM. Oxidized LDL regulates macrophage gene expression through ligand activation of PPARγ. Cell 1998;93:229–40.

[37] Chinetti G, Lestavel S, Bocher V, Remaley AT, Neve B, Torra IP, et al. PPARα and PPARγ activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. Nat Med 2001;53–8.

[38] DeLuca JG, Doebber TW, Kelly LJ, Kemp RK, Molon-Noblot S, Sahoo SP, et al. Evidence for peroxisome proliferator-activated receptor (PPAR)alpha-independent peroxisome proliferation: effects of PPARgamma/delta-specific agonists in PPARalpha-null mice. Mol Pharmacol 2000;58(3):470–6.

[39] Li AC, Brown KK, Silvestre MJ, Willson TM, Palinski W, Glass CK. Peroxisome proliferator-activated receptor gamma ligands inhibit development of atherosclerosis in LDL receptor-deficient mice. J Clin Invest 2000;106(4):523–31.

[40] Akiyama TE, Sakai S, Lambert G, Nicol CJ, Matsusue K, Pimprale S, et al. Conditional disruption of the peroxisome proliferator-activated receptor gamma gene in mice results in lowered expression of ABCA1, ABCG1, and apoE in macrophages and reduced cholesterol efflux. Mol Cell Biol 2002;22(8):2607–19.

[41] Gavrilova O, Haluzik M, Matsusue K, Cutson JJ, Johnson L, Dietz KR, et al. Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass. J Biol Chem 2003;278(36):34268–76.

[42] Kim H, Haluzik M, Asghar Z, Yau D, Joseph JW, Fernandez AM, et al. Peroxisome proliferator-activated receptor-alpha agonist treatment in a transgenic model of type 2 diabetes reverses the lipotoxic state and improves glucose homeostasis. Diabetes 2003; 52(7):1770–8.

[43] Lee Y, Yu X, Gonzales F, Mangelsdorf DJ, Wang MY, Richardson C, et al. PPAR alpha is necessary for the lipopenic action of hyperleptinemia on white adipose and liver tissue. Proc Natl Acad Sci U S A 2002;99(18):11848–53.

[44] Oliver Jr WR, Shenk JL, Snaith MR, Russell CS, Plunket KD, Bodkin NL, et al. A selective peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport. Proc Natl Acad Sci U S A 2001;98(9):5306–11.

[45] Chawla A, Lee CH, Barak Y, He W, Rosenfeld J, Liao D, et al. PPARdelta is a very low-density lipoprotein sensor in macrophages. Proc Natl Acad Sci U S A 2003;100(3):1268–73.

[46] Wang YX, Lee CH, Tiep S, Yu RT, Ham J, Kang H, et al. Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. Cell 2003;113(2):159–70.

[47] Moore KJ, Rosen ED, Fitzgerald ML, Randow F, Andersson LP, Altshuler D, et al. The role of PPAR-γ in macrophage differentiation and cholesterol uptake. Nat Med 2001;7:41–7.

[48] Chinetti G, Fruchart J-C, Staels B. Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. Inflamm Res 2000;49:497–505.

[49] Chawla A, Barak Y, Nagy L, Liao D, Tontonoz P, Evans RM. PPAR-gamma dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. Nat Med 2001; 7(1):48–52.

[50] Smith SA, Lister CA, Toseland CD, Buckingham RE. Rosiglitazone prevents the onset of hyperglycaemia and proteinuria in the Zucker diabetic fatty rat. Diabetes Obes Metab 2000;2:363–72.

[51] Mezei O, Banz WJ, Steger RW, Peluso MR, Winters TA, Shay N. Soy isoflavones exert antidiabetic and hypolipidemic effects through the PPAR pathways in obese Zucker rats and murine RAW 264.7 cells. J Nutr 2003;133:1238–43.

[52] Dang ZC, Audinot V, Papapoulos SE, Boutin JA, Lowik CW. PPARg as a molecular target for the soy phytoestrogen genistein. J Biol Chem 2003;278:962–7.

[53] Ferry G, Bruneau V, Beauverger P, Goussard M, Rodriguez M, Lamamy V, et al. Binding of prostaglandins to human PPARgamma: tool assessment and new natural ligands. Eur J Pharmacol 2001; 417(1–2):77–89.

[54] Barnes S. Evolution of the health benefits of soy isoflavones. Proc Soc Exp Biol Med 1998;217(3):386–92.

[55] Adlercreutz H, Mazur W. Phyto-oestrogens and Western diseases. Ann Med 1997;29(2):95–120.

[56] Setchell KD. Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. Am J Clin Nutr 1998;68(6 Suppl):1333S–46S.

[57] Iqbal MJ, Yaegashi S, Ahsan R, Lightfoot DA, Banz WJ. Differentially abundant mRNAs in rat liver in response to diets containing soy protein isolate. Physiol Genomics 2002;11:219–26.

[58] Banz WJ, Moustaind-Moussa N, Tebbe A, Kim Y-C, Cho KW, Shay NF, et al. Soy diets regulate adiposity, insulin sensitivity and lipogenic capacity in male ZDF rats. FASEB J 2004 [(abs) 369.3].

[59] Suetsugi M, Su L, Karlsberg K, Yuan YC, Chen S. Flavone and isoflavone phytoestrogens are agonists of estrogen-related receptors. Mol Cancer Res 2003;1(13):981–91.

[60] Eudy JD, Yao S, Weston MD, Ma-Edmonds M, Talmadge CB, Cheng JJ, et al. Isolation of a gene encoding a novel member of the nuclear receptor superfamily from the critical region of Usher syndrome type IIa at 1q41. Genomics 1998;50(3):382–4.

[61] Hong H, Yang L, Stallcup MR. Hormone-independent transcriptional activation and coactivator binding by novel orphan nuclear receptor ERR3. J Biol Chem 1999;274(32):22618–26.

[62] Yang N, Shigeta H, Shi H, Teng CT. Estrogen-related receptor, hER1, modulates estrogen receptor-mediated response of human lactoferrin gene promoter. J Biol Chem 1996;271:5795–804.

[63] Sladek R, Bader JA, Giguere V. The orphan nuclear receptor estrogen-related receptor alpha is a transcriptional regulator of the human medium-chain acyl coenzyme a dehydrogenase gene. Mol Cell Biol 1997;17(9):5400–9.

[64] Vega RB, Kelly DP. A role for estrogen-related receptor alpha in the control of mitochondrial fatty acid beta-oxidation during brown adipocyte differentiation. J Biol Chem 1997;272(50):31693–9.

[65] Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, et al. Genistein, a specific inhibitor of tyrosine-specific protein kinases. J Biol Chem 1987;262(12):5592–5.

[66] Paoletti R, Corsini A, Bellosta S. Pharmacological interactions of statins. Atherosclerosis 2002;3(1):35–40.